# Phytochemical and Pharmacological Studies on *Vitex altissima* and *Teramnus labialis*

**THESIS**

Submitted in partial fulfillment of
the requirements for the degree of
**DOCTOR OF PHILOSOPHY**

By
**CHENCHUGARI SRIDHAR**

Under the Supervision of
**Dr. G. V. SUBBARAJU**


**BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE**
**PILANI (RAJASTHAN) INDIA**
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# BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE
## PILANI (RAJASTHAN)

## CERTIFICATE

This is to certify that the thesis entitled **Phytochemical and Pharmacological Studies on *Vitex altissima* and *Teramnus labialis*** and submitted by **Mr. Chenchugari Sridhar** ID.No. **1998PHX F002** for the award of Ph.D. Degree of the Institute, embodies original work done by him under my supervision.

Signature in full of the Supervisor : G. V. Subba Raju

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Date
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# *ABBREVIATIONS*

| | |
|---|---|
| AA | : arachidonic acid |
| BHA | : butylated hydroxyanisole |
| BHT | : butylated hydroxytoluene |
| COSY | : correlation spectroscopy |
| COX | : cyclooxygenase |
| DEPT | : distortionless enhancement by polarization transfer |
| DMSO | : dimethyl sulfoxide |
| DPPH | : 1,1-Diphenyl-2-picrylhydrazyl |
| EtOAc | : ethyl acetate |
| g | : gram |
| HETE | : hydroxyeicosatetraenoic acid |
| HMBC | : heteronuclear multiple bond coherence |
| HMQC | : heteronuclear multiple quantum coherence |
| HPETE | : hydroperoxyeicosatetraenoic acid |
| HPLC | : high-performance liquid chromatography |
| HPTLC | : high-performance thin layer chromatography |
| 5-HT | : 5-hydroxytryptamine |
| IgE | : immunoglobulin E |
| IL | : interleukin |
| IR | : infrared |
| L | : litre |
| 5-LOX | : 5-lipoxygenase |
| LTs | : leukotrienes |
| MeOH | : methanol |
| MHz | : mega hertz |
| µg | : microgram |
| mg | : milligram |
| mL | : millilitre |
| mm | : millimeter |
| µm | : micrometer |

| | |
|---|---|
| μM | : micromole |
| mp | : melting point |
| NBT | : nitro blue tetrazolium |
| NDGA | : nordihydroguaiaretic acid |
| NO | : nitric oxide |
| NMR | : nuclear magnetic resonance |
| OD | : optical density |
| $PLA_2$ | : phospholipase $A_2$ |
| PGs | : prostaglandins |
| $R_f$ | : relative flow |
| ROS | : reactive oxygen species |
| SEM | : standard error mean |
| TNF-α | : tumor necrosis factor |
| TLC | : thin layer chromatography |
| $t_R$ | : retention time |
| TXs | : thromboxanes |
| UV | : ultraviolet |

# OBJECTIVES

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## Search for new anti-inflammatory agents

Medicinal plants represent the oldest known health care products that have been used by mankind all over the world in the form of folklore or traditional or ethnic medicines. The World Health Organization estimates that about 80% of the world's population still relies on herbal medicines as its major source of medicinal products.

India has an ancient heritage of traditional medicine. Indian traditional medicine is based on various systems including Ayurveda, Siddha and Unani. In almost all the traditional medicines, the medicinal plants play a major role and constitute the backbone of traditional medicine.

Chemical diversity in natural products serves as a rich source of novel pharmaceuticals, cosmetics, agrochemicals and other economically important chemicals. In fact, nearly 40% of all the currently prescribed drugs are directly or indirectly derived from plant sources.[1]

The earliest contribution of Ayurveda to modern drug development is reserpine, the anti hypertensive drug isolated from *Rauvolfia serpentina* (sarpagandha). It has received international attention and in a way rekindled interest of researchers in identifying possible leads from natural products.

In a search for bioactive compounds from plants, ethnobotanical observations offer the advantage, as many species are used in systems of traditional medicine. It has been estimated that nearly about 120 biologically active plant derived substances used were discovered by following up on leads from traditional medicine.[2]

Natural product research could be focused on a particular disease or disorder with the selection of a suitable in-vitro and in-vivo bioassay models used to monitor the crude extracts, chromatographic fractions and pure isolates of a plant or other organisms.

Over the last 10 years, a significant body of evidence has emerged that chemically diverse classes of naturally-occurring substances derived from higher plants are of potential interest for therapeutic interventions in several inflammatory diseases and a brief summary is presented in the following paragraphs.

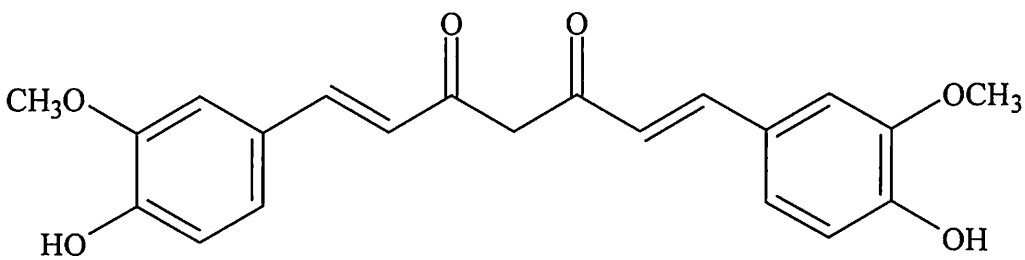
Inflammation can be defined as, normal essential protective and local tissue response to infections or injury by chemical or physical agents. Inflammation results from the complex series of actions and reactions triggered by body's immunological response to tissue damage. Many diseases as well physical trauma, including surgery, induce inflammatory reactions. These reactions, although necessary to start the healing process, too often leads to painful condition, which may even perpetuate the disease.[3]

A greatly increased understanding of the molecular and cellular mechanisms involved in the inflammation process has led to the discovery of many promising targets for the development of new drugs to treat chronic inflammatory diseases, such as rheumatoid arthritis, allergy, asthma, inflammatory bowl diseases and others.[3]

Most plant-derived secondary metabolites are known to interfere directly or indirectly with various inflammatory mediators such as, arachidonic acid metabolites, mediated by COX and LOX enzymes, peptides, cytokines and excitatory amino acids.

A wide range of chemical components possessing anti-inflammatory activity are obtained from plant sources and are commercially available such as Boswellic acids (*Boswellia serrata*), Gugulu steroids (*Commiphora mukul*), Punarnavin (*Bohervia diffusa*) etc.

*Curcuma longa* (turmeric) is one of the oldest anti-inflammatory drugs used in Ayurvedic medicine. Curcumin (**1**) and its congeners, the yellow pigments obtained from the rhizomes of the *Curcuma longa* are used in the management of various inflammatory disorders and wound healing. Several mechanisms have been proposed to explain the reported anti-inflammatory action of curcumin, including the inhibition of lipoxygenase and cyclooxygenase-2, metabolite production via arachidonic acid pathways and inhibition of macrophage activation. The anti-inflammatory activity of curcumin has also been attributed to its inhibitory activity on the nitric oxide (NO) production and nitric oxide synthase (NOS) activity and expression in macrophages.[4]

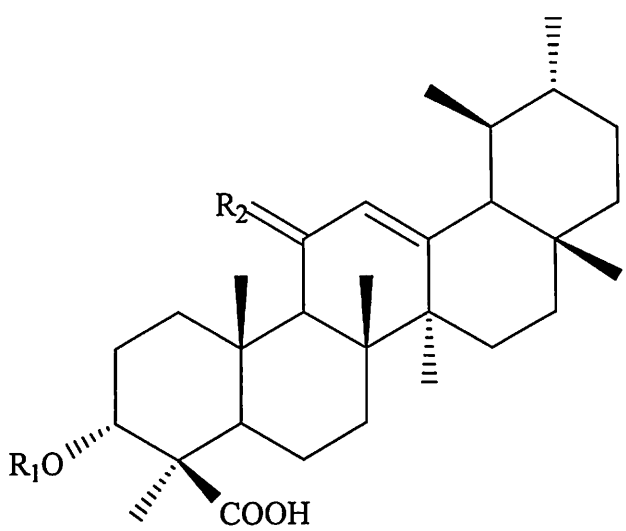



**1.** Curcumin

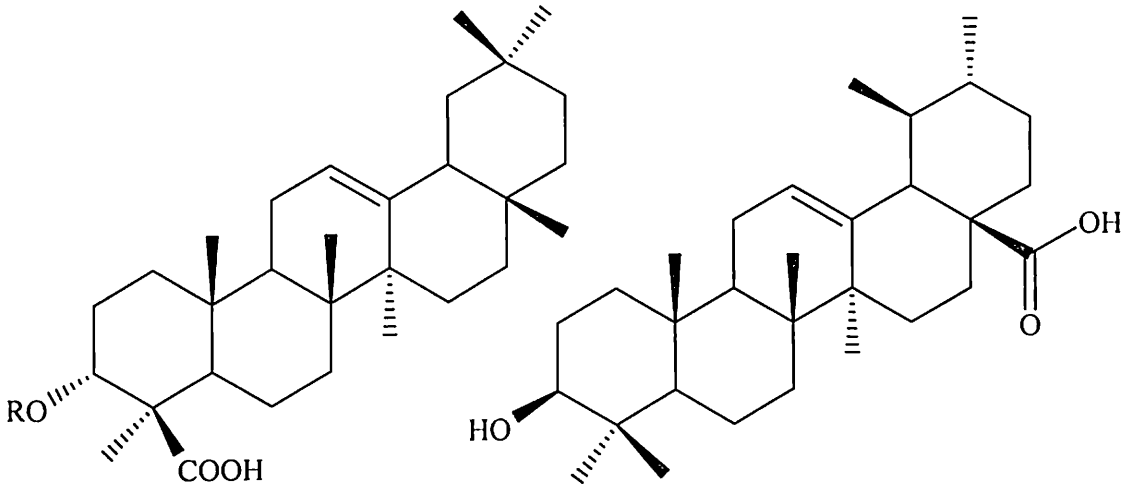
Pentacyclic triterpenoids constitute a class of naturally occurring substances that are widely distributed in plants and possess a broad range of biological actions, including significant anti-inflammatory effects.

The gum resin of *Boswellia serrata* has been used in the treatment of several inflammatory conditions. The oleogum resin mainly contains pentacyclic triterpene acids, commonly known as boswellic

acids (**2**-**7**).[5] Boswellic acids have exhibited significant inhibitory activity in various inflammatory models and acetyl-11-keto-*β*-boswellic acid (AKBA, **5**) has been reported as the most potent component of boswellic acids. The anti-inflammatory activity of boswellic acids is attributed to their selective inhibitory effect on 5-lipoxygenase enzyme and thus preventing the formation of inflammatory leukotrienes.[6] Acetyl-11-keto-*β*-boswellic acid (AKBA) inhibits the 5-LOX in non-competitive and specific manner ($IC_{50}$-1.5μM). A study on different analogues of AKBA revealed that the presence of pentacyclic skeleton is necessary for 5-LOX inhibition.[7]



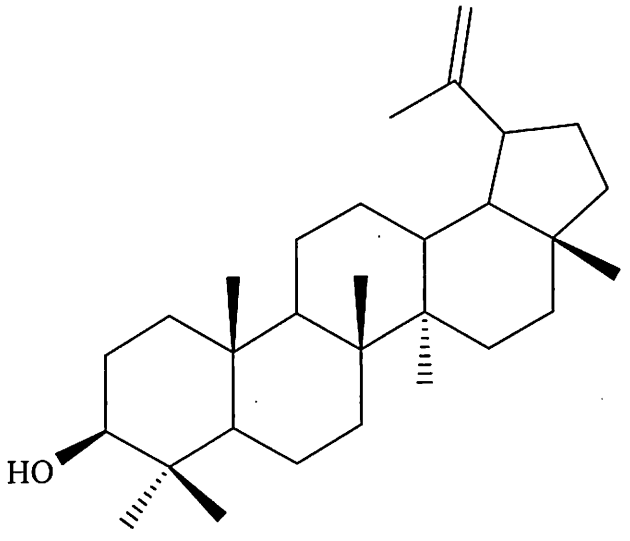

**2.** $R_1$=H; $R_2$=2H β-Boswellic acid
**3.** $R_1$=H; $R_2$=O 11-Keto-β-boswellic acid
**4.** $R_1$=Ac; $R_2$=2H 3-*O*-Acetyl-β-boswellic acid
**5.** $R_1$=Ac; $R_2$=O 3-*O*-Acetyl-11-keto-β-boswellic acid




**6.** R=H α-Boswellic acid **8.** Ursolic acid
**7.** R=Ac 3-*O*-Acetyl-α-boswellic acid

Ursolic acid (**8**) is ubiquitous in plant kingdom and it is a potent anti-inflammatory agent. Ursolic acid inhibits the cyclooxygenase and lipoxygenase enzymes.[8]

Lupeol (**9**) is another triterpene of plant origin, which acts as an anti-inflammatory agent. Lupeol prevented the generation of $PGE_2$ in macrophages with out affecting the levels of $LTC_4$.[9]




**9.** Lupeol

Several phenolic compounds of plant origin have been reported to exhibit significant anti-inflammatory activity.

Resveratrol (**10**), is a polyphenol present in grape seeds and other plants, is known to possess a wide range of biological actions including, anti-inflammatory, antiallergic and antioxidant. Resveratrol inhibits both COX-1 and COX-2 enzymes.[10]

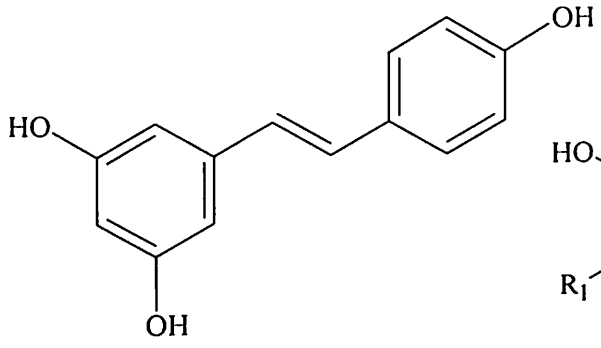
Flavonoids are polyphenolic compounds, widely distributed in plants. Several flavonoids have been reported to display marked anti-inflammatory properties.

Luteolin (**11**) isolated from *Perilla frutescens* exhibited significant inhibition of serum necrosis factor-α production, inhibition of arachidonic acid-induced ear edema, inhibition of 12-O-tetradecanoylphorbol-13-acetate-induced ear edema and inhibition of oxazolone-induced allergic edema.[11] Luteolin has also been reported to inhibit NO production.

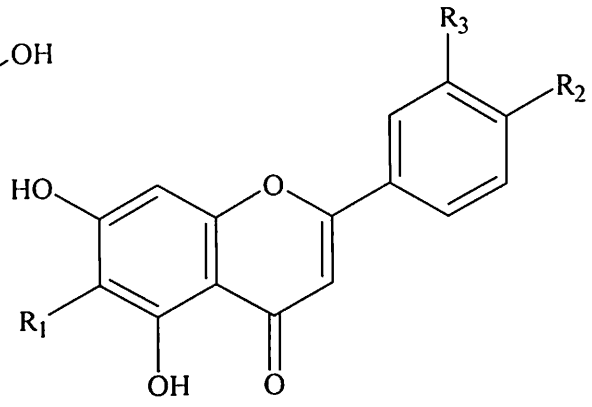
Baicalein (**12**) a flavonoid, isolated from roots of *Scutellaria baicalensis* is a selective inhibitor of 5-LOX and $LTC_4$.[12] Luteolin, quercetin (**13**) and baicalein (**12**) inhibited IgE-mediated TNF-α and IL-6 production from bone marrow-derived cultured murine mast cells (BMMC).

Other flavonoids, such as rutin (**14**), wogonin, (**15**) and naringenin (**16**) have been reported to exhibit anti-inflammatory activity through inhibition of COX-2 expression and activity in macrophages: Oroxylin-A, (**17**) baicalein (**12**) and wogonin (**15**) inhibited 12-LOX without affecting the levels of cyclooxygenase in human platelets.[13]

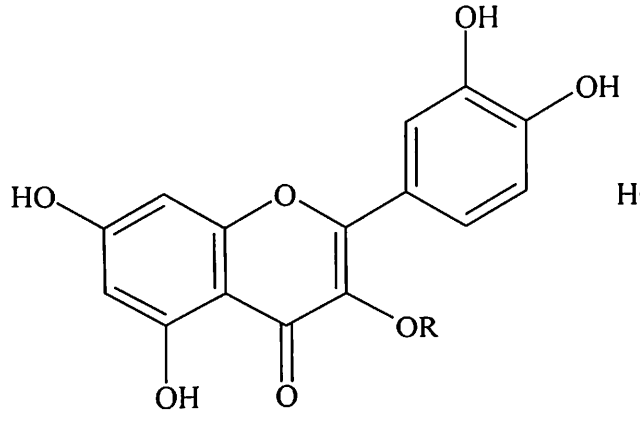
Cirsiliol, (**18**) is a potent inhibitor of 5-LOX.[14] Baicalin (**19**) has been reported to inhibit the production of prostaglandin $E_2$ and leukotriene $B_4$ biosynthesis. Other anti-inflammatory flavonoids such as, chrysin (**20**), apigenin (**21**), and phloretin (**22**) have been reported to inhibit COX-2 expression and platelet aggregation.[15]



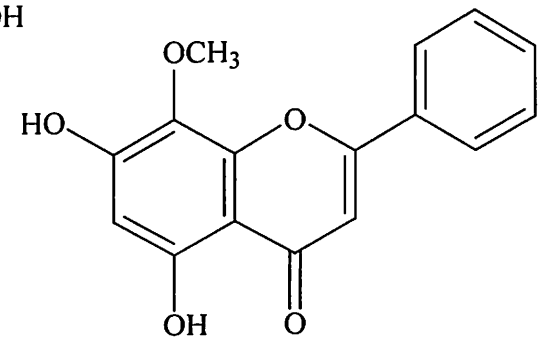

**10**. Resveratrol



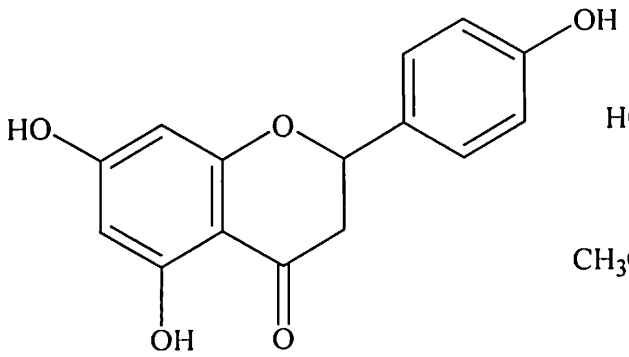

**11.** $R_1$=H; $R_2$=$R_3$=OH Luteolin
**12.** $R_1$=OH; $R_2$=$R_3$=H Baicalein
**20.** $R_1$=$R_2$=$R_3$=H Chrysin
**21.** $R_1$=$R_3$=H; $R_2$=OH Apigenin



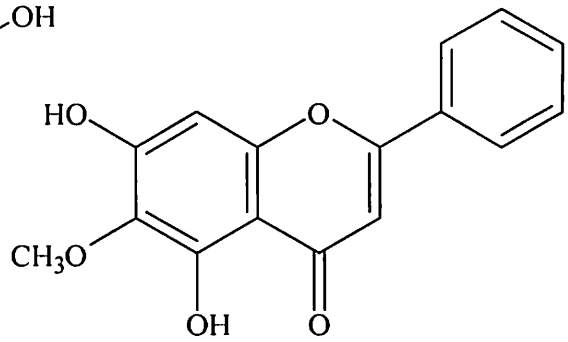

**13.** R=H Quercetin
**14.** R=Glu-Rha Rutin



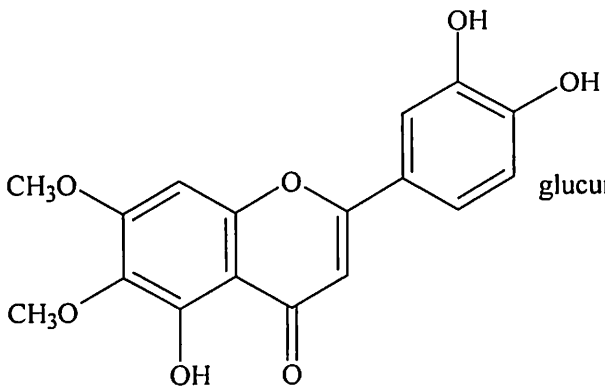

**15.** Wogonin



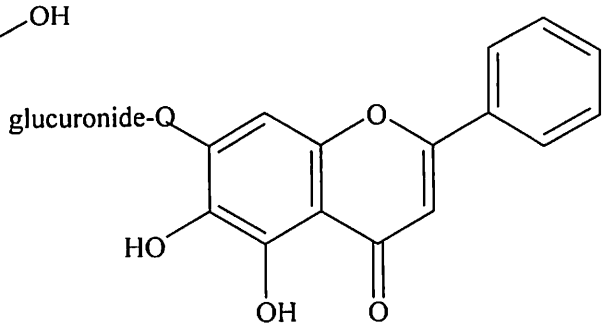

**16.** Naringenin



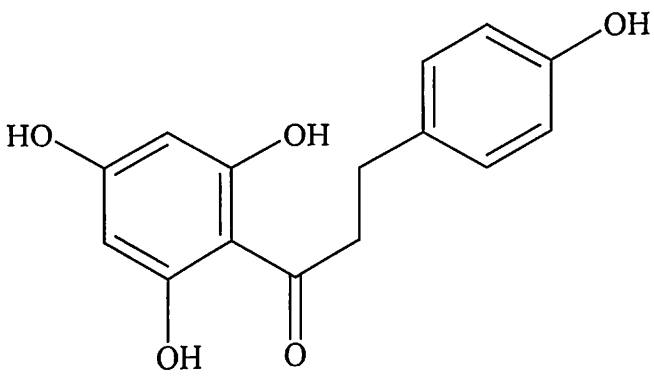

**17.** Oroxylin-A




**18.** Cirsiliol




**19.** Baicalin




**22.** Phloretin

An overview of the recent literature reveals that a great number of plant derived constituents exhibit a wide range of anti-inflammatory actions. Pharmacological and enzymatic studies have revealed that different chemical classes of plant-derived substances show promising anti-inflammatory activity by interacting with important cellular targets.

Thus, further investigation in this area may lead to the development of more safer and efficacious drugs for the management of inflammation and other related disorders.

In view of the reported folklore use of *Vitex altissima* and *Teramnus labialis*, in inflammatory conditions, the author has made a modest effort to screen these medicinal plants for their anti-inflammatory activity and isolate and characterize the chemical constituents responsible for the activity, to provide a scientific evidence for their folklore use in inflammatory conditions.

These two plants have not been studied earlier and are available locally in the Tirumala Hills. The results borne out of the detailed phytochemical and anti-inflammatory activity studies on these two medicinal plants have been presented in the following chapters. In **chapter-1**, a brief review on phytochemical and pharmacological studies on various species belonging to *Vitex* and *Teramnus* genus has been presented. The details of isolation, purification and characterization of several secondary metabolites of *V. altissima* and *T. labialis* have been presented in **chapters 2-7**. The details of anti-inflammatory activity studies on crude extracts, 5-lipoxygenase inhibitory activity and antioxidant activity studies on different isolates of *V. altissima* and *T. labialis* have been incorporated in **chapter-8**.

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# INTRODUCTION

The genus ***Vitex*** (n.o. Verbenaceae) consists largely of trees or shrubs and are widely distributed in the tropics and warm temperature regions of both the hemispheres.[1] About 250 species are known to occur worldwide and 14 species have been recorded in India.[2,3] Several species, including a few that are available in parts of the Deccan Peninsula and North-East India, yield timbers of commercial importance. About 8 species are used in the indigenous systems of Medicine, the Ayurveda and Unani. A large number of chemical constituents have been reported from different species and these include sterols, flavonoids, di and triterpenoids, and iridoids. Phytochemical studies on 21 species have been reported in literature and a brief summary is presented in the following pages.

## ***Vitex negundo* Linn**

A large aromatic shrub found throughout the greater part of India and is commonly known as sambhalu or nirgundi.[1-3] Different parts of the plant have been used for various medicinal purposes in the Ayurvedic and Unani systems of Medicine.[4] The leaves and roots are reported to be useful as anti-inflammatory agents.[1-5]

The leaves of *V. negundo* have been found to possess significant anti-inflammatory activity in carrageenan-induced hind paw edema in rats, in comparison with prednisolone.[6]

Telang *et al.*,[7] have screened the leaf extract for its analgesic and anti-inflammatory activities. The leaf extract, significantly increased the reaction time and decreased the writhing movement in mice in acetic acid-induced writhing test and also a significant increase in the reaction time in tail immersion test, supporting the peripheral and central analgesic effects of the leaf extract.

The extract showed weak anti-inflammatory activity in carrageenan-induced paw edema and highly significant activity in carrageenan-induced granuloma pouch model, revealing that the extract possesses better anti-inflammatory activity against sub-acute than acute inflammatory conditions. The analgesic and anti-inflammatory action of the extract is attributed to its flavonoid components, which are known to act through inhibition of prostaglandin biosynthesis.

*V. negundo* leaves extract exhibited significant inhibitory effect on cotton pellet induced granuloma in rats, when administered at a dose of 1000 mg/kg, for seven days.[8] It reduced weight of granulation tissue.

The anti-inflammatory activity was potentiated in presence of promethazine and cyproheptadine.[8]

During the course of experimental studies on anti-arthritic effects of Indigenous medicinal plants, Chaturvedi and Singh[9] have found that the extracts of the leaves of *V. negundo* prevented the development of swelling of joints in formalin induced experimental arthritis.

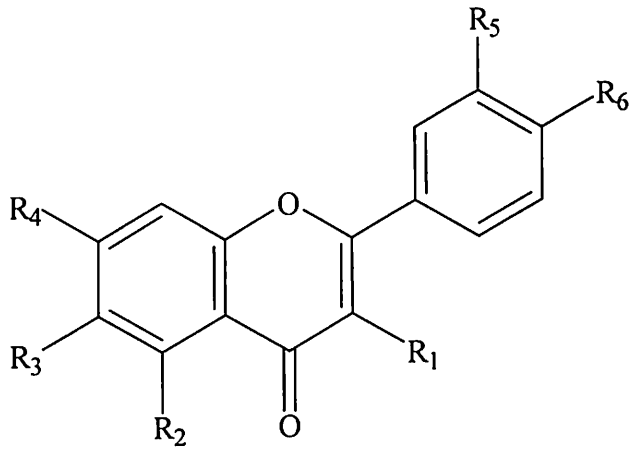
Methanol extractives of the leaves have been reported to potentiate the sleeping time induced by pentobarbitone sodium, diazepam, and chlorpromazine in mice and it also possesses analgesic properties and potentiated analgesia induced by morphine and pethidine. The methanol extract also showed significant protection against strychnine and leptazole induced convulsions.[10]

Oil obtained from steam distillate of leaves showed mosquito repellent activity against *Aedes* aegypti and bio-activity guided fractionation of the oil, led to an active fraction consisting of a mixture of mono and sesquiterpenes.[11] The leaf oil also showed significant antifungal activity against *Trichoderma viridae* and *Helminthosporium.*[12]

Methanolic extract of the leaves showed significant antihistamine property and bronchial releasing activity in induced cats trachea.[13] The active compounds in the extracts were identified as casticin (**1.01**), chrysosplenol D (**1.02**), luteolin (**1.03**), *p*-hydroxybenzoic acid and isoorientin (**1.04**).

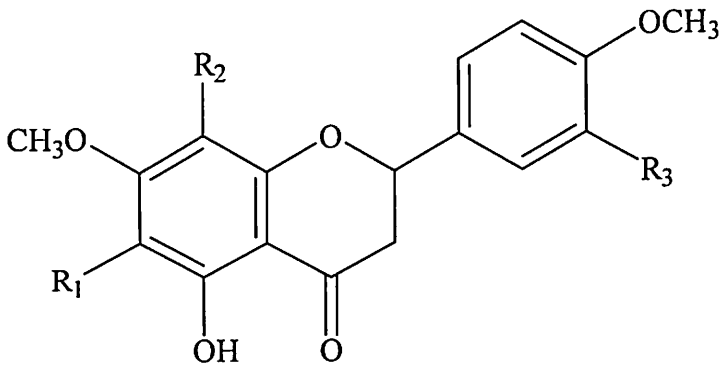
Hansel *et al.*,[14] have reported the presence of 5,3′-dihydroxy-3,6,7,4′-tetramethoxyflavone (casticin, **1.01**) in the leaves.

Cytotoxicity guided fractionation of the chloroform extract of leaves of *V. negundo* resulted in the isolation of flavone, casticin (**1.01**), which exhibited broad cytotoxicity in human cancer cell line panel.[15]



**1.01** $R_1$=$R_3$=$R_4$=$R_6$=$OCH_3$; $R_2$=$R_5$=OH
**1.02** $R_1$=$R_3$=$R_4$=$OCH_3$; $R_2$=$R_5$=$R_6$=OH
**1.03** $R_1$=$R_3$=H; $R_2$=$R_4$=$R_5$=$R_6$=OH
**1.04** $R_1$=H; $R_2$=$R_4$=$R_5$=$R_6$=OH; $R_3$=-*C*-glu

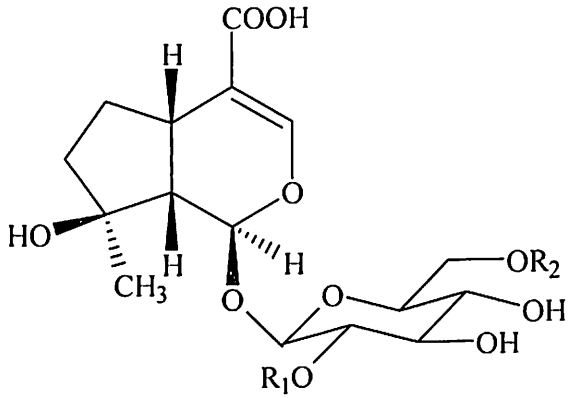
Achari *et al.*,[16] have isolated two isomeric flavanones, 5,3'-dihydroxy-7,8,4'-trimethoxyflavonone (**1.05**) and 5,3'-dihydroxy-6,7,4'-trimethoxyflavonone (**1.06**) from the leaves.



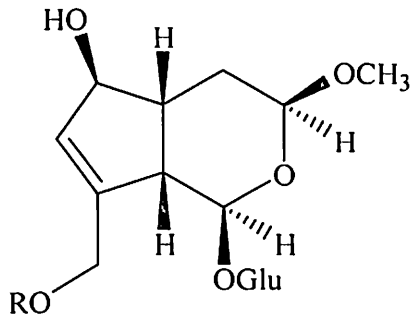
**1.05** $R_1$=H; $R_3$=OH; $R_2$=$OCH_3$
**1.06** $R_1$=$OCH_3$; $R_2$=H; $R_3$=OH

A new iridoid, 2'-*O*-*p*-hydroxybenzoylmussaenosidic acid (negundoside, **1.07**) was isolated from the leaves by Sehgal *et al.*,[17] and further investigation by the same authors[18] led to the isolation of 6'-*O*-*p*-hydroxybenzoylmussaenosidic acid (**1.08**) from the ethanolic extractives of the leaves.

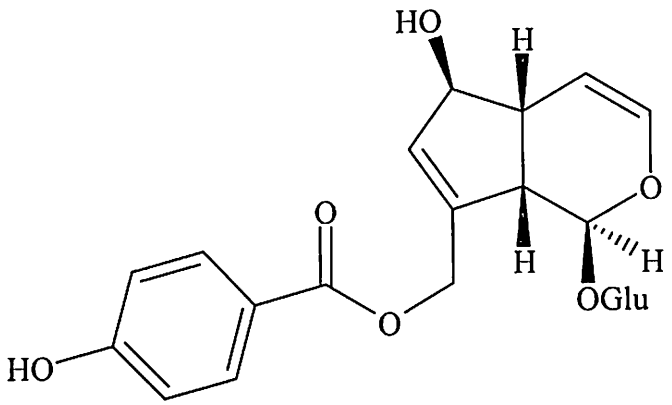
Dutta *et al.*,[19] have isolated negundoside (**1.07**), nishindaside (**1.09**), and agnuside (**1.10**), from the methanolic extractives of leaves of *V. negundo.*




**1.07** $R_1$=*p*-hydroxybenzoyl; $R_2$=H
**1.08** $R_1$=H; $R_2$=*p*-hydroxybenzoyl




**1.09** R=*p*-hydroxybenzoyl

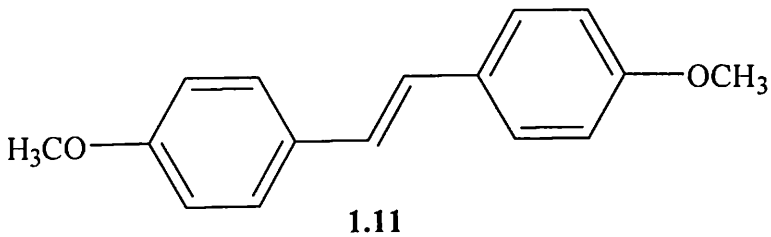



**1.10**

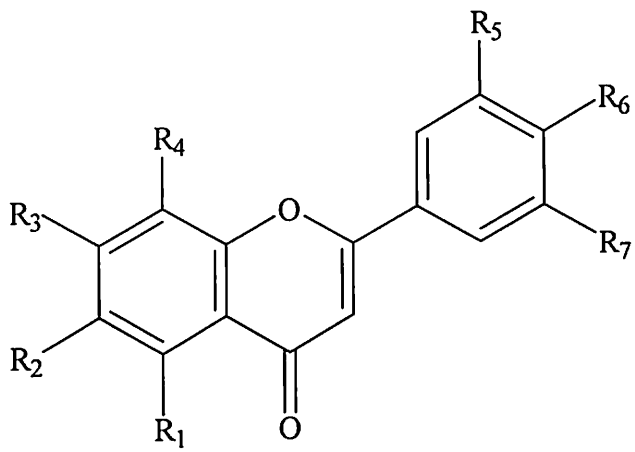
The leaves are reported to contain *p*-hydroxybenzoic acid, 5-hydroxyisophthalic acid and 3,4-dihydroxybenzoic acid.[20] Non-flavonoidal compounds, β-sitosterol, β-sitosterol acetate, and stigmasterol were isolated from the leaves by Mukherjee *et al.*[21]

Banerji *et al.*,[22] have isolated 4,4′-dimethoxy-*trans*-stilbene (**1.11**) along with five flavones, 5,6,7,8,3′,4′,5′-heptamethoxyflavone (**1.12**), 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone [5-O-desmethylnobiletin, **1.13**), 5-hydroxy-6,7,8,3′,4′,5′-hexamethoxyflavone [gardenin A, **1.14**), 5-hydroxy-6,7,8,4′-tetramethoxyflavone [gardenin B, **1.15**), 5-hydroxy-

7,3',4',5'-tetramethoxyflavone [corymbosin, **1.16**) from the leaves and twigs.

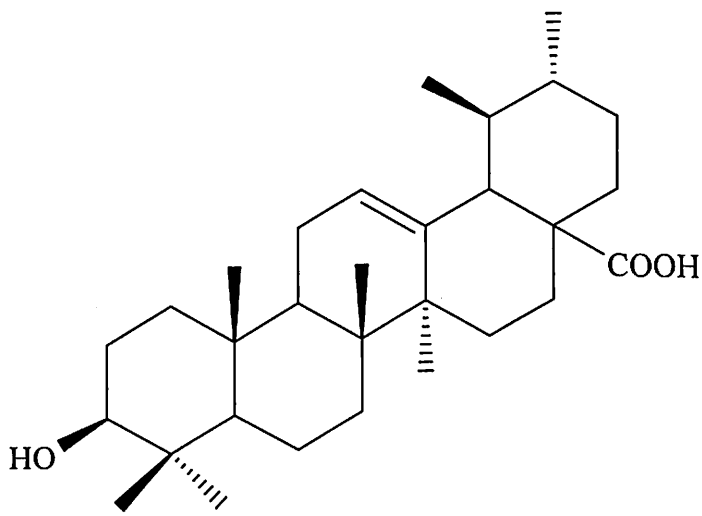


**1.11**

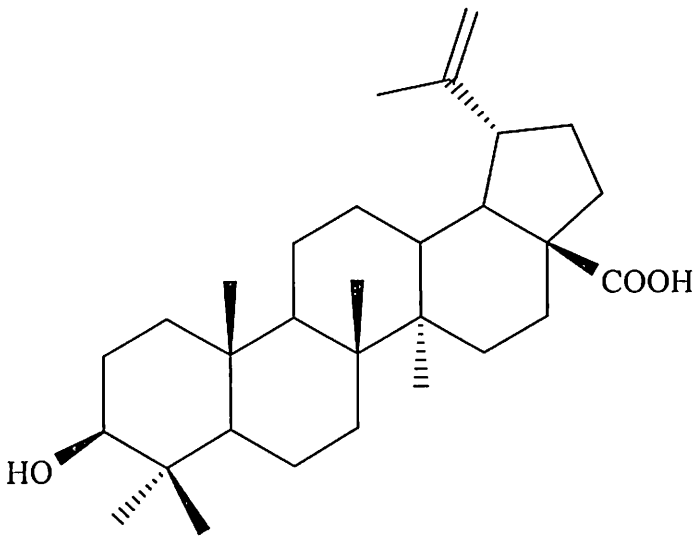


**1.12** $R_1=R_2=R_3=R_4=R_5=R_6=R_7=OCH_3$
**1.13** $R_1=OH$; $R_2=R_3=R_4=R_5=R_6=OCH_3$; $R_7=H$
**1.14** $R_1=OH$; $R_2=R_3=R_4=R_5=R_6=R_7=OCH_3$
**1.15** $R_1=OH$; $R_2=R_3=R_4=R_6=OCH_3$; $R_5=R_7=H$
**1.16** $R_1=OH$; $R_3=R_5=R_6=R_7=OCH_3$; $R_2=R_4=H$

Chandramu *et al.*,[23] have isolated ursolic acid (**1.17**) and betulinic acid (**1.18**) along with β-sitosterol and *p*-hydroxybenzoic acid from the leaves of *V. negundo*. Compounds **1.17** and **1.18** showed antifeedant activity against *Achoea janata*.



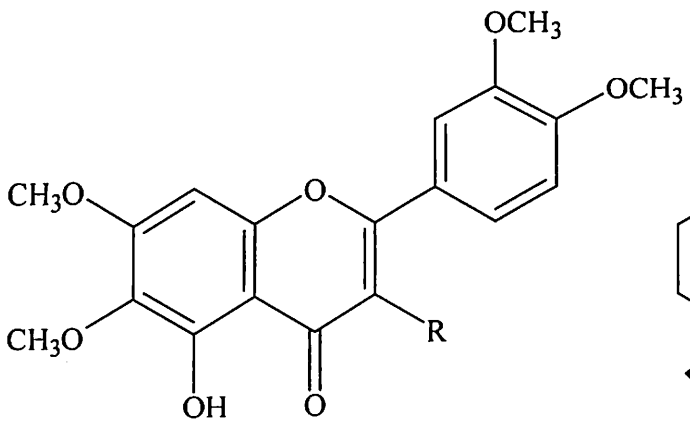
**1.17**



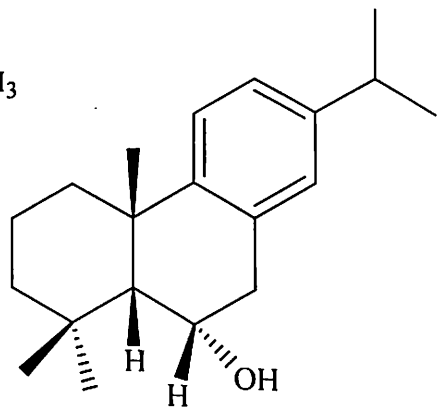

**1.18**

Banerji *et al.*[24], have reported the presence of 5-hydroxy-3,6, 7,3′,4′-pentamethoxyflavone (artemetin, **1.19**) from the leaves. Ferdous *et al.*,[25] have isolated **1.19**, and 3,5-dihydroxy-3′,4′,6,7-tetramethoxyflavanol (**1.20**).

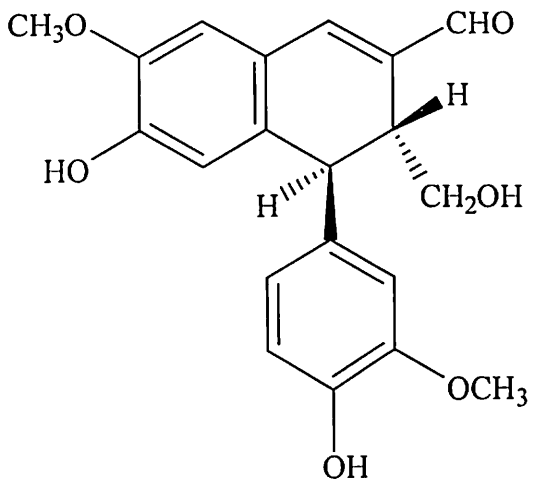
During the course of chemical investigation and anti-inflammatory activity studies on *V. negundo* seeds Chawla *et al.*,[26] have isolated a new anti-inflammatory diterpene, 5β-hydro-8,11,13-abietatrien-6α-ol (**1.21**) and **1.19** from the active fractions.




**1.19** R=$OCH_3$
**1.20** R=OH



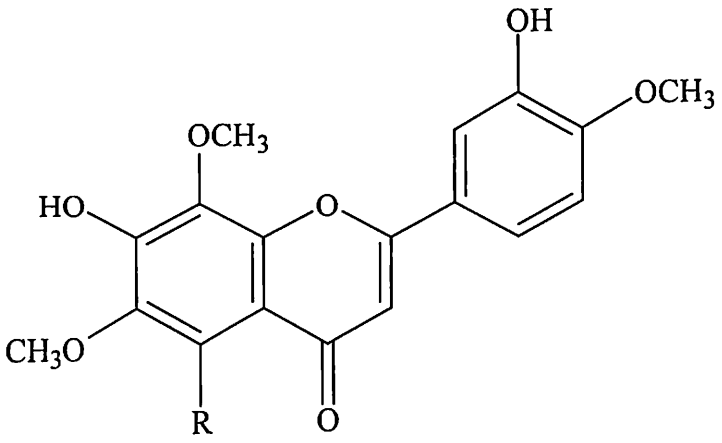

**1.21**




**1.26**

Alcoholic extract of the seeds of *V. negundo* was found to be effective in preventing the liver damage, induced by carbon tetrachloride in experimental animals.[29]

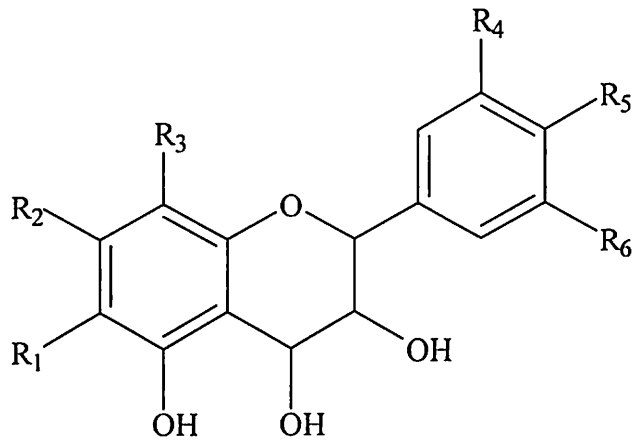
Bhargava,[30,31] had reported the antifertility and estrogenic activities of the extracts of seeds of *V. negundo* and isolated, flavonoids, 3′,5,7-trihydroxy-4′6,8-trimethoxyflavone (**1.27**) and its 5-*O*-glucoside (**1.128**) from the active fractions.



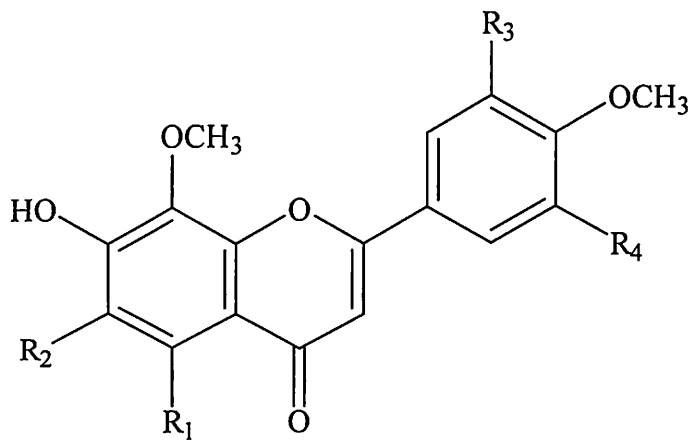

**1.27** R=OH
**1.28** R=OGlu

Kodanda Rao *et al.*,[32] have isolated β-sitosterol, vanillic acid, and luteolin (**1.03**) from the bark of *V. negundo*. Leucoanthocyanidins,

methyl ether of leucodelphindin (**1.29**), and leucocyanidin-7-*O*-rhamnoglucoside (**1.30**], were isolated from the stem bark by Subramanian and Misra.[33] Further investigation by the same authors led to the isolation of 6-*C*-glucosyl-5-*O*-rhamnopyranosyltrimethoxywogonin (**1.31**), and acerosin-5-*O*-glucoside monoacetate (**1.32**).[34]

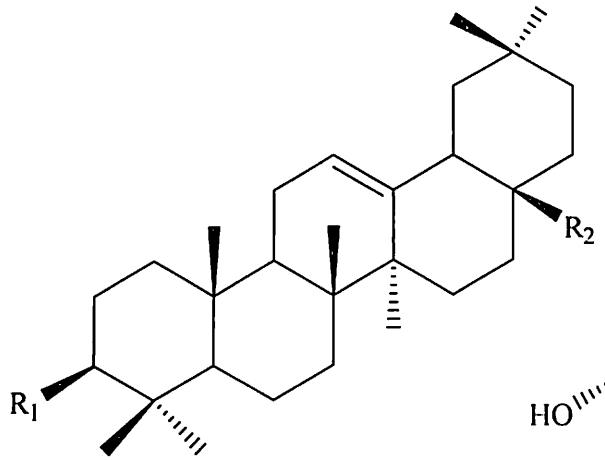


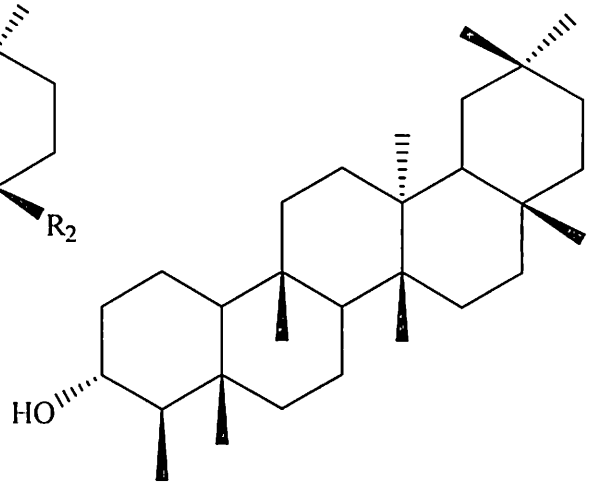

**1.29** $R_1$=$R_3$=$OCH_3$; $R_2$=$R_4$=$R_5$=$R_6$=OH
**1.30** $R_1$=$R_3$=$R_4$=H; $R_4$=$R_5$=$OCH_3$; $R_2$=-*O*-rhamnoglucoside




**1.31** $R_1$=*O*-rha; $R_2$=*C*-glu; $R_3$=$R_4$=$OCH_3$
**1.32** $R_1$=-*O*-[6-acetyl]glu; $R_2$=$OCH_3$; $R_3$=OH; $R_4$=H

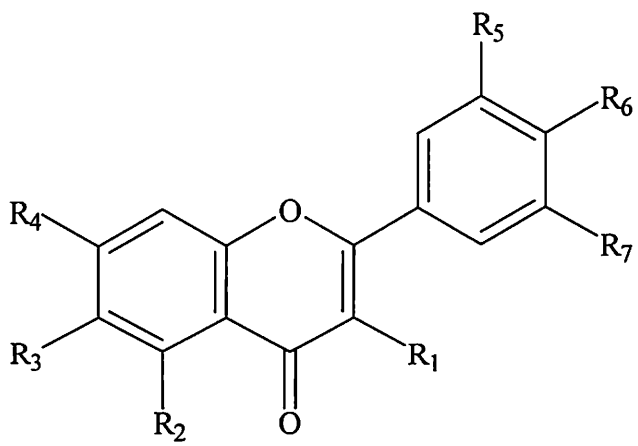
During the course of isolation of triterpenoids from the Indigenous plants, Vivek *et al.*,[35] have isolated β-amyrin (**1.33**), epifriedelinol (**1.34**) and oleanolic acid (**1.35**) from the heart wood of *V. negundo.*



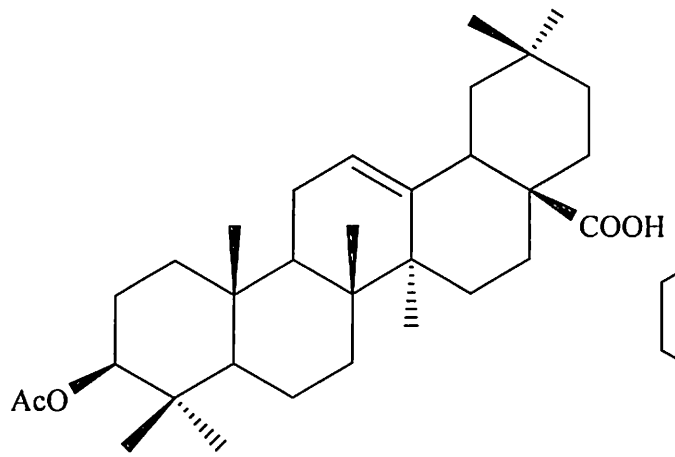

**1.33** $R_1$=OH; $R_2$=$CH_3$ **1.34**
**1.35** $R_1$=OH; $R_2$=COOH

From the ethyl acetate extract of the stem bark, Misra *et al.*,[36] have isolated 3,6,7,3′,4′-pentamethoxyflavone-5-*O*-glucopyranosyl rhamnoside (**1.36**), 4′-O-methylmyrecetin-3-*O*-(4″-*O*-β-D-galactosyl)-β-D-galactopyranoside (**1.37**).

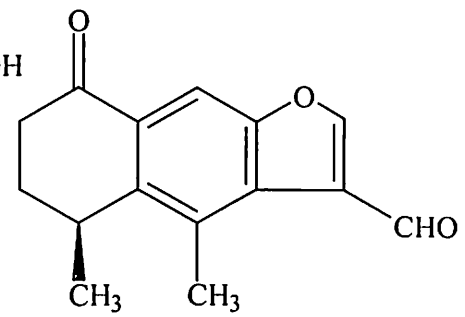



**1.36** $R_1$=$R_3$=$R_4$=$R_5$=$R_6$=$OCH_3$; $R_2$=-*O*-α-L-rha(1→4)-*O*-β-D-glu; $R_7$=H
**1.37** $R_1$=-*O*-gal(1→4)galactoside; $R_2$=$R_4$=$R_5$=$R_7$=OH; $R_3$=H; $R_6$=$OCH_3$

Vishnoi *et al.*,[37] have isolated acetyl oleanolic acid (**1.38**), β-sitosterol and a sesquiterpene, furanoeremophilane (**1.39**) from the ethanol extractives of roots of *V. negundo*.




**1.38**




**1.39**

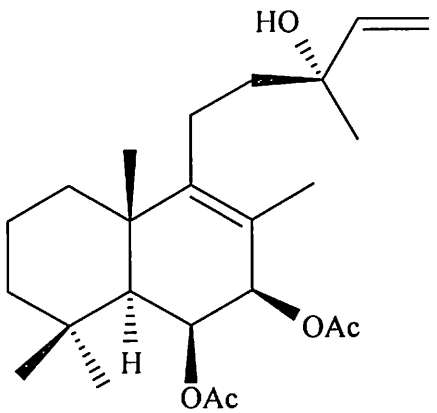
## *Vitex agnus-castus* Linn.

It is a small tree or shrub, commonly known as chaste tree and is distributed in the Mediterranean region, central Asia and southern Europe.[1-3,38] The fruits have been reported to possess important medicinal properties and are used in the treatment of premenstrual problems and hyperprolactinemia, because of their hormone like effects.[39,40] There is a long tradition on the use of different preparations of drugs of *V. agnus-castus* in complementary medicine in Europe.[41]

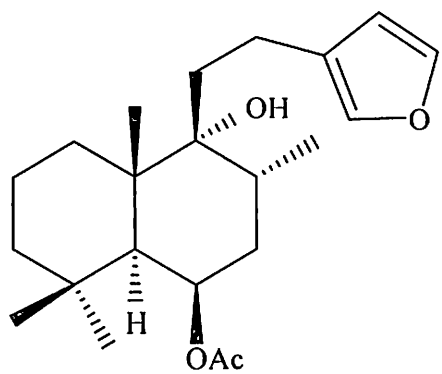
During the course of evaluation of estrogenic activity of plant extracts for the potential treatment of menopausal symptoms, Liu *et al.*,[42] had found that the methanol extract of *V. agnus-castus* fruits stimulated the progesterone receptor expression, but, no induction of alkaline phosphatase activity. Several experimental studies on the extracts of the fruits of *V. agnus-castus* supported its efficacy in the treatment of premenstrual symptoms.[43-48] The treatment of premenstrual symptoms by the *V. agnus-castus* extracts was reported to reduce the serum prolactin levels by exerting dopaminergic activity.[49]

Hoberg *et al.*,[50] have isolated three labdane-type diterpenoids, 6β,7β-diacetoxy-13-hydroxy-labda-8,14-diene (**1.40**), rotundifuran (**1.41**) and vitexilactone (**1.42**) from the hexane extract of fruits of *V. agnus-castus*. Compounds **1.40** and **1.41** were found to inhibit the secretion of prolactin by exerting their action on dopamine-$D_2$-receptor.

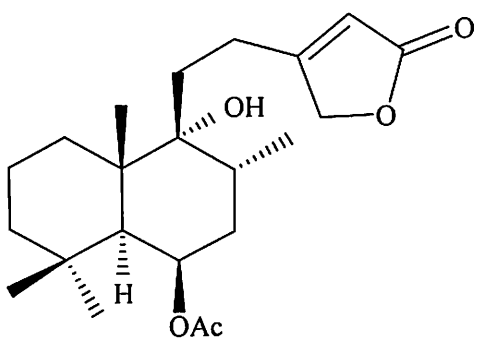
Li *et al.*,[51] have isolated a diterpene alkaloid, vitexlactam A (6β-acetoxy-9α-hydroxy-13-labden-16,15-amide, **1.43)** from the hexane extract of the fruits.



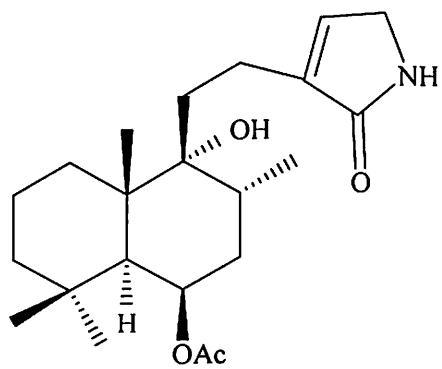

**1.40**




**1.41**



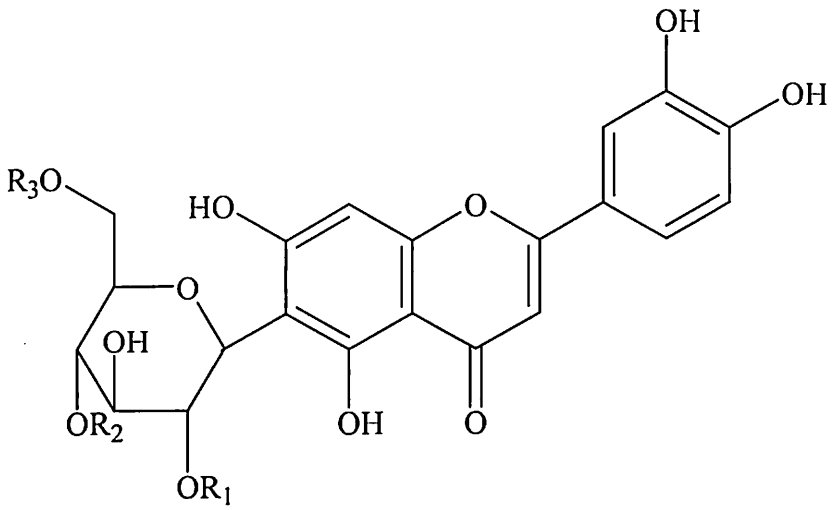

**1.42**



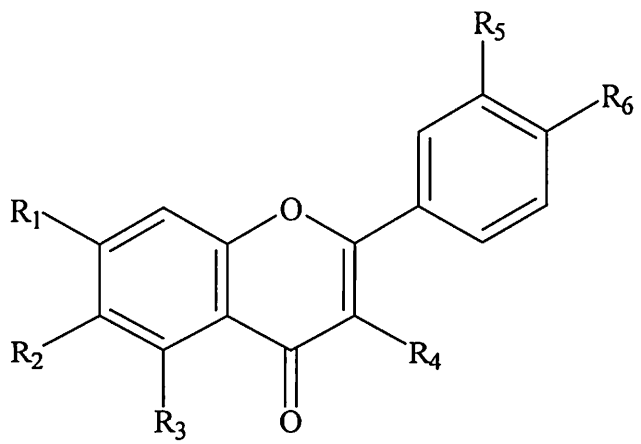

**1.43**

During the course of a study on cytotoxic principles of *V. agnus-castus*, Hirobe *et al.*,[52] have isolated four new flavonoids, namely, luteolin 6-*C*-(4"-methyl-6"-*O*-*trans*-caffeoylglucoside) (**1.44**), luteolin 6-*C*-(6"-*O*-*trans*-caffeoylglucoside) (**1.45**), luteolin 6-*C*-(2"-*O*-*trans*-caffeoylglucoside), (**1.46**), luteolin 7-*O*-(6"-*p*-hydroxybenzoylglucoside),

(**1.47**) along with the known 4′,5-dihydroxy-3,3′,6,7-tetramethoxyflavone (**1.48**), luteolin (**1.03**), artemetin (**1.19**) and isorhamnetin (**1.49**) from the fruits. Among these, **1.48** exhibited potent cytotoxic activity ($IC_{50}$ 0.1$\mu$g/ml) against P 388 lymphocytic leukemia cells.



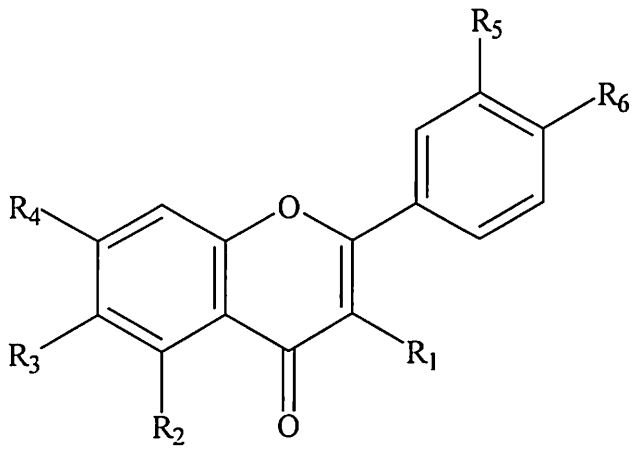

**1.44** $R_1$=H; $R_2$=$CH_3$; $R_3$= *trans*-caffeoyl
**1.45** $R_1$=$R_2$=H; $R_3$= *trans*-caffeoyl
**1.46** $R_1$= *trans*-caffeoyl; $R_2$=$R_3$=H




**1.47** $R_1$=-*O*-(6″-*p*-hydroxybenzoyl) glu; $R_2$=$R_4$=H; $R_3$=$R_5$=$R_6$=OH
**1.48** $R_1$=$R_2$=$R_4$=$R_5$=$OCH_3$; $R_3$=$R_6$=OH
**1.49** $R_1$=$R_3$=$R_4$=$R_6$=OH; $R_5$=$OCH_3$; $R_2$=H

Wollenweber *et al.*,[53] have isolated casticin (**1.01**), penduletin (**1.50**), chrysosplenol D (**1.02**), 5-hydroxy-3,6,7,4′-tetramethoxyflavone (**1.51**) from the fruits of *V. agnus-castus*.

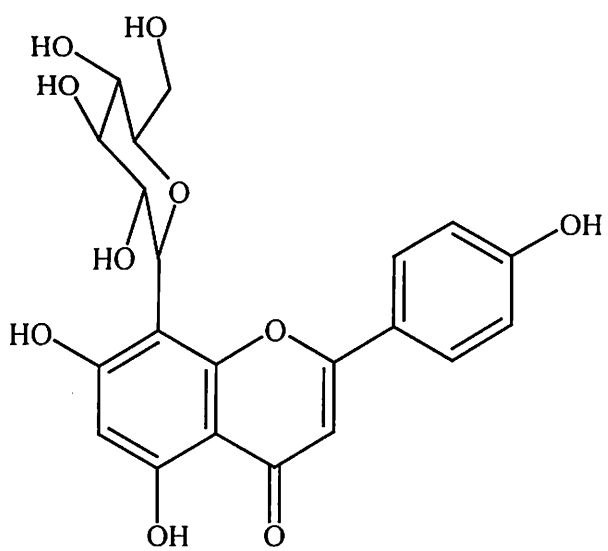
Flavonoids such as casticin (**1.01**) and luteolin-7-*O*-glucoside (**1.52**) were isolated from the leaves by Sirait *et al.*[54]



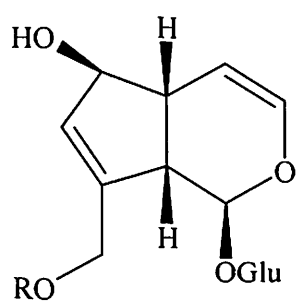
**1.50** $R_1$=$R_3$=$R_4$=$OCH_3$; $R_2$=$R_6$=OH; $R_5$=H
**1.51** $R_1$=$R_3$=$R_4$=$R_6$=$OCH_3$; $R_2$=OH; $R_5$=H
**1.52** $R_1$=$R_3$=H; $R_2$=$R_5$=$R_6$=OH; $R_4$=*O*-glu

During the course of chemical investigation on different *Vitex* species, Rao,[55] had isolated a *C*-glucosyl flavonoid, vitexin (**1.53**) from the leaves of *V. agnus-castus*. Hansel and Winde[56] reported the presence of agnuside (**1.10**) in the leaves and fruits.

Iridoids, aucubin (**1.54**), agnuside (**1.10**), and eurotoside (**1.55**) were also isolated from the leaves of this plant by Goerler *et al.*[57]



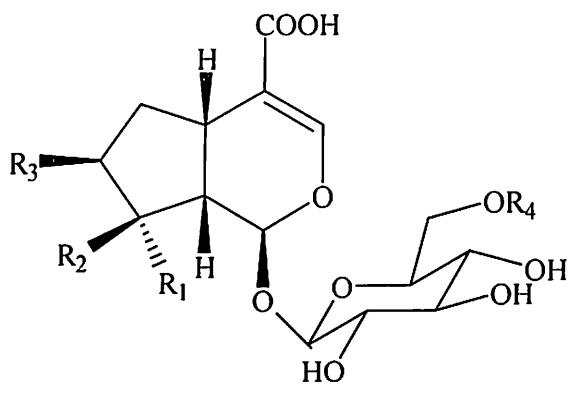
**1.53**

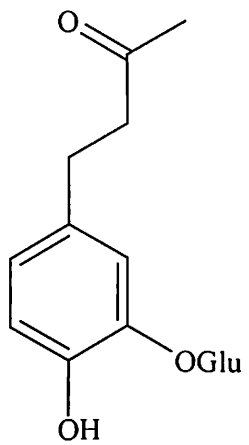


**1.54** R=H
**1.55** R=*p*-coumaryl

Casticin (**1.01**) was isolated from the seeds of *V. agnus-castus* by Belic *et al.*[58]

During the chemical investigation on the glycosides in flowering stems of *V. agnus-castus*, Kuruuzum-Uz *et al.*,[59] have isolated three new iridoids, namely, 6′-*O*-foliamenthoylmussaenosidic acid (agnucastoside A, **1.56**), 6′-*O*-(6,7-dihydrofoliamenthoyl) mussaenosidic acid (agnucastoside B, **1.57**), 7-*O*-trans-*p*-coumaroyl-6′-*O*-*trans*-caffeoyl-8-epiloganic acid (agnucastoside C, **1.58**), along with four known iridoids, aucubin (**1.54**), agnuside (**1.10**), mussaenosidic acid (**1.59**), 6′-*O*-*p*-hydroxybenzoylmussaenosidic acid (**1.08**) and a phenylbutanone glucoside (myzodendrone, **1.60**) from the methanol extract. The glucosides isolated did not exhibit significant antimicrobial or cytotoxic activities.



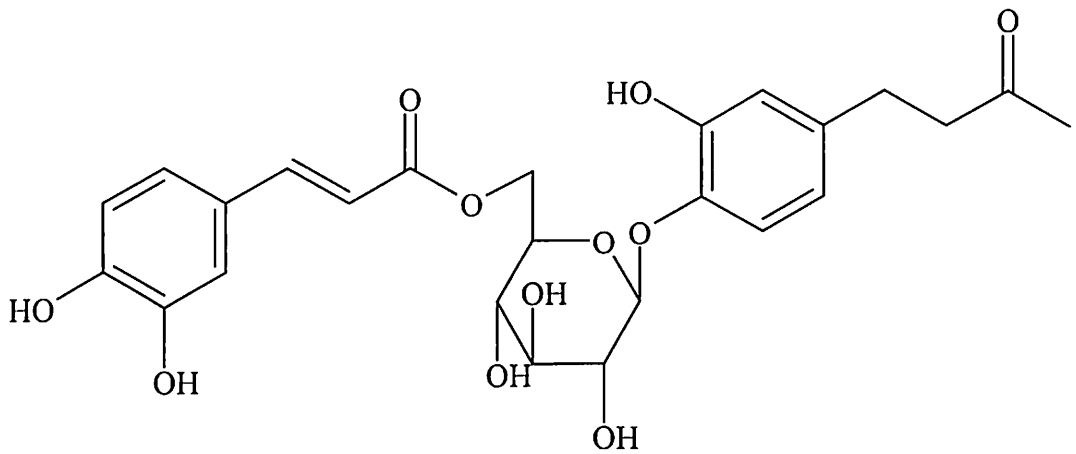

**1.60**

**1.56** $R_1$=$CH_3$; $R_2$=OH; $R_3$=H; $R_4$=foliamenthoyl
**1.57** $R_1$=$CH_3$; $R_2$=OH; $R_3$=H; $R_4$=6,7-dihydrofoliamenthoyl
**1.58** $R_1$=$CH_3$; $R_2$=H; $R_3$=-*O*-*p*-coumaryl; $R_4$=-*trans*-caffeoyl
**1.59** $R_1$=$CH_3$; $R_2$=OH; $R_3$=H; $R_4$=H

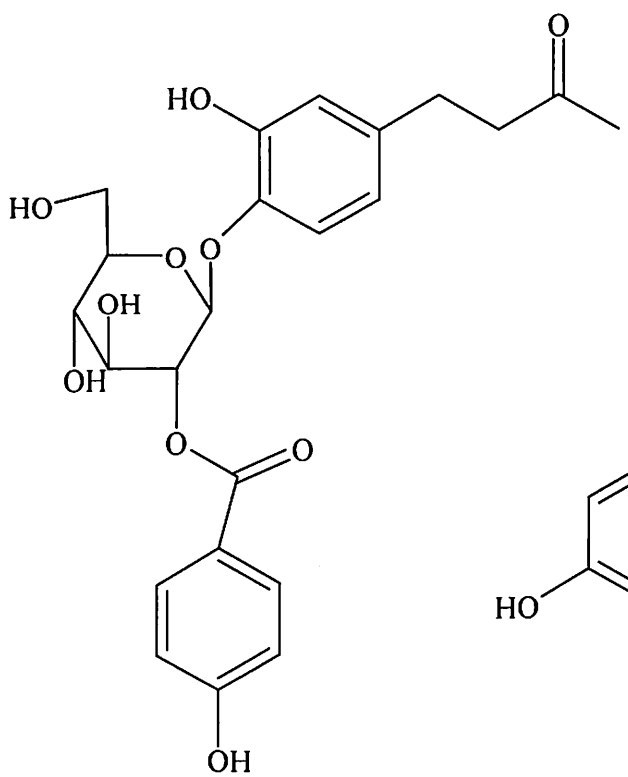
## *Vitex rotundifolia* Linn

It is widely distributed in Japan and Southeast Asia and its fruits (Viticis Fructus) are used as folk medicine in China and Japan for headaches, cold, migraine, eye-pain, and other inflammatory conditions.[60] The leaves are reported to contain casticin (**1.01**).[61]

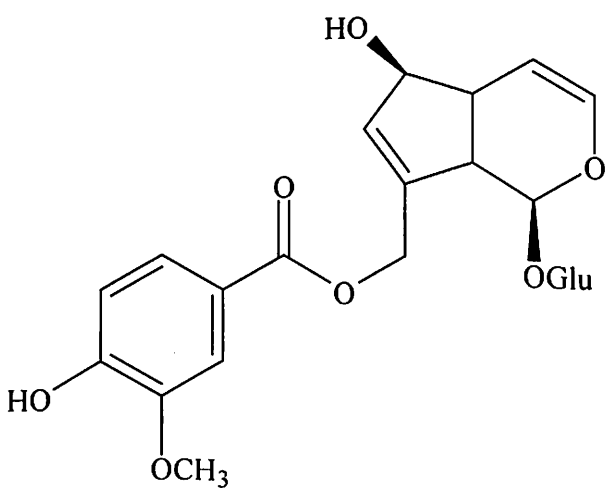
Bio-assay guided fractionation based on analgesic activity of methanolic extractives of fruits of *V. rotundifolia* yielded vitexifolin A (**1.61**), vitexifolin B (**1.62**), vitexifolin C (**1.63**) 10-O-vanilloylaucubin (**1.64**), dihydrodehydrodiconiferylalcohol-β-D-(2'-O-*p*-hydroxybenzoyl) glucoside (**1.65**), vanilloyl-β-D-(2'-O-*p*-hydroxybenzoyl) glucoside (**1.66**), agnuside (**1.10**), *erythro*-guaiacylglycerol (**1.67**) and *threo*-guaiacylglycerol (**1.68**) from the active fraction.[62]



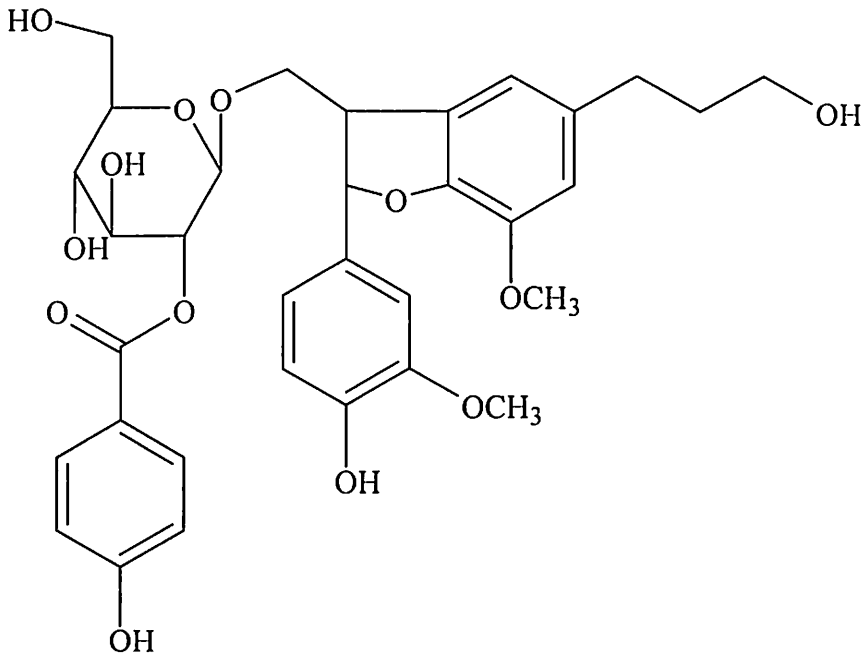
**1.61** (7",8"-*trans*)
**1.62** (7",8" -*cis*)



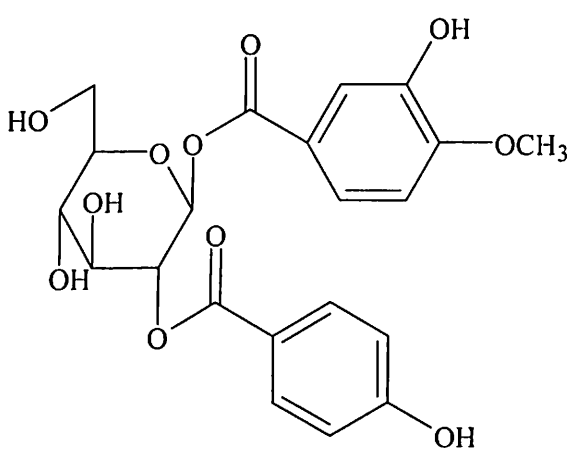
**1.63**



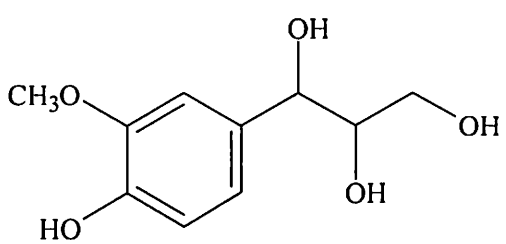
**1.64**



**1.65**

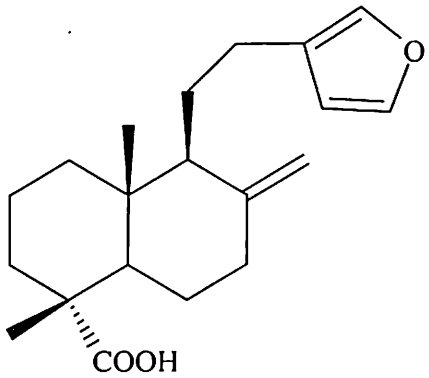


**1.66**

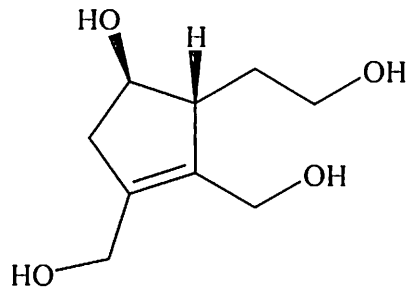


**1.67** and **1.68**
*erythro* and *threo*

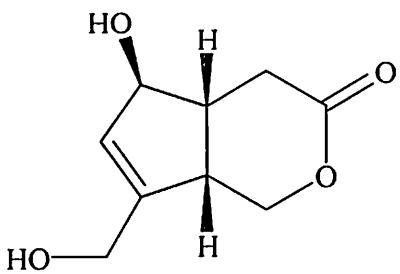
Methanolic extract of *V. rotundifolia* exhibited antimutagenic activity and the active fraction yielded (+)-polyalthic acid (**1.69**).[63] During the course of studies on natural antioxidants, Ono *et al.*,[64] have isolated several iridoids, namely, eucommiol (**1.70**), iridolactone (**1.71**), pedicularis-lactone (**1.72**), 1-oxo-eucommiol (**1.73**), 10-O-vanilloyl aucubin (**1.64**), agnuside (**1.10**), viteoid I (**1.74**) and viteoid-II (**1.75**) from the methanolic extractives of fruits of *V. rotundifolia.* These compounds showed potent antioxidative activity in comparison with tert-butylhydroxyanisole.



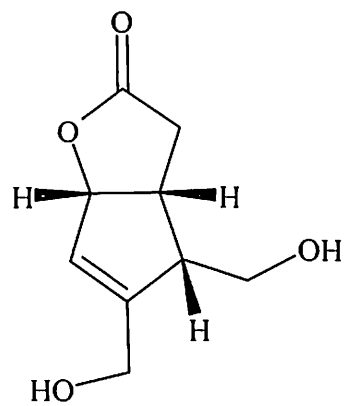

**1.69**



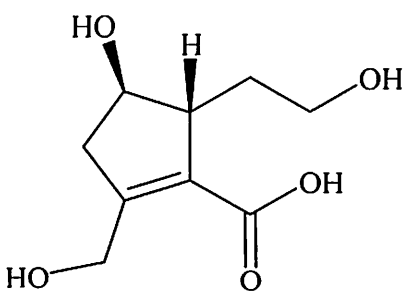

**1.70**



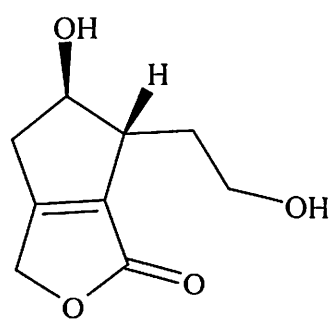

**1.71**



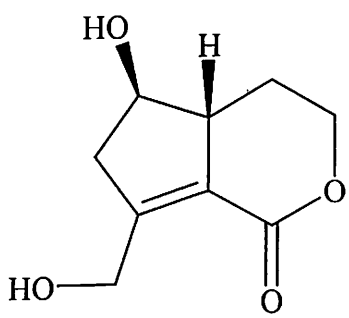

**1.72**




**1.73**




**1.74**

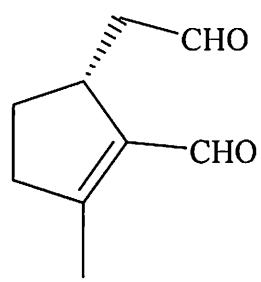



**1.75**

An ether extract of the fruits, exhibited potent inhibition of rat lens aldose reductase activity in vitro and the active fraction yielded luteolin (**1.03**).[65]

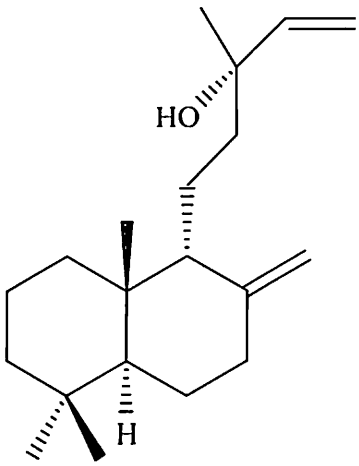
Shin *et al.*,[66] have investigated the effect of aqueous extract of the fruits on the immediate-type allergic reactions in vivo and in vitro. The extracts have reduced the plasma histamine levels in a dose-dependent manner when employed in a systemic allergic reaction test. It has also produced a significant inhibitory effect on anti-DNP IgE-induced tumor necrosis factor-α production from rat peritoneal mast cells. These results indicated that *V. rotundifolia* fruit extract may be beneficial in the regulation of immediate-type allergic reaction.

Watanabe *et al.*,[67] have isolated a new natural mosquito repellent monoterpene, rotundial (**1.76**) from the fresh leaves of *V. rotundifolia.* Rotundial showed potent repelling activity against *Aedes aegypti.*

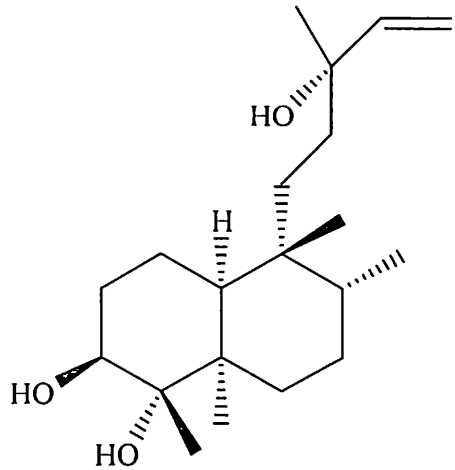



**1.76**

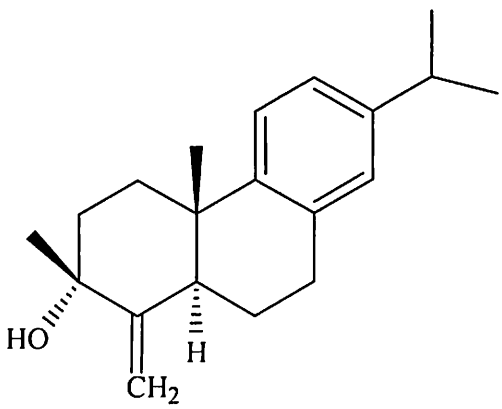
Ono *et al.*,[68] have isolated a labdane-type diterpene, vitexifolin A (**1.77**), a new clerodane-type diterpene, vitexifolin B (**1.78**), abeoabietane-type diterpene, vitexifolin C (**1.79**), two norlabdane-type diterpenes, vitexifolin D (**1.80**), vitexifolin E (**1.81**), a halimane type diterpene, vitetrifolin D (**1.82**), trisnor-γ-lactone (**1.83**), iso-ambreinolide (**1.84**), and three flavonoids, namely, casticin (**1.01**), artemetin (**1.19**), and 5,3′-dihydroxy-6,7,4′-trimethoxyflavanone (**1.06**) from the methanolic extract of fruits. Among the isolated compounds, casticin exhibited considerable growth inhibitory activity against human lung cancer cells (PC-12) and human colon cancer cells (HCT116).



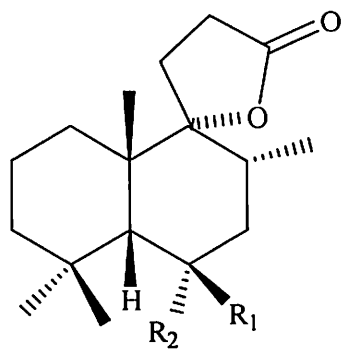

**1.77**



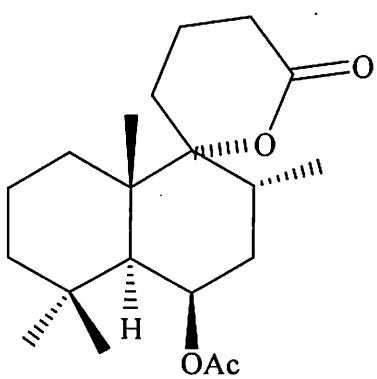

**1.78**



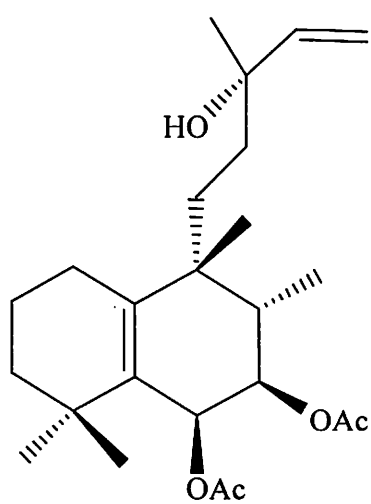

**1.79**




**1.80** $R_1$=H; $R_2$=OAc
**1.83** $R_1$=OAc; $R_2$=H
**1.84** $R_1$=$R_2$=H



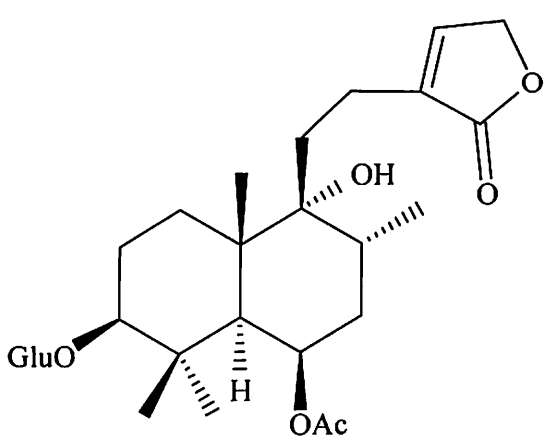

**1.81**



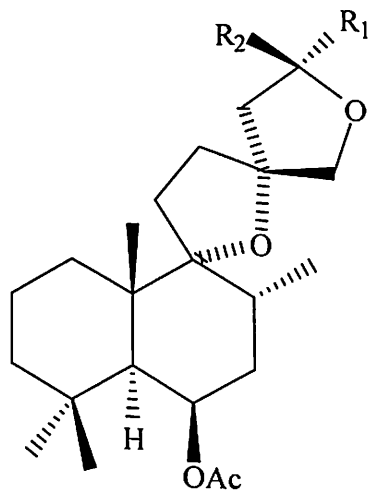

**1.82**

During the course of study on inhibitory effect of traditional herbal medicines on the infectivity of rotavirus, Kim *et al.*,[69] have reported the antiviral activity of *V. rotundifolia* fruit extract against rotavirus (25% inhibition).

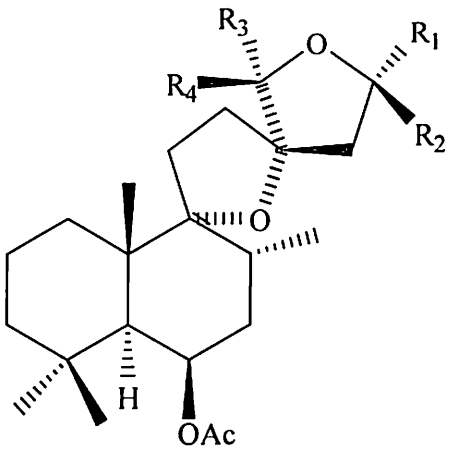
Ono *et al.*,[70,71] have isolated nine new labdane-type diterpenoids, namely, viteoside A (**1.85**), (rel 5S,6R, 8R, 9R, 10S, 13R, 15R)-6-acetoxy-9,15;15,16-diepoxy-15-methoxylabdane (**1.86**), (rel 5S,6R, 8R, 9R, 10S, 13R, 15S)-6-acetoxy-9,15;15,16-diepoxy-15-methoxylabdane (**1.87**), (rel 5S,6R, 8R, 9R, 10S, 13S, 15S)-6-acetoxy-9,15;15,16-diepoxy-15-methoxylabdane (**1.88**), (rel 5S,6R, 8R, 9R, 10S, 13S, 15R)-6-acetoxy-9,15;15,16-diepoxy-15-methoxylabdane (**1.89**), (rel 5S,6R, 8R, 9R, 10S, 13S, 15S, 16R)-6-acetoxy-9,13;15,16-diepoxy-15,16-dimethoxylabdane (**1.90**), (rel 5S,6R, 8R, 9R, 10S, 13S, 15R, 16R)-6-acetoxy-9,13;15,16-diepoxy-15,16-dimethoxylabdane (**1.91**), (rel 5S,6R, 8R, 9R, 10S, 13S, 15S, 16S)-6-acetoxy-9,13;15,16-diepoxy-15,16-dimethoxylabdane (**1.92**), and (rel 5S,6R, 8R, 9R, 10S, 13S, 15R, 16S)-6-acetoxy-9,13;15,16-diepoxy-15,16-dimethoxylabdane (**1.93**), along with two known abietane-type diterpenoids, ferruginol (**1.94**), and abietatrien-3β-ol (**1.95**) from the fruits of *V. rotundifolia.*

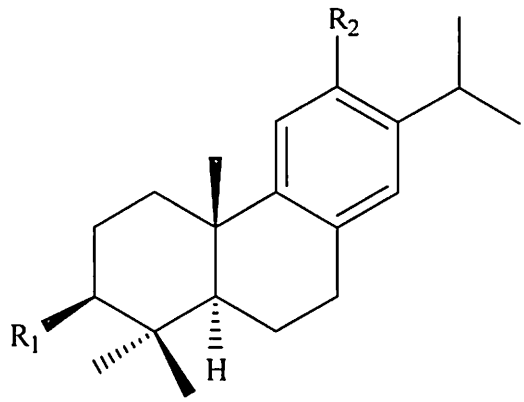



**1.85**




**1.86** $R_1$=$OCH_3$; $R_2$=H
**1.87** $R_1$=H; $R_2$=$OCH_3$

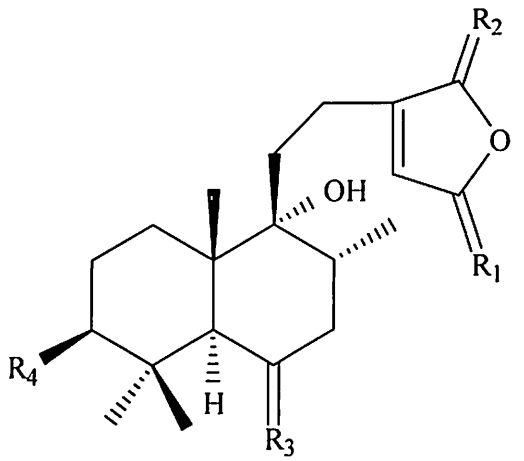


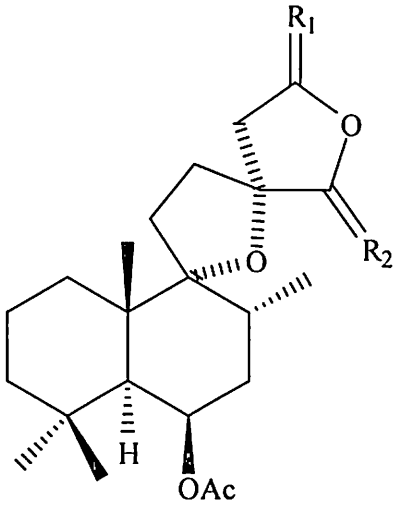

**1.88** $R_1$=$OCH_3$; $R_2$=$R_3$=$R_4$=H
**1.89** $R_1$=$R_3$=$R_4$=H; $R_2$=$OCH_3$
**1.90** $R_1$=$R_3$=$OCH_3$; $R_2$=$R_4$=H
**1.91** $R_1$=$R_4$=H; $R_2$=$R_3$=$OCH_3$
**1.92** $R_1$=$R_4$=$OCH_3$; $R_2$=$R_3$=H
**1.93** $R_1$=$R_3$=H; $R_2$=$R_4$=$OCH_3$

**1.94** $R_1$=H; $R_2$=OH
**1.95** $R_1$=OH; $R_2$=H

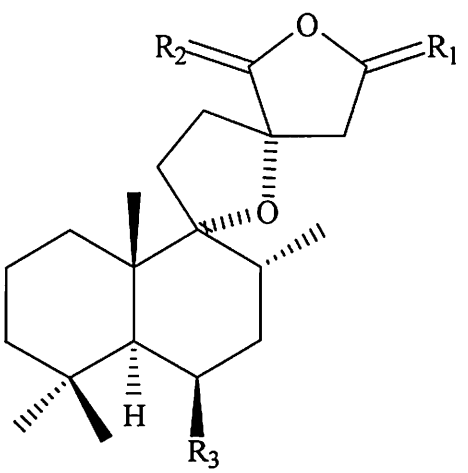
Further investigation by the same authors[72] led to the isolation of ten new labdane-type diterpenoids from the methanolic extractives of fruits of *V. rotundifolia*, namely, (rel 5S,6R,8R,9R,10S)-6-acetoxy-9-hydroxy-13(14)-labden-16,15-olide (**1.96**), (rel 5S,6S,8R,9R,10S)-6-acetoxy-9-hydroxy-13(14)-labden-16,15-olide (**1.97**), (rel 5S,6R,8R,9R,10S)-6-acetoxy-9-hydroxy-15-methoxy-13(14)-labden-16,15-olide (**1.98**), (rel 5S,6R,8R,9R,10S,13S,16S)-6-acetoxy-9,13-epoxy-16-methoxy-labden-15,16-olide (**1.99**), (rel 5S,6R,8R,9R,10S,13R,16S)-6-acetoxy-9,13-epoxy-15-methoxy-labdan-16,15-olide (**1.100**), (rel 5S,6R,8R,9R,10S,13S)-6-acetoxy-9,13-epoxy-16-methoxy-labden-15,16-olide (**1.101**), (rel 5S,6R,8R,9R,10S,13R)-6-acetoxy-9,13-epoxy-15-methoxy-labdan-16,15-olide (**1.102**), (rel 5S,8R,9R,10S,13S,15S,16R)-9,13;15,16-diepoxy-15,16-dimethoxy-labdane (**1.103**), (rel 5S,8R,9R,10S,13S,15R,16S)-9,13;15,16-diepoxy-15,16-dimethoxy-labdane (**1.104**), (rel 5S,8R,9R,10S,13S,15R,16R)-9,13;15,16-diepoxy-15,16-dimethoxy-labdane (**1.105**), and vitexilactone (**1.42**).




**1.96** $R_1$ (=$H_2$); $R_2$=O; $R_3$=α-H,β-OAc; $R_4$=H
**1.97** $R_1$ (=$H_2$); $R_2$=O; $R_3$=α-OAc,β-H; $R_4$=H
**1.98** $R_1$=H,$OCH_3$; $R_2$=O; $R_3$=α-H,β-OAc; $R_4$=H



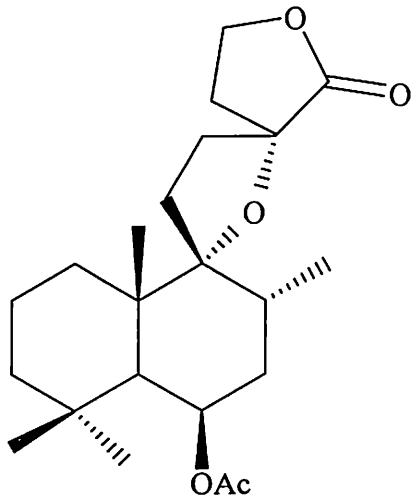

**1.100** $R_1$=O; $R_2$=α-$OCH_3$,β-H
**1.102** $R_1$=H,$OCH_3$; $R_2$=O



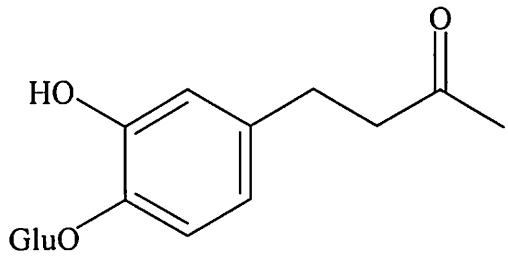

**1.99** $R_1$=O; $R_2$=β-$OCH_3$,α-H; $R_3$=OAc
**1.101** $R_1$=$OCH_3$,H; $R_2$=O; $R_3$=OAc
**1.103** $R_1$=α-$OCH_3$,β-H; $R_2$=α-$OCH_3$,β-H; $R_3$=H
**1.104** $R_1$=β-$OCH_3$,α-H; $R_2$=β-$OCH_3$,α-H; $R_3$=H
**1.105** $R_1$=β-$OCH_3$,α-H; $R_2$=β-H,α-$OCH_3$; $R_3$=H

Kondo *et al.*,[73] have isolated vitexilactone (**1.42**), previtexilactone (**1.106**), luteolin (**1.03**), casticin (**1.01**), artemetin (**1.19**), vanillic acid and *p*-hydroxybenzoic acid from the fruits of *V. rotundifolia*. From the

methanol extractives of the leaves of *V. rotundifolia* Kouno *et al.*,[74] have isolated iridoid glucosides, agnuside (**1.10**), eurostoside (**1.55**) and a phenylbutanone glucoside (**1.107**).

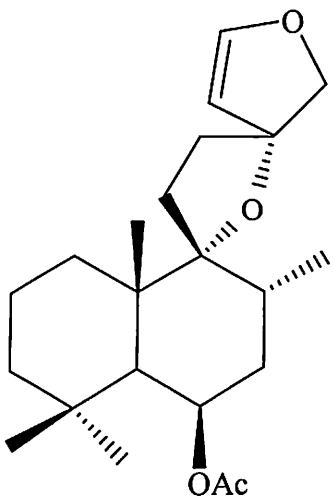



**1.106**

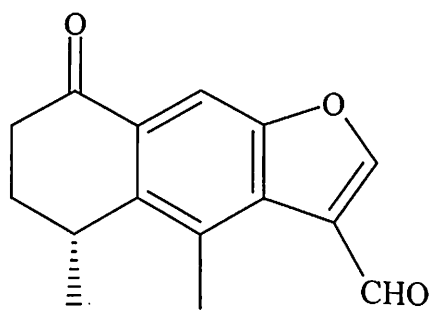



**1.107**

Aska *et al.*,[75] have isolated diterpenoids, rotundifuran (**1.41**), and prerotundifuran (**1.108**) from the leaves. During the phytochemical survey on Japanese medicinal plants, Tada and Yasuda[76] have isolated a benzofuran derivative, (–)-vitiralone (**1.109**) from the creeping stem of *V. rotundifolia.*



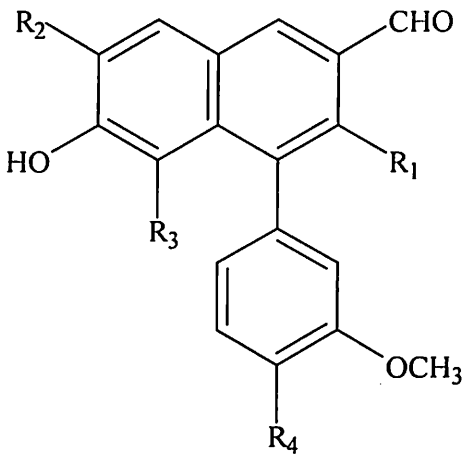

**1.108**

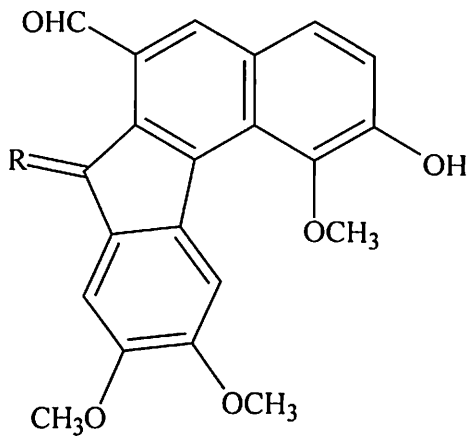



**1.109**

Phytochemical investigations on the subterranean parts of *V. rotundifolia* by Kawazoe *et al.*,[77,78] have led to the isolation of nine

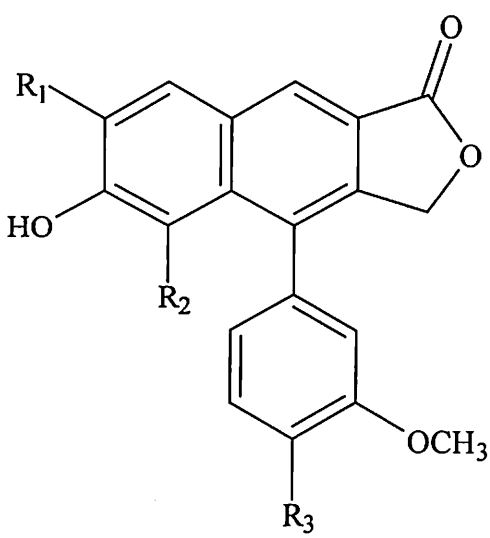
lignans having 1-phenylnaphthalene-type skeleton, namely, vitrofolal A (**1.110**), vitrofolal B (**1.111**), vitrofolal C (**1.112**), vitrofolal D (**1.113**), vitrofolal E (**1.114**), vitrofolal F (**1.115**), vitrofolal G (**1.116**), vitrofolal H (**1.117**) and detetrahydroconidendrin (**1.118**). These compounds showed antibacterial activity against methicillin-resistant *Staphylococcus aureus*.




**1.110** $R_1$=$R_2$=H; $R_3$=$R_4$=$OCH_3$
**1.111** $R_1$=OH; $R_2$=H; $R_3$=$R_4$=$OCH_3$
**1.114** $R_1$=$R_3$=H; $R_2$=$OCH_3$; $R_4$=OH
**1.115** $R_1$=$R_4$=OH; $R_2$=$OCH_3$; $R_3$=H

**1.112** R=OH,H
**1.113** R=O




**1.116** $R_1$=H; $R_2$=$R_3$=$OCH_3$
**1.117** $R_1$=$R_3$=$OCH_3$; $R_2$=H
**1.118** $R_1$=$OCH_3$; $R_2$=H; $R_3$=OH

## *Vitex leucoxylon* Linn. F.

A small to large size tree, found throughout the Deccan Peninsula. The root and bark are astringent, the roots are reported to be used as a febrifuge. The leaves are used for relieving headache and catarrh.[1] During the course of comparative phytochmical studies on *Vitex* species, Rao *et al.*,[79] have isolated β-sitisterol, dimethylterephthalate, vitexin (**1.53**) isovitexin (**1.119**), agnuside (**1.10**) and aucubin (**1.54**) from the leaves and bark of *V. leucoxylon*. The authors have also reported the hepatoprotective activity of the extracts.

Sarma et al.,[80] have studied the anti-inflammatory and wound healing activities of the crude alcoholic extract of the leaves and flavonoids isolated and reported that in acute inflammation model, the crude extract as well as mixture of flavonoids of *V. leucoxylon* showed anti-inflammatory activity. However, the extracts or flavonoids did not show any effect on chronic inflammation. The crude extract significantly reduced the wound breaking strength.

During the general pharmacological screening of leaf extracts, Makwana *et al.*,[81] have studied various pharmacological activities such as acute toxicity and general behavior, spontaneous motor activity, antipsychotic activity, antidepressant activity, antiparkinsonian activity, anticonvulsant activity, analgesic activity, anti-inflammatory activity and antimicrobial activity of ethanol and aqueous extracts. The authors reported that the analgesic and anti-inflammatory activities of *V. leucoxylon* were comparable with those of ethanol extractives of leaves of *V. negundo*.

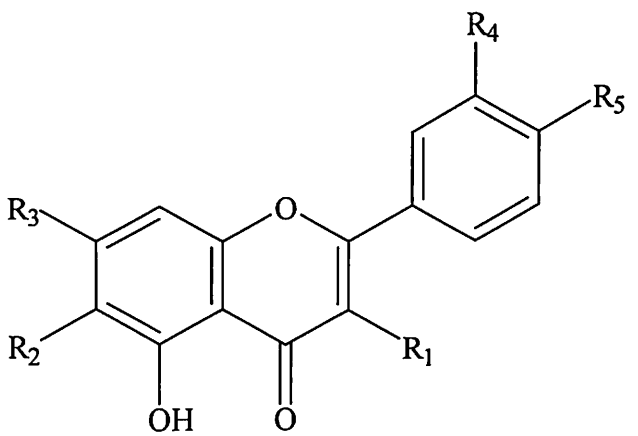
## *Vitex trifolia* L.

A stout, aromatic shrub or a small tree, found in the Himalayas and the greater part of India.[1] The shrub closely resembles *V. negundo*

and has similar distribution, uses and vernacular name. The leaves are reported to be useful in poultices for rheumatic pains, inflammation and sprains and to possess insecticidal and antibacterial properties.[1] An extract of the leaves is reported to be useful in the treatment of tuberculosis whereas the decoction of the roots are used as a febrifuge in Philippines.[1]

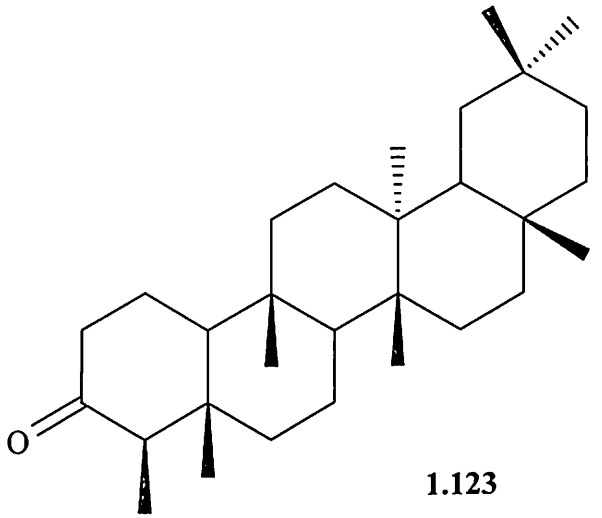
During the course of screening of the Indonesian medicinal plants for inhibitory effect on histamine release from RBL-2H3 cells, Ikawati *et al.*,[82] have reported the inhibitory effect of extracts of *V. trifolia* on mast-cell degranulation.

Sirait and Liemtswanhoo[83] have isolated agnuside (**1.10**) from the leaves of *V. trifolia*. Ramesh *et al.*,[84] have isolated luteolin-7-*O*-β-D-glucuronide (**1.120**), luteolin-3'-*O*-β-D-glucuronide (**1.121**) and isoorientin (**1.04**) from the alcoholic extractives of the leaves. Nair *et al.*,[85] have isolated artemetin (**1.19**), and 7-desmethylartemetin (**1.122**) also from the leaves.



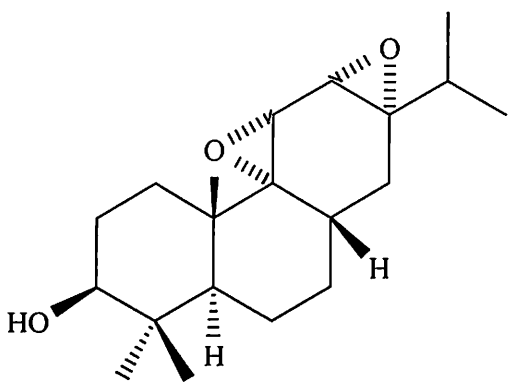

**1.119** $R_1$= $R_4$=H; $R_2$=*C*-glu; $R_3$=$R_5$=OH
**1.120** $R_1$=$R_2$=H; $R_3$=O-β-glucuronide; $R_4$=$R_5$=OH
**1.121** $R_1$=$R_2$=H; $R_3$= $R_5$=OH; $R_4$= O-β-glucuronide
**1.122** $R_1$=$R_2$=$R_4$=$R_5$=$OCH_3$; $R_3$=OH

Vedantham and Subramanian[86] have reported the isolation of friedelin (**1.123**), β-sitosterol and β-sitosterol glucoside from the leaves.

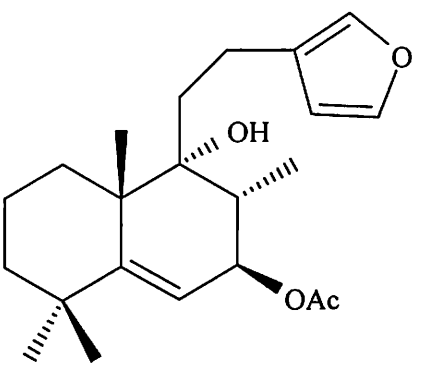



**1.123**

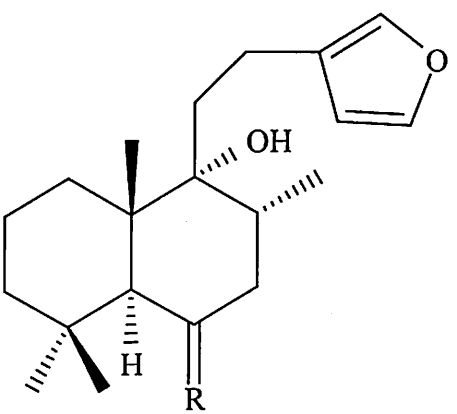
From the acetone extract of fruits, Ono *et al.*,[87] have isolated an abietane-type diterpene, vitetrifolin A, (**1.124**) two labdane–type diterpenes, vitetrifolin B (**1.125**), vitetrifolin C (**1.126**), along with rotundifuran (**1.41**), dihydrosolidagenone (**1.127**) and abietatriene 3β-ol (**1.95**)




**1.124**

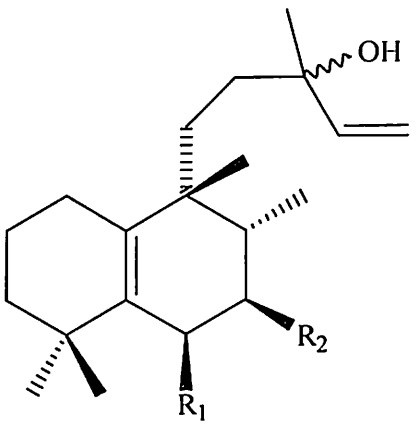



**1.126**

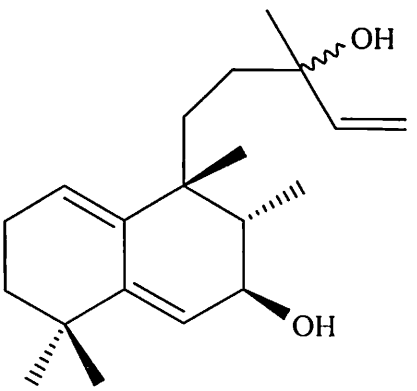



**1.125** R=α-OAc,β-H
**1.127** R=O

Further investigation by the same authors[88] led to the isolation of four halimane-type diterpenes, vitetrifolin D (**1.82**), vitetrifolin E (**1.128**), vitetrifolin F (**1.129**), vitetrifolin G (**1.130**) from the acetone extract of fruits of *V. trifolia.*



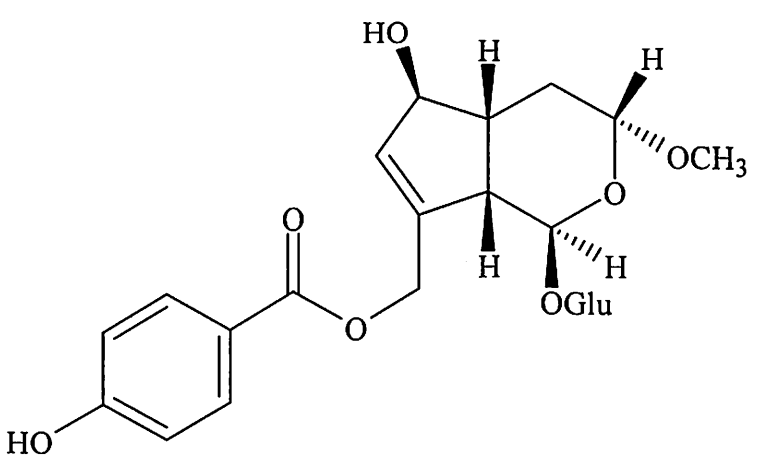

**1.128** $R_1$=OAc; $R_2$=H
**1.129** $R_1$=H; $R_2$=OAc




**1.130**

## *Vitex cannabifolia*

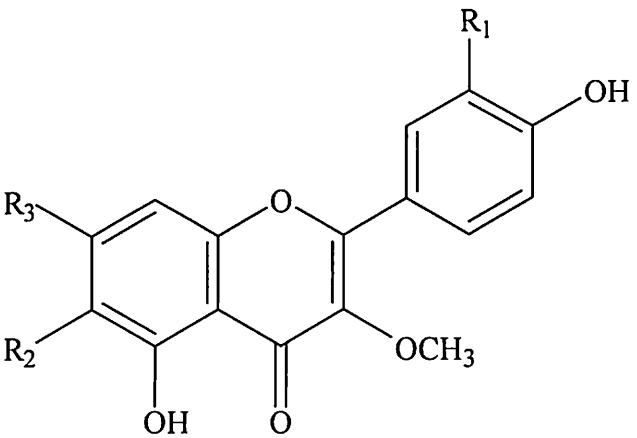
It is a deciduous tree native to China. Its fruits possess anti-inflammatory properties and have been used in traditional Chinese medicine under the name of Bokeishi.[89] Taguchi[90] had isolated vitexilactone (**1.42**), agnuside (**1.10**), artemetin (**1.19**) and *p*-hydroxybenzoic acid, from the leaves of *V. cannabifolia.* Iwagawa *et al.*,[89] have isolated iridoids, nishindaside (**1.09**) and isonishindaside (**1.131**) from the leaves.



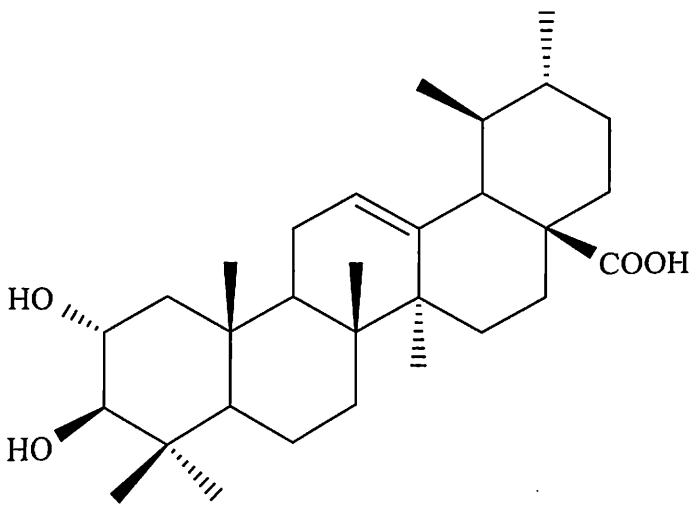

**1.131**

## *Vitex peduncularis Wall.*

A small to moderate–sized tree, found in the Eastern Himalayas, Assam, Bihar, Orissa and Andhra Pradesh.[1] An infusion of the leaves or stem bark is reported to be useful in malarial and black water fever.[1] The leaves are reported to contain vitexin (**1.53**).[91,92] Sahu *et al.*,[93] have isolated pachypodol (**1.132**), peduncularison (5,4'-dihydroxy-3,6-dimethoxyflavone, **1.133**) and triterpenoids, namely ursolic acid (**1.17**) and corosolic acid (**1.134**).



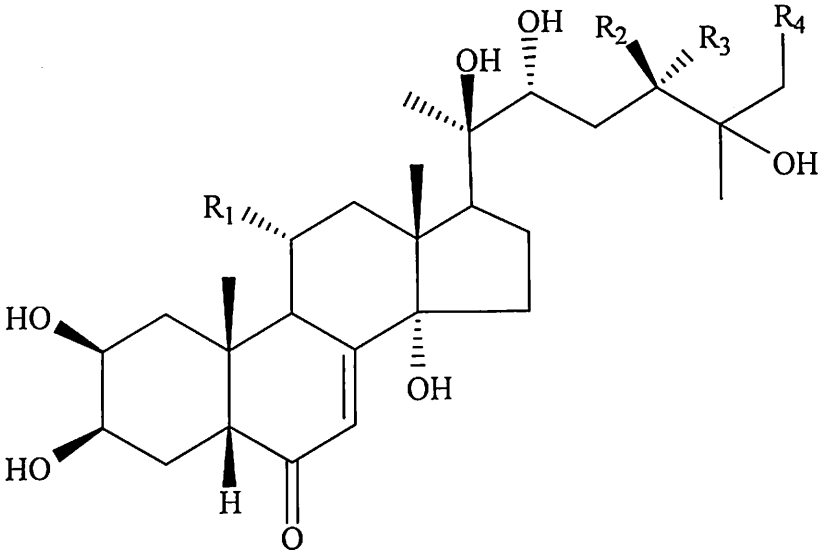

**1.132** $R_1$=$R_3$=$OCH_3$; $R_2$=H
**1.133** $R_2$=$OCH_3$; $R_1$=$R_3$=H



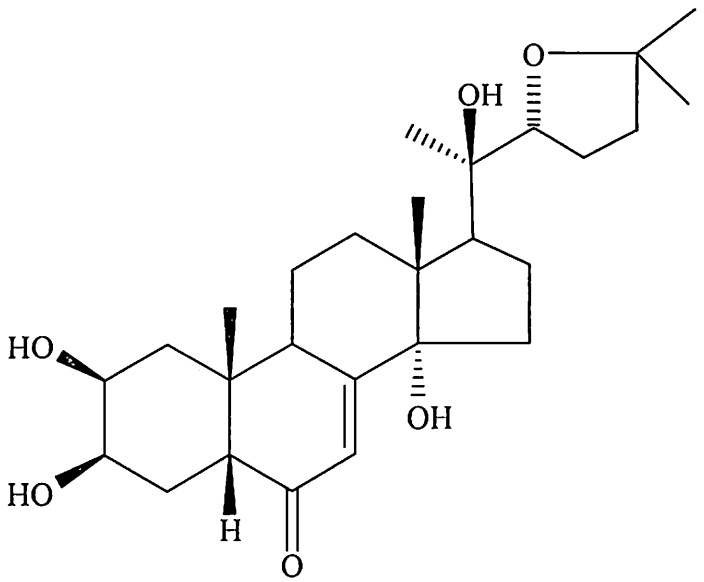

**1.134**

## *Vitex canescens*

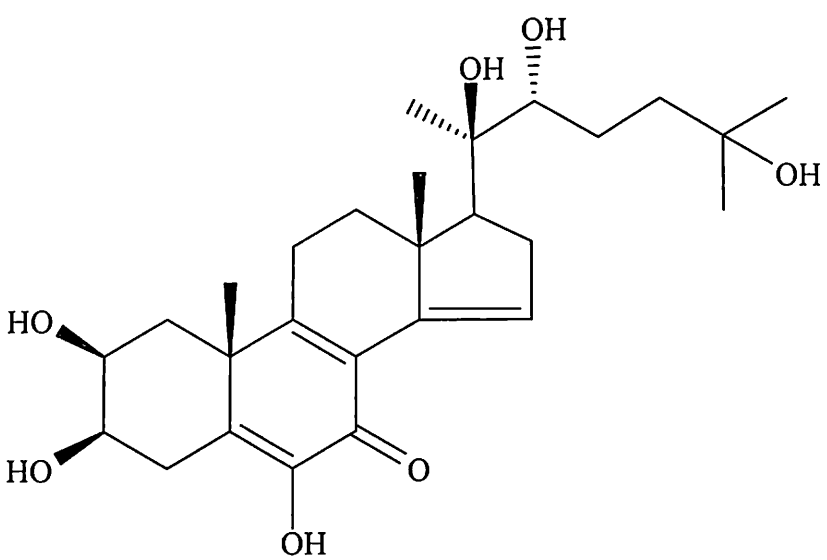
It is a moderate–sized tree found in Assam and the neighbouring states.[1] As a part of comparative phytochemical studies on the *vitex* species of Thailand, Suksamrarn *et al.*,[94-96] have isolated several ecdysteroids from the root bark of *V. canescens*, namely, 20-hydroxyecdysone (**1.135**), turkesterone (**1.136**), 24-*epi*-abutasterone (**1.137**), 24-*epi*-makisterone A (**1.138**), (24R)-11α,20,24-trihydroxyecdysone (**1.139**), abutasterone (**1.140**), 20,26-dihydroxyecdysone (**1.141**), 11α,20,26-trihydroxyecdysone (**1.142**), shidasterone (**1.143**) and calonysterone (**1.144**).




**1.135** $R_1=R_2=R_3=R_4=H$
**1.136** $R_1=OH$; $R_2=R_3=R_4=H$
**1.137** $R_1=R_3=R_4=H$; $R_2=OH$
**1.138** $R_1=R_2=R_4=H$; $R_3=CH_3$
**1.139** $R_1=R_2=OH$; $R_3=R_4=H$
**1.140** $R_1=R_2=R_4=H$; $R_3=OH$
**1.141** $R_1=R_2=R_3=H$; $R_4=OH$
**1.142** $R_1=R_4=OH$; $R_2=R_3=H$



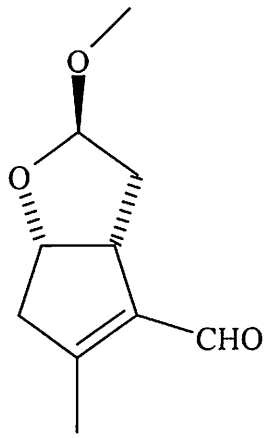

**1.143**



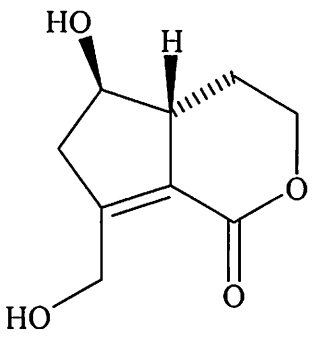

**1.144**

## *Vitex cymosa*

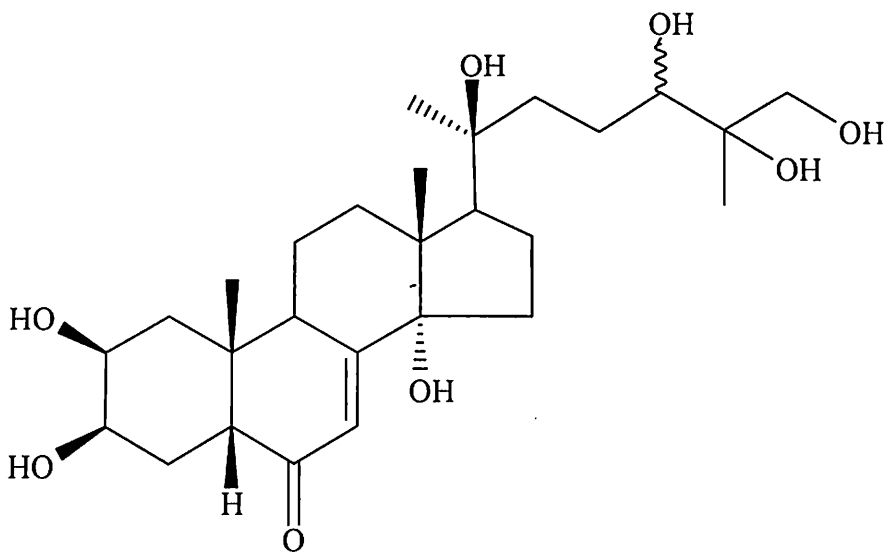
It is a small tree, widely distributed in the Central and Amazon regions of Brazil. It is popularly known as Taruma-do-Igapo.[97] Dos santos *et al.*,[97,98] have isolated iridoids, namely, tarumal (**1.145**), viteoid (**1.146**), agnuside (**1.10**) from the leaves and ecdysteroids, 26-hydroxypinnatasterone (**1.147**) and 20-hydroxyecdysone (**1.135**) from the stem bark of *V. cymosa*.




**1.145**




**1.146**

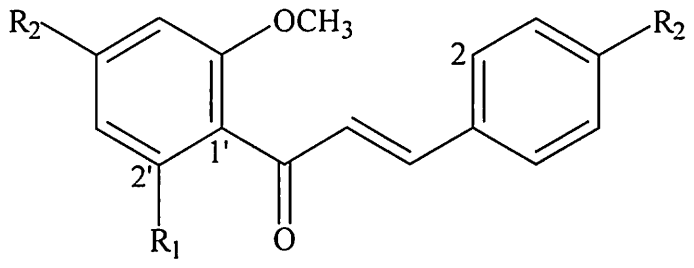



**1.147**

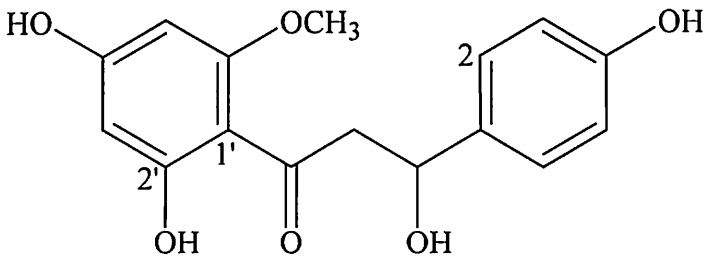
***Vitex leptobotrys***

A small shrub growing up to 4-6 m, is found in Vietnam. During the general phytochemical studies on Vietnamese plants, Thuy *et al.*,[99] have isolated several chalcones, namely, 2′,4′-dihydroxy-6′-methoxychalcone (**1.148**), 2′,4′,4-trihydroxy-6′-methoxychalcone (**1.149**), 2′,4′-dihydroxy-4,6′-dimethoxychalcone (**1.150**), 4,4′-dihydroxy-2′,6′-dimethoxychalcone (**1.151**), 4′-hydroxy-4,2′,6′-trimethoxychalcone (**1.152**) and 4,2′,4′,β-tetrahydroxy-6′-methoxy-α,β-dihydrochalcone (**1.153**) and ecdysteroids, ecdysterone (**1.135**), 24(28)-

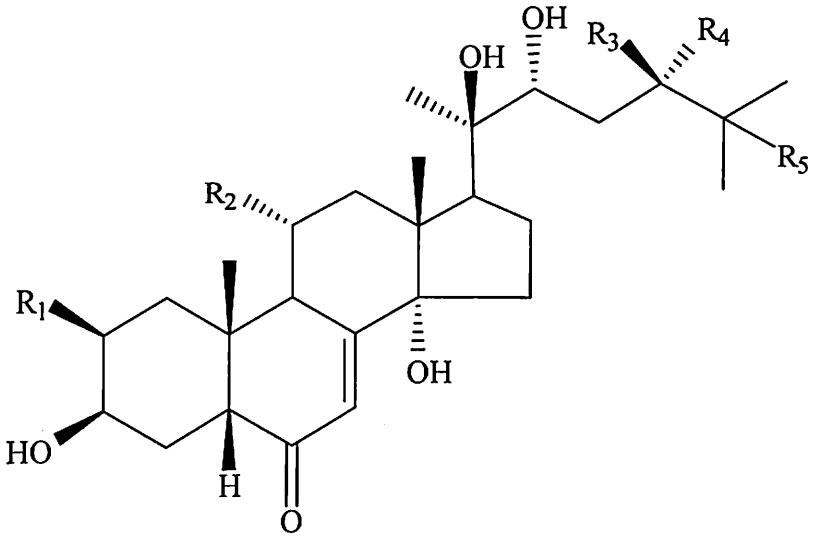
dehydromakisterone A (**1.154**), 24-*epi*-makisterone A (**1.138**), ajugasterone C, (**1.155**) 2-deoxy-20-hydroxyecdysone (**1.156**) and pinnatasterone (**1.157**) from the aerial parts of *V. leptobotrys*.




**1.148** $R_1$=OH; $R_2$=H
**1.149** $R_1$=$R_2$=OH
**1.150** $R_1$=OH; $R_2$=$OCH_3$
**1.151** $R_2$=OH; $R_1$=$OCH_3$
**1.152** $R_1$=$R_2$=$OCH_3$




**1.153**

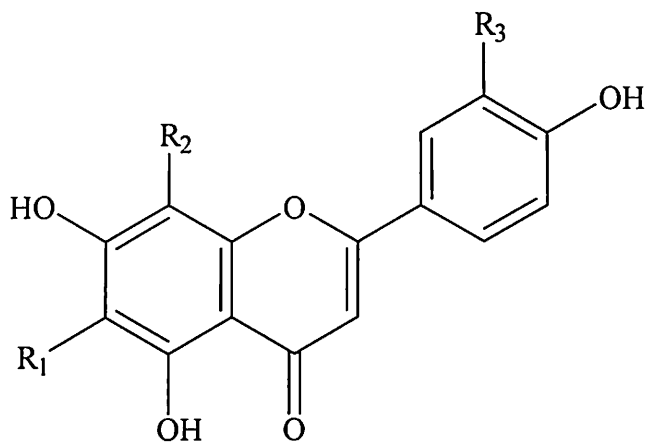



**1.154** $R_1$=$R_5$=OH; $R_2$=H; $R_3$,$R_4$= (=$CH_2$)
**1.155** $R_1$=$R_2$=OH; $R_3$=$R_4$=$R_5$=H
**1.156** $R_1$=$R_2$=$R_4$=H; $R_3$=$R_5$=OH
**1.157** $R_1$=$R_4$=$R_5$=OH; $R_2$= $R_3$=H

## *Vitex lucens* T. Kirk ( Syn. *V. littoralis* A. Cunn.)

It is native to New Zealand, ornamental tree with pink flowers and attractive hanging bunches of marble-sized, rose-red, rounded drupes.[1]

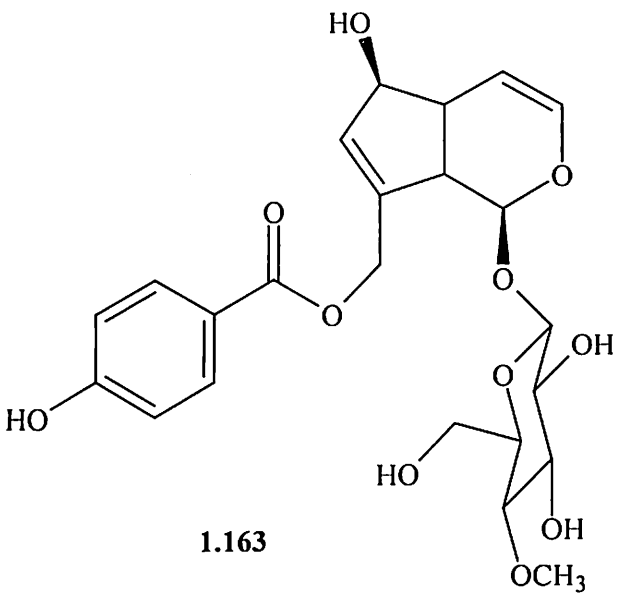
An infusion of the leaves is reported to be used for sprains, back-ache, ulcers and sore throat,[1] by the Maori natives of New Zealand. Wood of *V. lucens* is a rich source of glycoflavonoid compounds. Cambie[100] had reported the presence of vitexin (**1.53**) and β-sitosterol from the leaves of *V. lucens*. Seikel *et al.*,[101,102] have isolated several *C*-glycosyl flavonoids, namely vitexin (**1.53**), isovitexin (**1.119**), orientin (**1.158**), isoorientin, (**1.04**), vitexin-*O*-xyloside (**1.159**), orientin-*O*-xyloside (**1.160**), lucenin-1 (**1.161**) and vicenin-1 (**1.162**) from the wood of *V. lucens*.



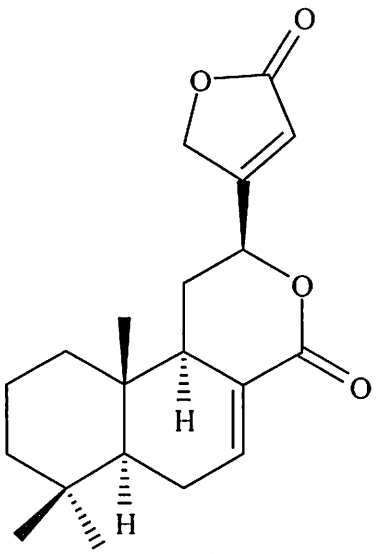

**1.158** $R_1$=H; $R_2$=*C*-glu; $R_3$=OH
**1.159** $R_1$=$R_3$=H; $R_2$=-[β-*O*-xylosyl(1-2)]*C*-glu; =H
**1.160** $R_1$=H; $R_2$=-[β-*O*-xylosyl(1-2)]*C*-glu; $R_3$=OH
**1.161** $R_1$=$R_2$=*C*-glu; $R_3$=OH
**1.162** $R_1$=$R_2$=*C*-glu; $R_3$=H

## *Vitex limnifolia*

Suksamrarn *et al.*,[103] have isolated iridoids, agnuside (**1.02**), limoniside (**1.163**) from the bark of *V. limnifolia*. Aphaijitt *et al.*,[104] have isolated a ladane diterpene, limonidilactone (**1.164**] from the leaves.



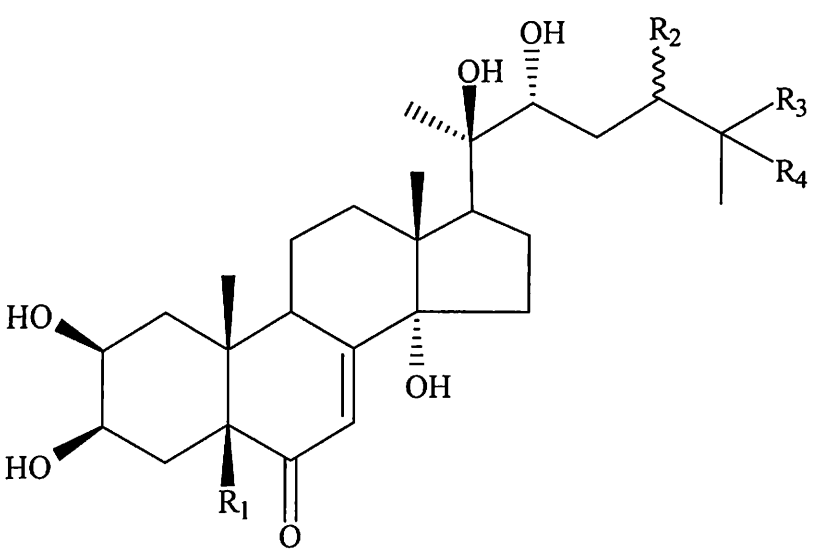
1.163



1.164

## *Vitex megapotamica*

Rimpler[105-107] had isolated several ecdysteroids, namely, ecdysterone (**1.135**), inokosterone (**1.165**), polypodin B (**1.166**), pterosterone (**1.167**) and viticosterone E [**1.168**] in addition to agnusude [**1.10**] from the wood of *V. megapotamica.*



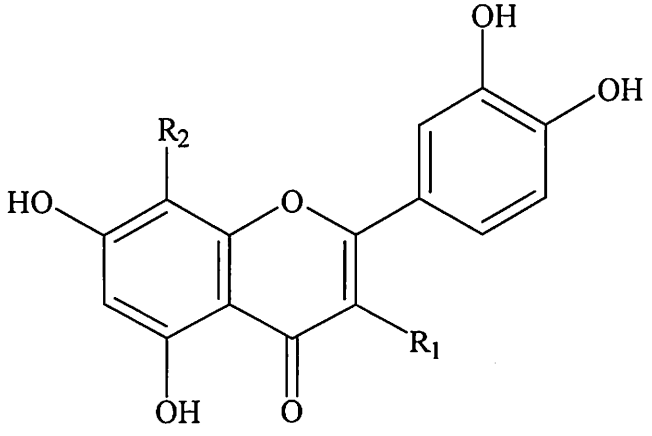
**1.165** $R_1$=$R_2$=$R_4$=H; $R_3$=$CH_2OH$
**1.166** $R_1$=$R_4$=OH; $R_2$=H; $R_3$=$CH_3$
**1.167** $R_1$=$R_4$=H; $R_2$=OH; $R_3$=$CH_3$
**1.168** $R_1$=$R_2$=H; $R_3$=$CH_3$; $R_4$=$OCOCH_3$

## *Vitex pinnata* Linn (syn *V. pubescens* Vahl.)

It is a moderate-sized tree found in tropical Asia.[1,2] A decoction of the bark is given to relieve stomach-ache, and poultice of the leaves is used in fever and to treat wounds.[108] Suksamrarn and Sommechai[109] have isolated 20-hydroxyecdysone (**1.135**), pinnatasterone (**1.157**), and turkesterone (**1.136**) from the ethanolic extract of the bark of *V. pinnata.*

## *Vitex polygama* Cham.

It is a moderate-size tree found in Brazil. The bark and fruits of this plant have been to be useful in traditional medicine as emenagogue and diuretic. *Leitao et al.*,[110] have isolated several flavonoids, namely, 2"-O-caffeoyl-orientin (**1.169**), orientin (**1.158**), isoorientin (**1.04**), vitexin (**1.53**), isovitexin (**1.119**), luteolin (**1.03**), quercetin (**1.170**) and quercetin 3-methyl ether (**1.171**), in addition to *p*-hydroxybenzoic acid.




**1.169** $R_1$=H; $R_2$= *C*-(2-*O*-caffeoyl)glu
**1.170** $R_1$=OH; $R_2$=H
**1.171** $R_1$=$OCH_3$; $R_2$=H

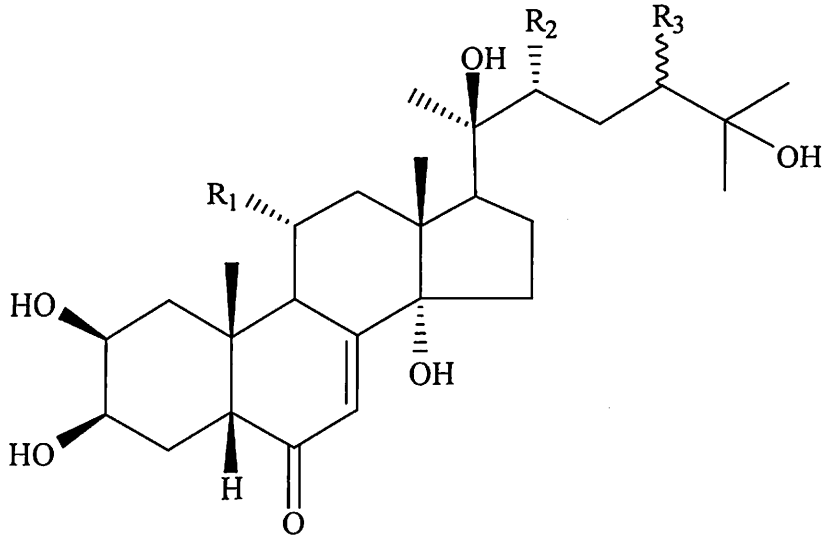
Extracts and flavonoid rich fractions from the fruits and leaves of *V. polygama* exhibited a dose dependent antiviral activity against acyclover resistant herpes simplex virus.[111]

## *Vitex glabrata*

A moderate sized tree found in Assam, Meghalaya and Rajmahal hills in Bihar.[1] It is known as Kai Nao in Thailand.[112] The bark and root have been reported to be useful as astringents. The bark has also been claimed to be an anthelmentic and a remedy for gastro-intestinal disorders.[113,114] Werawattanametin *et al.*,[115] have isolated 20-hydroxyecdysone (**1.135**) and turkesterone (**1.136**) from the bark of *V. glabrata.*

## *Vitex scabra* Wall

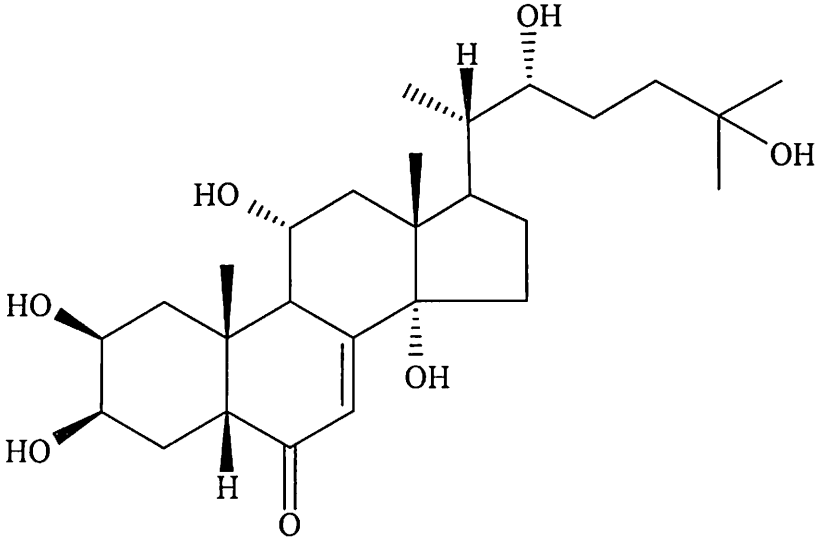
It is a moderate-sized tree, endemic to the northeastern part of Thailand. Suksamrarn *et al.*,[116] have isolated two new ecdysteroids, 24-epi-pinnatasterone (**1.172**) and scabrasterone (**1.173**) along with 11 known ecdysteroids, calonysterone (**1.144**), pterosterone (**1.167**), 24-epi-makisterone A (**1.138**), 20-hydroxyecdysone (**1.135**), polypodin B (**1.166**), ajugasterone C (**1.155**), pinnatasterone (**1.157**), 11α-hydroxyecdysone (**1.163**), 24-epi-abutasterone (**1.137**), 20,26-dihydroxyecdysone (**1.141**) and turkesterone (**1.136**) from the stem bark of *V. scabra.*



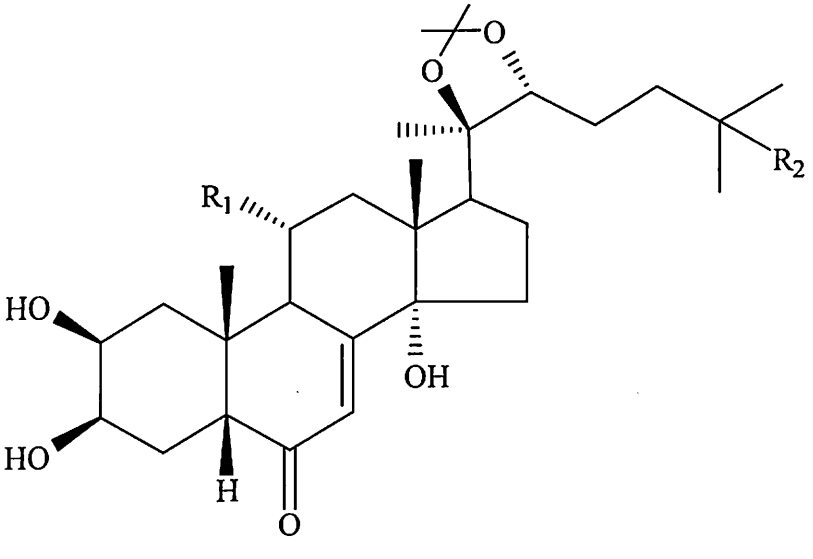

**1.172** $R_1$=$R_2$=H; $R_3$=OH
**1.173** $R_1$=OH; $R_2$=$R_3$=H

## *Vitex strickeri*

Zhang *etal.*,[117] have isolated six phytoecdysteroids from the root bark of *V. strickeri* using a combination of rotation locular countercurrent chromatography (RLCC) and recycling high performance liquid chromatography (R-HPLC). The compounds were identified as 20-hydroxyecdysone (**1.135**), ajugasterone C (**1.155**), abutasterone (**1.140**), 11α-hydroxyecdysone (**1.174**), 20-hydroxyecdysone-20,22-monoacetonide (**1.175**) and ajugasterone C-20,22-monoacetonide (**1.176**).




**1.174**



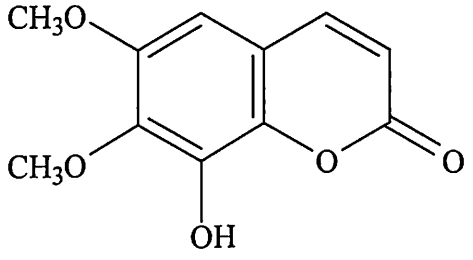

**1.175** $R_1$=H; $R_2$=OH
**1.176** $R_1$=OH; $R_2$=H

From the foregoing, it is evident that, a large number of ***Vitex*** species are used in traditional systems of medicine in India, China, Japan, Europe, Brazil and Thailand, due to the broad range of biological activities exhibited by the various ***Vitex*** species. Plants belonging to the genus ***Vitex*** are a rich source of diverse chemical constituents such as iridoids, terpenoids, lignans, and flavonoids.

## TERAMNUS

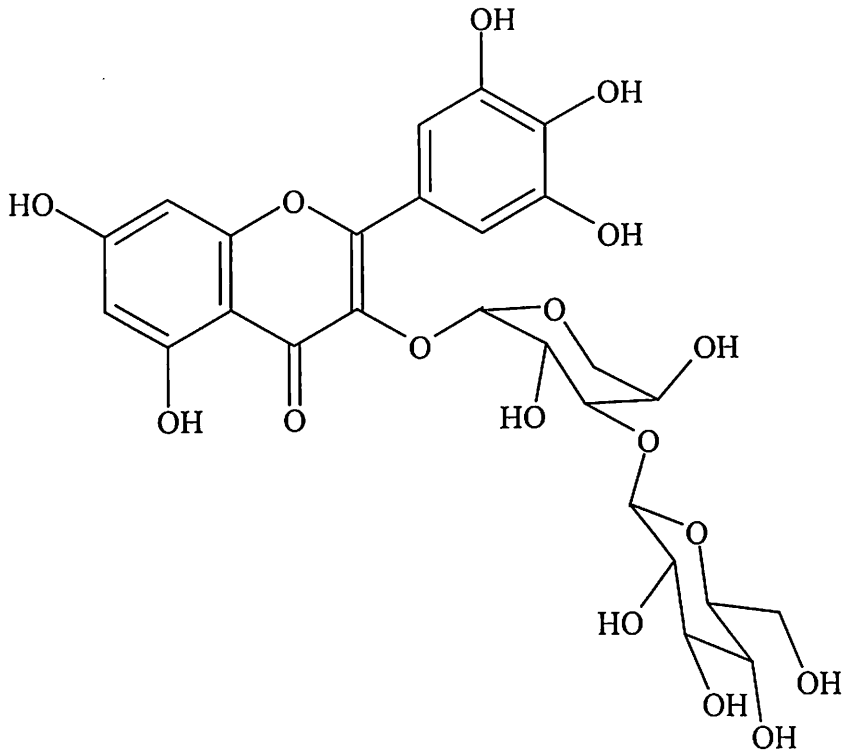
*Teramnus* (n.o. Leguminosae) is a small genus of twining herbs, distributed in the tropic regions. Two species, *Teramnus labialis* and *T. mollis* are available in India.[118] *Teramnus labialis* spreng. is a herb. It is commonly known as mashaparni (Sanskrit) and mashavan (Hindi). It is a well-known Medicinal plant in the Ayurvedic system of medicine. Traditionally, different parts of the plant have been reported to be useful in treating rheumatism, tuberculosis, nerve disorders, paralysis and catarrhs.[118,119] The fruits are reported as astringent, stomachic and possess febrifugal properties.[120]

Phytochemical investigation on the seeds of *T. labialis*, yielded a water soluble gallactomannan.[121] Bio-assay guided fractionation, based on antihyperglycemic activity of aqueous alcoholic extract of *T. labialis*, yielded fraxidin (**1.177**) as the major active constituent.[122]




**1.177**

Recently, a flavonol glycoside 3,5,7,3′,4′,5′-hexahydroxyflavone-3-O-β-D-glucopyranosyl(1→3)-O-α-L-arabinopyranoside (**1.178**), has been isolated from the ethanolic extractives of the stems of *T. labialis*.[123]



**1.178**

In view of the reported traditional use of *Vitex altissima* and *Teramnus labialis* in rheumatism and lack of earlier phytochemical investigations on these species, the author has chosen to study the anti-inflammatory activity of the leaves of *V. altissima* and aerial parts of *T. labialis* and characterize the active constituents. The plants chosen for the study are available locally on the Tirumala Hill ranges.

Tirumala Hill ranges (Fig. 1.01) are the parts of Eastern Ghats of India, more particularly, extensions of Velikonda Ranges, locally called as Seshachala Hills. These Hills are located in Chittoor district of Andhra Pradesh (Fig. 1.02) and geographically, it is situated between east longitudes 79° 24′ 29″ and north latitudes 13° 36′ 54″ and 13° 39′ 12″. Tirumala Hills are rich in medicinal flora.

Why photography of medicinal plants discussed were not included in Kuleś

Fig. 1.01 Tirumala Hill Range

A Famous Temple on the Tirumala Hills

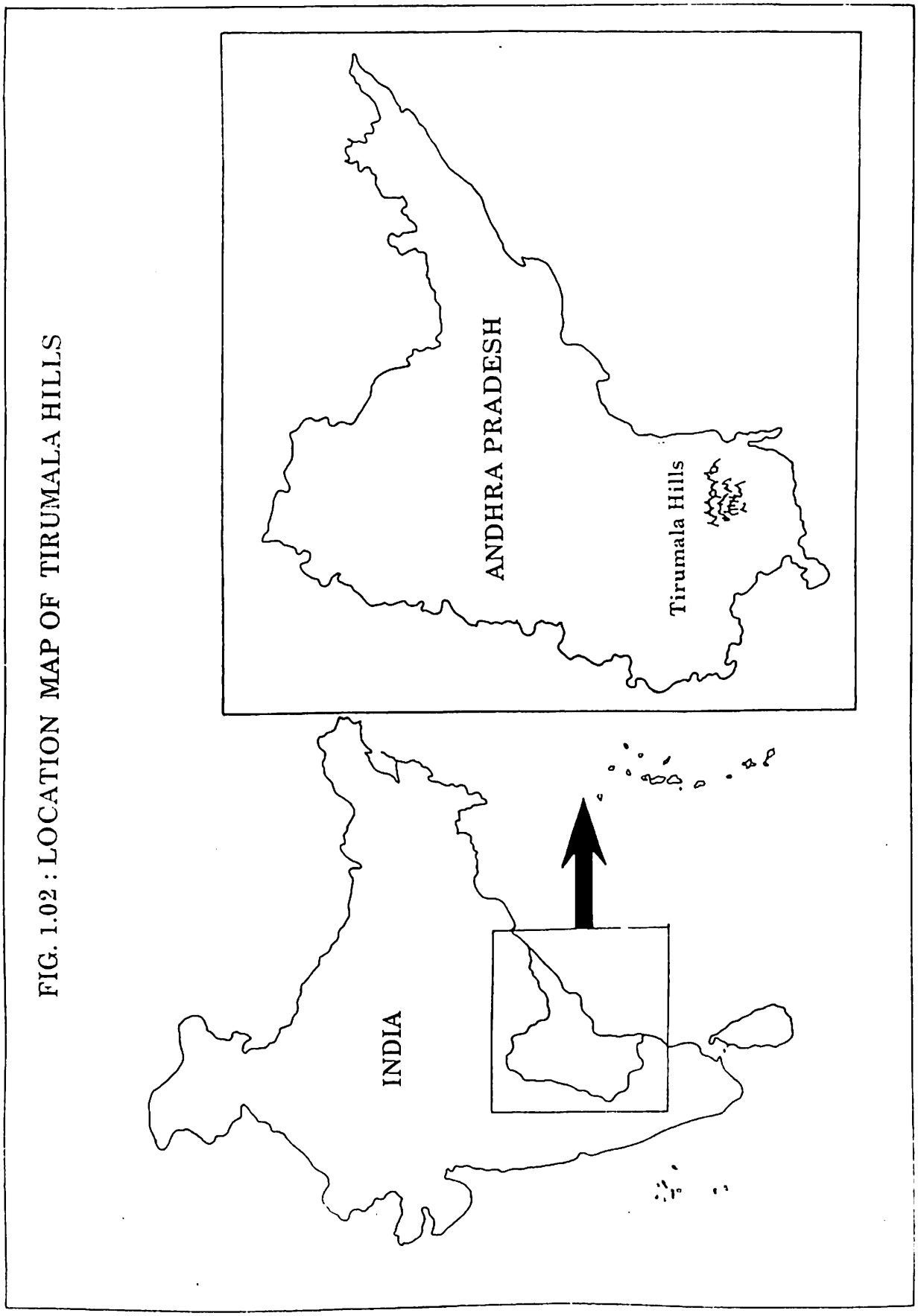



FIG. 1.02 : LOCATION MAP OF TIRUMALA HILLS

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# Chemical examination of *Vitex altissima* and *Teramnus labialis*

The details of isolation and purification of different chemical constituents of *Vitex altissima* and *Teramnus labialis* have been discussed in the following pages under **section-A** and **section-B**, respectively.

## Materials and Methods:

### Collection of the plants

The leaves of *V. altissima* and the aerial parts of *T. labialis* were collected from the Sesachalam Hill ranges of the Tirumala Forest, chittoor district, India, during January 2001 and dried under shade.

### Identification

The specimens of the plants were authenticated as *Vitex altissima* Linn. and *Teramnus labialis* Spreng by Dr. K. Madhava chetty, Department of Botany, Sri Venkateswara University, Tirupati, India. Voucher specimens (VA-010222 and TL-01211) are on deposit at the Herbarium, Department of Botany, Sri Venkateswara University, Tirupati, India.

### General experimental procedures

- Melting points were recorded on a MEL-Temp apparatus and are uncorrected.
- Ultra-violet (UV) spectra were recorded on Varian (Cary-50) spectrometer.
- Infrared (IR) spectra were recorded on Perkin-Elmer BX FT-IR spectrophotometer with potassium bromide pellets.
- $^{1}H$ and $^{13}C$ NMR spectra were recorded on Varian Inova 600 MHz, Varian Inova 500 MHz, Bruker AMX 400 MHz and 300 MHz spectrometers, using tetramethylsilane (TMS) as the internal standard. Chemical shifts are given in $\delta$ ppm units downfield from TMS.
- The Optical rotations were measured on Jasco Dip 360 Digital Polarimeter.
- LC-MS mass spectral data were recorded on Agilent 1100 Series LC-MSD.

- C, H analyses were performed on Vario EL Elementar.
- Preparative HPLC was carried out on Shimadzu HPLC system (LC-8A Pump, SPD-10A UV-Visible detector) using Luna (10 μM, 21.2 × 250 mm, Phenomenex) column.
- Analytical HPLC was carried out on Shimadzu HPLC (PDA-M10 AVP, CLASS-VP software, LC-10AT VP Pumps) equipped with CTO-10 ASVP column oven, using Luna $C_{18}$ (5 μ, 4.6 × 250 mm, Phenomenex) columns.
- HPTLC was performed on CAMAG (TLC Scanner-III, equipped with Auto applicator Linomat-IV)
- All solvents and reagents used for extraction, chromatography and recrystallisation are of laboratory reagent grade. Solvents were purified further by standard methods. Column chromatography was carried out using silica gel (ACME, 100-200 mesh/finer than 200 mesh), Silica gel G and Merck pre-coated silica gel-60 $F_{254}$ plates were used for thin layer chromatography. Chromatograms were visualized under UV light or by spraying with methanolic $H_2SO_4$ (10%) followed by heating at 100 °C for 3 minutes.

## Section-A

### Isolation of chemical constituents from *V. altissima*

*Vitex altissima* Linn. is a moderate to large–sized tree found in the Eastern Ghats and Deccan plateau in India.[1-4] The leaves are reported to be useful in the treatment of rheumatism.[5] The stem bark has been reported to possess antimicrobial activity[6] and paste of stem bark is applied externally for various skin diseases by the tribals of the Eastern Ghats.[6]

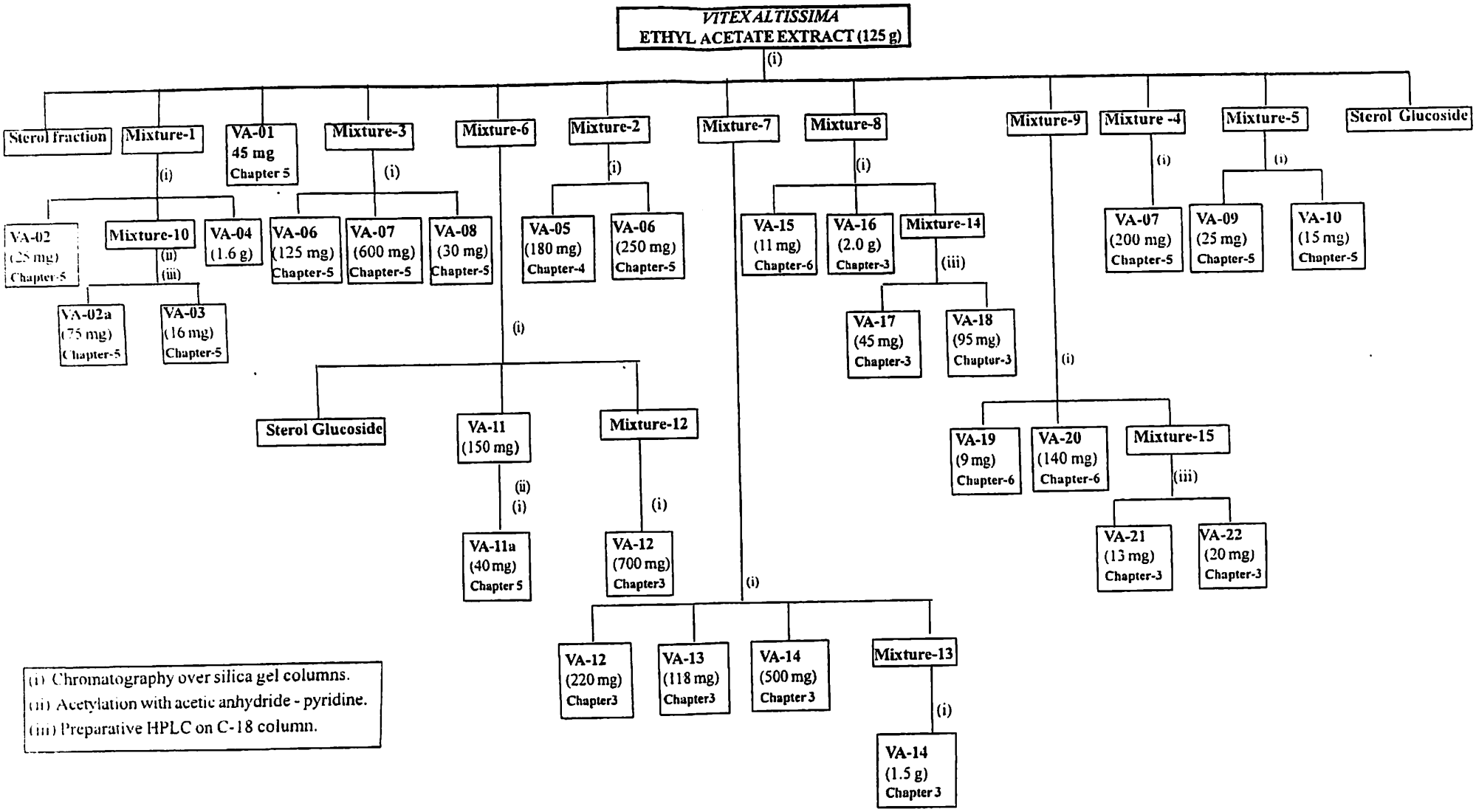
**Extraction**

The dried leaves of *V. altissima* were cut into small pieces and milled to a coarse powder. The powder (ca 2.8 kg) was extracted, successively, with *n*-hexane (5 × 10 L), ethyl acetate (5 × 10 L), methanol (5 × 10 L) and 70% aqueous methanol (5 × 10 L) in a soxhlet apparatus. The combined extracts of each solvent were concentrated under reduced pressure to yield residues, from *n*-hexane extractives (60 g), ethyl acetate extractives (150 g), methanol extractives (400 g), and 70% aqueous methanol solubles (275 g). These extracts were screened for anti-inflammatory activity by carrageenan-induced rat paw edema model and the details are presented in **chapter-8**. The ethyl acetate extract which exhibited potent anti-inflammatory activity was chromatographed over silica gel column. The details of isolation and purification of chemical constituents from the ethyl acetate extractives are noted in the following paragraphs.

The summary of the procedure of isolation of chemical constituents from ethyl acetate extract of *V. altissima* has been presented in chart **2.01**

About 125 g of the residue from the ethyl acetate extract was chromatographed over silica gel (1500 g) column and eluted with hexane and mixtures of hexane and ethyl acetate followed by mixtures of chloroform and methanol, with increasing polarity. Fractions of 1000 mL were collected and monitored over silica gel thin layers. The results of chromatography are noted in table 2.01.

## CHART 2.01: SCHEME OF ISOLATION OF METABOLITES FROM ETHYL ACETATE EXTRACTIVES OF *VITEX ALTISSIMA*



## TABLE 2.01

**Chromatography of ethyl acetate extract of *V. altissima***

| Eluant | Fractions | Compounds | $R_f$ | Yield |
|---|---|---|---|---|
| Hexane: ethyl acetate[a] | | | | |
| 100:0 | 1-15 | Oily residue[b] | -- | -- |
| 90:10 | 16-22 | **Sterol fraction** | 0.79[c] | 25 mg |
| 80:20 | 23-38 | **VA-01** | 0.73[d] | 45 mg |
| 70:30 | 39-53 | **Mixture-1** | -- | 6.0 g |
| 60:40 | 54-69 | **Mixture-2** | -- | 5.0 g |
| 50:50 | 70-75 | **Mixture-3** | -- | 5.0 g |
| 50:50 | 76-95 | **Mixture-4** | -- | 4.0 g |
| 25:75 | 96-105 | **Mixture-5** | -- | 160 mg |
| Chloroform: methanol[a] | | | | |
| 90:10 | 106-107 | **Sterol glucoside** | 0.73[e] | 100 mg |
| 90:10 | 108-121 | **Mixture-6** | -- | 13.0 g |
| 80:20 | 122-125 | **Mixture-7** | -- | 20.0 g |
| 80:20 | 126-131 | **Mixture-8** | -- | 15.0 g |
| 80:20 | 132-144 | **Mixture-9** | -- | 13.0 g |
| 50:50 | 145-150 | Intractable gum | -- | -- |

[a]Values refer to the ratio of solvents by volume.

[b]Low polar material and moves along with the solvent front on TLC.

[c]Solvent system used for TLC: Hexane: ethyl acetate, 7:3.

[d]Solvent system used for TLC: Chloroform: acetone, 8:2.

[e]Solvent system used for TLC: Chloroform: methanol, 8:2.

**Sterol fraction (25 mg)**

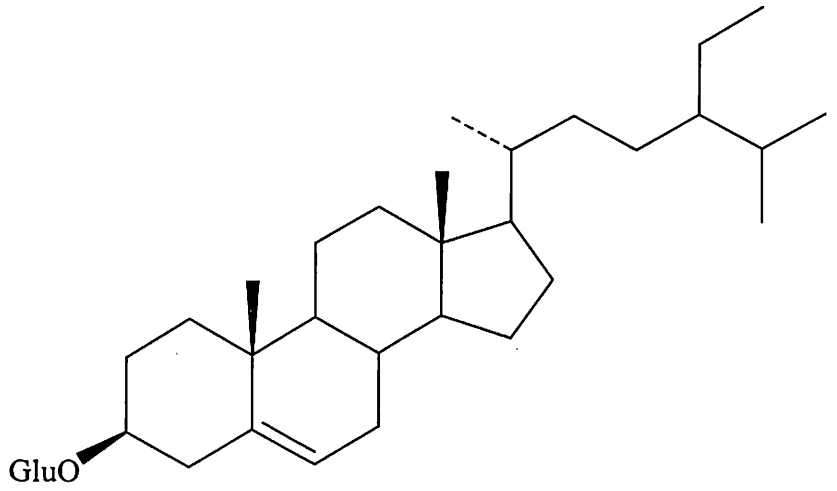
It was obtained as colorless needles from pet. ether and ethyl acetate, m.p. 136-138 °C. It gave positive Liebermann-Burchard test with play of colors, characteristic of phytosterols. The phytosterol fraction was not examined further.

**Ursolic acid (VA-01, 45 mg)**

It was obtained as white amorphous powder from methanol, m.p. 285-287 °C. It gave positive Liebermann-Burchard test for triterpenoids and its structure elucidation has been discussed in **Chapter-5**.

**Sitosterol-3-*O*-β-D-glucoside (2.01, 100 mg)**

It was obtained as colorless crystals from chloroform-methanol, m.p. 290-292 °C. It gave positive Liebermann-Burchard test with play of colors, characteristic of steroids. It was identified as sitosterol-3-*O*-*β*-D-glucoside (**2.01**) by direct comparison with authentic sample, isolated from *Justicia diffusa* willd through co-TLC and undepressed mixed melting point.[7]




**2.01**

**Separation of mixture-1 (6.0 g)**

Mixture-1 (6.0 g) showed two major spots on silica gel thin layers ($R_f$ 0.61 and 0.32; Chloroform: acetone, 8:2). The mixture was chromatographed over silica gel column (200 g) using mixtures of chloroform and methanol with increasing polarity, for elution. Fractions of 200 mL were collected and monitored over silica gel thin layers. The details of chromatography are noted in table 2.02.

**TABLE 2.02**

**Chromatography of mixture-1**

| Eluant (Chloroform: methanol)[a] | Fractions | Compounds | $R_f$ | Yield |
|---|---|---|---|---|
| 100:0 | 1-10 | Oily residue[b] | -- | -- |
| 98:2 | 11-15 | Green oily residue | -- | -- |
| 98:2 | 16-20 | **VA-02** | 0.61[c] | 25 mg |
| 98:2 | 21-35 | **Mixture-10** | 0.61[c] | 800 mg |
| 96:4 | 36-40 | Intractable gum | -- | --- |
| 94:6 | 41-55 | **VA-04** | 0.32[c] -- | 1.6 g |
| 90:10 | 56-60 | Intractable gum | -- | -- |

[a]Values refer to the ratio of solvents by volume.

[b]Low polar material and moves along with the solvent front on TLC.

[c]Solvent system used for TLC: Chloroform: acetone, 8:2.

**Epicorosolic acid (VA-02, 25 mg)**

It was obtained as a white amorphous powder from methanol, m.p. 195-197 °C. It gave positive Liebermann-Burchard test for triterpenoids and its structure elucidation has been discussed in **chapter-5**.

**Separation of mixture-10**

Mixture-10 appeared as a single spot identical to VA-02, on silica gel thin layers, but, HPLC on reversed-phase column showed the presence of two peaks. The mixture could not be resolved even after repeated chromatography over normal silica gel columns. So, the mixture was acetylated with acetic anhydride-pyridine and the residue obtained after usual workup, was subjected to column chromatography on silica gel using mixtures of hexane and ethyl acetate, with increasing polarity, for elution. But these attempts were also failed to resolve the acetylated compounds. Then, 150 mg of the acetylated mixture was subjected to preparative HPLC on a reversed-phase $C_{18}$ column (Luna $C_{18}$, 10 μm, 250 × 21.2 mm, 20 mL/min) and eluted with isocratic mixture of water and acetonitrile (15: 85), to give **VA-02a** ($t_R$ 14.6 min) and **VA-03** ($t_R$ 12.9 min).

**2α,3α-diacetoxyurs-12-en-28-oic acid (VA-02a, 75 mg)**

It was obtained as white amorphous powder. The details of its structure elucidation have been presented in **chapter-5**.

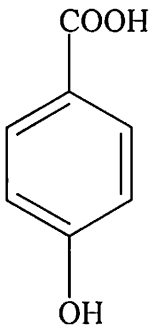
**2α,3α-diacetoxyolean-12-en-28-oic acid (VA-03, 16 mg)**

It was obtained as white amorphous solid. It gave positive LB test for triterpenoids. Its structure elucidation has been discussed in **chapter-5**.

***p*-Hydroxybenzoic acid (VA-04, 2.02, 1.6 g)**

It was obtained as colorless solid from chloroform, m.p. 214-216 °C. Its IR (KBr) spectrum showed bands at $\nu_{max}$ 3382 (hydroxyl), 1676 (Ar-COOH), 1593, 1447 $cm^{-1}$ (aromatic). The $^1H$ NMR spectrum of VA-04 showed the presence of two AB doublets at δ 7.76 (2H, d, $J$ = 8.6 Hz) and δ 6.78 (2H, d, $J$ = 8.6 Hz) characteristic of *p*-hydroxybenzoic acid. This was supported by the $^{13}C$ NMR spectrum which showed signal at δc 189.3 (COOH), 172.2, 161.2, 131.5 and

114.9. The identity of VA-04 as *p*-hydroxybenzoic acid was ascertained further by direct comparison with the authentic sample (co-HPTLC and undepressed mixed melting point).




**2.02**

**Separation of mixture-2**

Mixture-2 (5.0 g) showed two major spots on silica gel thin layers ($R_f$ 0.82 and 0.57; Chloroform: acetone, 8:2). The mixture was chromatographed over silica gel column (200 g) using mixtures of chloroform and acetone with increasing polarity, for elution. Fractions of 200 mL were collected and monitored over silica gel thin layers. The details of chromatography are noted in table 2.03.

**TABLE 2.03**

**Chromotography of mixture-2**

| Eluant (chloroform: acetone)[a] | Fractions | Compounds | $R_f$ | Yield |
|---|---|---|---|---|
| 95:5 | 1-10 | Oily residue[b] | -- | -- |
| 92:8 | 11-15 | **VA-05** | 0.82[c] | 180 mg |
| 90:10 | 16-20 | Green oily residue | -- | -- |
| 88:12 | 21-30 | **VA-06** | 0.57[c] | 250 mg |
| 86:14 | 31-35 | Intractable gum | -- | -- |

[a]Values refer to the ratio of solvents by volume.

[b]Low polar material and moves along with the solvent front on TLC.

[c]Solvent system used for TLC: Chloroform: acetone, 8:2.

**Altissinone (VA-05, 180 mg)**

It was obtained as pale green flakes from hexane and acetone, m.p 151-152 °C. It was found to be a new tetrahydrofuranoid lignan and its structure elucidation has been discussed in **chapter-4.**

**Euscaphic acid (VA-06, 250 mg)**

It was obtained as white amorphous powder from methanol, m.p. 268-270 °C. It gave positive LB test for triterpenoids. The details of its structure elucidation have been presented in **chapter-5.**

**Separation of mixture-3**

Mixture-3 (5.0 g) exhibited three major spots on silica gel thin layers ($R_f$ 0.57, 0.38 and 0.37; Chloroform: acetone, 8:2). The mixture was chromatographed over silica gel column (200 g) using mixtures of chloroform and methanol with increasing polarity, for elution. Fractions of 200 mL were collected and monitored over silica gel thin layers. The details of chromatography are noted in table 2.04.

**TABLE 2.04**

**Chromatography of mixture-3**

| Eluant (chloroform: methanol)[a] | Fractions | Compounds | $R_f$ | Yield |
|---|---|---|---|---|
| 98:2 | 1-5 | Oily residue[b] | -- | -- |
| 98:2 | 6-20 | **VA-06** | 0.57[c] | 125 mg |
| 98:2 | 21-40 | **VA-07** | 0.38[c] | 600 mg |
| 98:2 | 41-45 | **VA-07+VA-08** | | 80 mg |
| 98:2 | 46-50 | **VA-08** | 0.37 | 30 mg |
| 98:2 | 51-55 | Intractable gum | -- | -- |

[a]Values refer to the ratio of solvents by volume.

[b]Low polar material and moves along with the solvent front on TLC.

[c]Solvent system used for TLC: Chloroform: acetone, 8:2.

**Corosolic acid (VA-07, 600 mg)**

It was obtained as white amorphous powder from methanol, m.p. 243-245 °C. It gave positive LB test for triterpenoids. Its structure elucidation has been discussed in **chapter-5**.

**Maslinic acid (VA-08, 30 mg)**

It was obtained as white amorphous powder from methanol, m.p. 252-254 °C. It gave positive LB test for triterpenoids and its structure elucidation has been discussed in **chapter-5.**

**Separation of mixture-4**

Mixture-4 (4.0 g) showed two major spots on silica gel thin layers ($R_f$ 0.38 and 0.37; Chloroform: acetone, 8:2). The mixture was chromatographed over silica gel column (200 g) using mixtures of chloroform and methanol with increasing polarity, for elution. Fractions of 200 mL were collected and monitored over silica gel thin layers. The details of chromatography are noted in table 2.05.

**TABLE 2.05**

**Chromatography of mixture-4**

| **Eluant (chloroform: methanol)[a]** | **Fractions** | **Compounds** | **$R_f$** | **Yield** |
|---|---|---|---|---|
| 98:2 | 1-5 | Oily residue[b] | -- | -- |
| 98:2 | 6-20 | **VA-07** | 0.38[c] | 200 mg |
| 98:2 | 21-40 | **Mixture-11** | 0.37[c] | 600 mg |
| 98:2 | 41-45 | Intractable gum | | |

[a]Values refer to the ratio of solvents by volume.

[b]Low polar material and moves along with the solvent front on TLC.

[c]Solvent system used for TLC: Chloroform: acetone, 8:2.

**Separation of mixture-11**

Mixture-11 appeared as a single spot identical to VA-07 on silica gel thin layers, but HPLC on reversed phase column showed the presence of three peaks. The mixture could not be resolved on normal silica gel column and attempts to resolve on reversed phase preparative HPLC were also unsuccessful. The mixture was not studied further.

**Separation of mixture-5**

Mixture-5 appeared as a single spot on silica gel thin layers, but, HPLC showed the presence of two compounds. These compounds could not be resolved on normal silica gel columns. So, the mixture was subjected to preparative HPLC on reversed-phase $C_{18}$ column (Luna $C_{18}$, 10 μm, 250 × 21.2 mm, 20 mL/min) using water and acetonitrile (1:1) mixture as an eluent. This yielded **VA-09** ($t_R$ 10.7 min) and **VA-10** ($t_R$ 8.0 min).

**2α,3α,24-Trihydroxyurs-12-en-28-oic acid (VA-09, 25 mg)**

It was obtained as colorless amorphous powder from methanol, m.p. 278-281 °C. It was found to be a triterpenoid. Its structure elucidation has been discussed in **chapter-5.**

**2α,3α,24-Trihydroxyurs-12,20(30)-dien-28-oic acid (VA-10, 15 mg)**

It was obtained as colorless amorphous powder, m.p. 258-260 °C. It was found to be a triterpenoid and the details of its structure elucidation have been presented in **chapter-5**.

**Separation of mixture-6**

Mixture-6 (13.0 g) showed two major spots on silica gel thin layers ($R_f$ 0.80 and 0.72; Ethyl acetate: methanol, 9.4:0.6). The mixture was chromatographed over silica gel column (300 g) using mixtures of chloroform and methanol, with increasing polarity, for elution.

Fractions of 250 mL were collected and monitored over silica gel thin layers. The details of chromatography are noted in table 2.06.

**TABLE 2.06**

**Chromatography of mixture-6**

| **Eluant (chloroform: methanol)[a]** | **Fractions** | **Compounds** | **$R_f$** | **Yield** |
|---|---|---|---|---|
| 100:0 | 1-15 | Oily residue[b] | -- | -- |
| 94:6 | 16-25 | Green oily residue | -- | -- |
| 90:10 | 26-30 | **Sterol glucoside** | 0.73[c] | 30 mg |
| 90:10 | 31-35 | **VA-11** | 0.80[d] | 150 mg |
| 90:10 | 36-45 | Intractable gum | -- | -- |
| 85:15 | 46-50 | **Mixture-12** | 0.72[d] | 3.0 g |
| 85:15 | 51-60 | Intractable gum | -- | -- |

[a]Values refer to the ratio of solvents by volume.

[b]Low polar material and moves along with the solvent front on TLC.

[c]Solvent system used for TLC: Chloroform: methanol, 8:2.

[d]Solvent system used for TLC: Ethyl acetate: methanol, 9.4: 0.6.

**VA-11**

VA-11 mainly contained one major spot on silica gel thin layers along with some impurities. Attempted crystallizations and column chromatography on silica gel column failed to yield the pure compound. So, the mixture was acetylated with acetic anhydride-pyridine and the viscous mass obtained after usual workup was purified by column chromatography on silica gel using mixtures of hexane and ethyl acetate (75:25) as eluents to yield **VA-11a.**

**Euscaphic acid ester glucoside hexaacetate (VA-11a, 40 mg)**

It was obtained as a white amorphous powder. It was found to be a triterpenoid and its structure elucidation details have been presented in **chapter-5**.

**Separation of mixture-12**

Mixture-12 showed one major UV active spot on silica gel thin layers ($R_f$ 0.72, Ethyl acetate: methanol, 9.4:0.6). The mixture was chromatographed over silica gel column (150 g) and eluted with ethyl acetate and mixtures of ethyl acetate and methanol. Fractions of 150 mL were collected and monitored over silica gel thin layers. The details of chromatography are noted in table 2.07.

**TABLE 2.07**

**Chromatography of mixture-12**

| **Eluant** (Ethyl acetate: methanol)[a] | **Fractions** | **Compounds** | **$R_f$** | **Yield** |
|---|---|---|---|---|
| 100:0 | 1-10 | Green oily residue | -- | -- |
| 100:0 | 11-25 | **VA-12** | 0.72[b] | 700 mg |
| 98:2 | 26-30 | Intractable gum | -- | -- |

[a]Values refer to the ratio of solvents by volume.

[b]Solvent system used for TLC: Ethyl acetate: methanol, 9.4: 0.6.

**6′-O-Feruloylnegundoside (VA-12, 700 mg)**

It was obtained as pale-yellow amorphous powder, m.p. 155-156 °C. It was found to be an iridoid glucoside and its structure elucidation has been discussed in **chapter-3**.

**Separation of mixture-7**

Mixture-7 (20 g) showed three major UV active spots on silica gel thin layers ($R_f$ 0.72, 0.67 and 0.45; Ethyl acetate: methanol, 9.4:0.6). The mixture was chromatographed over silica gel column (450 g) using mixtures of chloroform and methanol, with increasing polarity, for elution. Fractions of 250 mL were collected and monitored over silica gel thin layers. The details of chromatography are noted in table 2.08.

**TABLE 2.08**

**Chromatography of mixture-7**

| **Eluant (chloroform: methanol)**[a] | **Fractions** | **Compounds** | **$R_f$** | **Yield** |
|---|---|---|---|---|
| 95:5 | 1-10 | Oily residue[b] | -- | -- |
| 90:10 | 11-20 | Oily residue[b] | | |
| 85:15 | 25-35 | **VA-13** | 0.45[c] | 118 mg |
| 80:20 | 36-45 | **VA-12** | 0.72[c] | 220 mg |
| 80:20 | 46-60 | **VA-14** | 0.67[c] | 500 mg |
| 80:20 | 61-80 | **Mixture-13** | 0.67[c] | 4.0 g |
| 75:25 | 81-86 | Intractable gum | | |

[a]Values refer to the ratio of solvents by volume.

[b]Low polar material and moves along with the solvent front on TLC.

[c]Solvent system used for TLC: Ethyl acetate: methanol, 9.4: 0.6.

**Agnuside (VA-13, 118 mg)**

It was obtained as a brown amorphous powder from ethyl acetate, m.p. 151-153 °C. It was found to be an iridoid glucoside and its structure elucidation has been discussed in **chapter-3**.

**6'-O-Caffeoylnegundoside (VA-14, 500 mg)**

It was obtained as pale-yellow amorphous powder, m.p. 168-169 °C. It was found to be an iridoid glucoside and its structure elucidation details have been presented in **chapter-3**.

**Separation of mixture-13**

Mixture-13 was purified further by column chromatography on silica gel (200 g) using ethyl acetate and mixtures of ethyl acetate and methanol in increasing polarity, for elution. Fractions of 200 mL were collected and monitored over silica gel thin layers. The details of chromatography are noted in table 2.09.

**TABLE 2.09**

**Chromatography of mixture-13**

| **Eluant** (Ethylacetate: methanol)[a] | **Fractions** | **Compounds** | **$R_f$** | **Yield** |
|---|---|---|---|---|
| 100:0 | 1-15 | Green oily residue | -- | -- |
| 100:0 | 16-35 | **VA-14** | 0.67[b] | 1.5 g |
| 98:2 | 36-40 | Intractable gum | -- | -- |

[a]Values refer to the ratio of solvents by volume.

[b]Solvent system used for TLC: Ethyl acetate: methanol, 9.4:0.6.

**Separation of mixture -8**

Mixture-8 (15.0 g) showed three major UV active spots on silica gel thin layers ($R_f$ 0.64, 0.60 and 0.37; Ethyl acetate: methanol, 9.4:0.6). The mixture was chromatographed over silica gel column (400 g) using ethyl acetate and mixtures of ethyl acetate and methanol, with increasing polarity, for elution. Fractions of 250 mL were collected and

**6'-O-Caffeoylnegundoside (VA-14, 500 mg)**

It was obtained as pale-yellow amorphous powder, m.p. 168-169 °C. It was found to be an iridoid glucoside and its structure elucidation details have been presented in **chapter-3**.

**Separation of mixture-13**

Mixture-13 was purified further by column chromatography on silica gel (200 g) using ethyl acetate and mixtures of ethyl acetate and methanol in increasing polarity, for elution. Fractions of 200 mL were collected and monitored over silica gel thin layers. The details of chromatography are noted in table 2.09.

**TABLE 2.09**

**Chromatography of mixture-13**

| **Eluant** (Ethylacetate: methanol)[a] | **Fractions** | **Compounds** | **$R_f$** | **Yield** |
|---|---|---|---|---|
| 100:0 | 1-15 | Green oily residue | -- | -- |
| 100:0 | 16-35 | **VA-14** | 0.67[b] | 1.5 g |
| 98:2 | 36-40 | Intractable gum | -- | -- |

[a]Values refer to the ratio of solvents by volume.

[b]Solvent system used for TLC: Ethyl acetate: methanol, 9.4:0.6.

**Separation of mixture -8**

Mixture-8 (15.0 g) showed three major UV active spots on silica gel thin layers ($R_f$ 0.64, 0.60 and 0.37; Ethyl acetate: methanol, 9.4:0.6). The mixture was chromatographed over silica gel column (400 g) using ethyl acetate and mixtures of ethyl acetate and methanol, with increasing polarity, for elution. Fractions of 250 mL were collected and

monitored over silica gel thin layers. The details of chromatography are noted in table 2.10.

**TABLE 2.10**

**Chromatography of mixture-8**

| **Eluant (Ethyl acetate: methanol)[a]** | **Fractions** | **Compounds** | **$R_f$** | **Yield** |
|---|---|---|---|---|
| 100:0 | 1-5 | Oily residue[b] | -- | -- |
| 100:0 | 6-25 | **Mixture-14** | 0.64[c] | 1.0 g |
| 100:0 | 26-30 | **VA-15** | 0.60[c] | 11 mg |
| 100:0 | 31-50 | **VA-16** | 0.37[c] | 2.0 g |
| 98:2 | 51-60 | Intractable gum | | |

[a]Values refer to the ratio of solvents by volume

[b]Low polar material and moves along with the solvent front on TLC.

[c]Solvent system used for TLC: Ethyl acetate: methanol, 9.4:0.6.

**Vitexin (VA-15, 11 mg)**

It was obtained as yellow amorphous powder from methanol, m.p. 275-277 °C. It was found to be a flavonoid and the details of its structure elucidation have been described in **chapter-6**.

**Negundoside (VA-16, 2.0 g)**

It was obtained as crystalline solid from methanol, m.p. 161-163 °C. It was found to be an iridoid glucoside. Its structure elucidation details have been presented in **chapter-3**.

**Separation of mixture-14**

Mixture-14 appeared as a single spot on silica gel thin layers, but, HPLC on reversed-phase column showed two peaks corresponding to two different compounds. These compounds could not be separated

on normal silica gel columns. So, a portion (200 mg) of the mixture was subjected to preparative HPLC on reversed-phase $C_{18}$ column (Luna $C_{18}$, 10 μm, 250 × 21.2 mm, 20 mL/min) using water and acetonitrile mixture (75:25) as eluant to yield **VA-17** ($t_R$ 11.2 min, 45 mg) and **VA-18** ($t_R$ 14.6 min, 95 mg).

**2′-*O*-*p*-Hydroxybenzoyl-6′-*O*-*trans*-caffeoylgardoside (VA-17, 45 mg)**

It was obtained as pale-yellow amorphous powder, m.p. 191-192 °C. It was found to be an iridoid glucoside and its structure elucidation has been discussed in **chapter-3**.

**2′-*O*-*p*-Hydroxybenzoyl-6′-*O*-*trans*-caffeoyl-8-epiloganic acid (VA-18, 95mg)**

It was obtained as a pale-yellow amorphous powder, m.p. 158-160 °C. It was found to be an iridoid glucoside. The details of its structure elucidation have been presented in **chapter-3**.

**Separation of mixture-9**

Mixture-9 (13.0 g) showed three major UV active spots on silica gel thin layers ($R_f$ 0.72, 0.38 and 0.32; Ethyl acetate: methanol, 9.4:0.6). The mixture was chromatographed over silica gel column (400 g) using ethyl acetate and mixtures of ethyl acetate and methanol, with increasing polarity, for elution. Fractions of 200 mL were collected and monitored over silica gel thin layers. The details of chromatography are noted in table 2.11.

## TABLE 2.11

**Chromatography of mixture-9**

| Eluant (Ethyl acetate: methanol)[a] | Fractions | Compounds | $R_f$ | Yield |
|---|---|---|---|---|
| 100:0 | 1-8 | Intractable gum | -- | -- |
| 100:0 | 9-15 | **VA-19** | 0.72[b] | 09 mg |
| 100:0 | 16-30 | **VA-20** | 0.42[b] | 140 mg |
| 100:0 | 31-40 | **Mixture-15** | 0.30[b] | 125 mg |
| 100:0 | 41-45 | Intractable gum | -- | -- |
| 98:2 | 46-50 | Intractable gum | -- | -- |

[a]Values refer to the ratio of solvents by volume.

[b]Solvent system used for TLC: Ethyl acetate: methanol, 9.4: 0.6.

**2″-*O*-*p*-Hydroxybenzoylorientin (VA-19, 09 mg)**

It was obtained as yellow amorphous powder from methanol. It was found to be a new flavonoid. The details of its structure elucidation have been presented in **chapter-6**.

**Luteolin-7-*O*-glucoside (VA-20, 140 mg)**

It was obtained as yellow amorphous powder from methanol, m.p. 253-255 °C. It was found to be a flavonoid and its structure elucidation has been discussed in **chapter-6**.

**Separation of mixture-15**

Mixture-15 appeared as a single spot on silica gel thin layers, but, HPLC on reversed-phase column showed the presence of two compounds. These compounds could not be resolved on normal silica gel columns. So, the mixture was subjected to preparative HPLC on reversed-phase $C_{18}$ column (Luna $C_{18}$, 10 μm, 250 × 21.2 mm, 20 mL/min) using water and acetonitrile (85:15) mixture as an eluent to yield **VA-21** ($t_R$ 8.8 min, 13 mg) and **VA-22** ($t_R$ 14.0 min, 20 mg).

**TABLE 2.11**

**Chromatography of mixture-9**

| **Eluant (Ethyl acetate: methanol)[a]** | **Fractions** | **Compounds** | **$R_f$** | **Yield** |
|---|---|---|---|---|
| 100:0 | 1-8 | Intractable gum | -- | -- |
| 100:0 | 9-15 | **VA-19** | 0.72[b] | 09 mg |
| 100:0 | 16-30 | **VA-20** | 0.42[b] | 140 mg |
| 100:0 | 31-40 | **Mixture-15** | 0.30[b] | 125 mg |
| 100:0 | 41-45 | Intractable gum | -- | -- |
| 98:2 | 46-50 | Intractable gum | -- | -- |

[a]Values refer to the ratio of solvents by volume.

[b]Solvent system used for TLC: Ethyl acetate: methanol, 9.4: 0.6.

**2″-*O*-*p*-Hydroxybenzoylorientin (VA-19, 09 mg)**

It was obtained as yellow amorphous powder from methanol. It was found to be a new flavonoid. The details of its structure elucidation have been presented in **chapter-6**.

**Luteolin-7-*O*-glucoside (VA-20, 140 mg)**

It was obtained as yellow amorphous powder from methanol, m.p. 253-255 °C. It was found to be a flavonoid and its structure elucidation has been discussed in **chapter-6**.

**Separation of mixture-15**

Mixture-15 appeared as a single spot on silica gel thin layers, but, HPLC on reversed-phase column showed the presence of two compounds. These compounds could not be resolved on normal silica gel columns. So, the mixture was subjected to preparative HPLC on reversed-phase $C_{18}$ column (Luna $C_{18}$, 10 μm, 250 × 21.2 mm, 20 mL/min) using water and acetonitrile (85:15) mixture as an eluent to yield **VA-21** ($t_R$ 8.8 min, 13 mg) and **VA-22** ($t_R$ 14.0 min, 20 mg).

**2′-*O*-*p*-Hydroxybenzoylgardoside (VA-21, 13 mg)**

It was obtained as colorless amorphous powder, m.p. 215-216 °C. It was found to be an iridoid glucoside and its structure elucidation has been discussed in **chapter-3**.

**2′-*O*-*p*-Hydroxybenzoyl-8-epiloganic acid (VA-22, 20 mg)**

It was obtained as colorless amorphous powder, m.p. 219-220 °C. It was found to be an iridoid glucoside. The details of its structure elucidation have been presented in **chapter-3**.

## Section-B

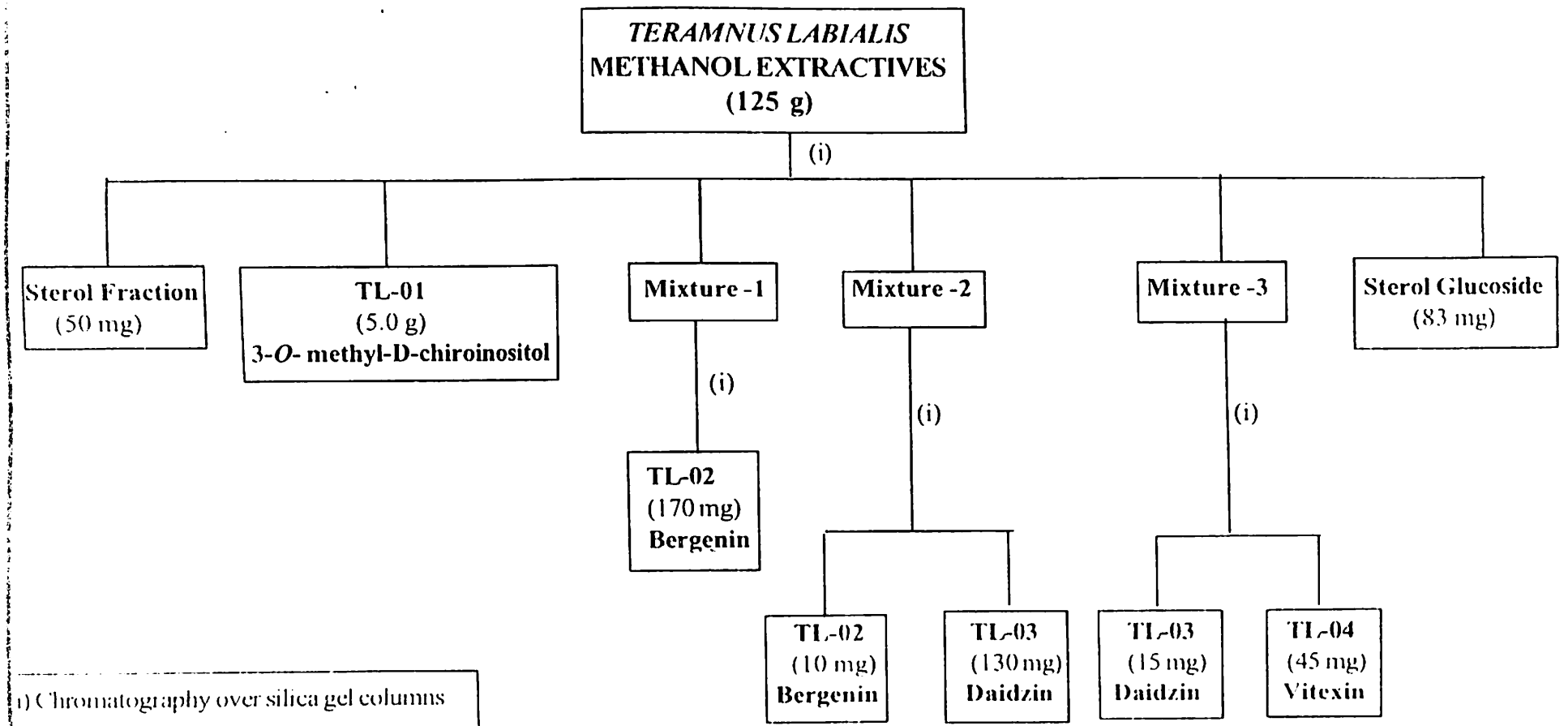
## Isolation of chemical constituents from *T. labialis*

**Extraction**

The aerial parts of *T. labialis* were cut into small pieces and milled to a coarse powder. The powder (ca 2.5 Kg) was extracted, successively, with hexane (5 × 10 L), ethyl acetate (5 × 10 L) and methanol (5 × 10 L), in a soxhlet apparatus. The combined extracts of each solvent were concentrated under reduced pressure to yield hexane (50 g), ethyl acetate (56 g) and methanol (150 g), residues. The extracts were screened for anti-inflammatory activity by carrageenan-induced rat paw edema model and the details are presented in **chapter-8**. The methanolic extractives which exhibited better anti-inflammatory activity were chromatographed over silica gel columns. The details of isolation of chemical constituents are presented in the following paragraphs.

The summary of the procedure of isolation of chemical constituents from methanolic extractives of *T. labialis* has been presented in chart **2.02**

CHART 2.02 SCHEME OF ISOLATION OF METABOLITES FROM METHANOL EXTRACTIVES OF *TERAMNUS LABIALIS*



The combined methanolic extractives were concentrated under reduced pressure to give a dark brown gummy residue (ca 150 g). About 125 g of the extract was chromatographed over silica gel (1000 g) column and eluted with chloroform and mixtures of chloroform and methanol, with increasing polarity. Fractions of 1000 mL were collected and monitored over silica gel thin layers. The results of chromatography are noted in Table 2.12.

**TABLE 2.12**

**Chromatography of methanolic extractives**

| Eluant (chloroform: methanol)[a] | Fractions | Compounds | $R_f$ | Yield |
|---|---|---|---|---|
| 100:0 | 1-21 | Oily residue[b] | | |
| 100:0 | 22-32 | **Sterol fraction** | 0.79[c] | 50 mg |
| 90:10 | 33-40 | **Sterol glucoside** | 0.73[d] | 83 mg |
| 90:10 | 41-55 | **Mixture-1** | | 4.0 g |
| 70:30 | 56-57 | **Mixture-2** | | 4.0 g |
| 70:30 | 58-59 | **Mixture-3** | | 6.0 g |
| 70:30 | 60-85 | **TL-01** | 0.3[e] | 5 g |
| 50:50 | 86-90 | Intractable gum | | |

[a]Values refer to the ratio of solvents by volume.

[b]Low polar material and moves along with the solvent front on TLC.

[c]Solvent system used for TLC: Hexane: ethyl acetate, 7:3.

[d]Solvent system used for TLC: Chloroform: methanol, 8:2.

[e]Solvent system used for TLC: *n*-Butanol: acetic acid: water, 6:2:2.

**Sterol fraction (50 mg)**

It was obtained as colorless needles from pet. ether and ethyl acetate, m.p. 137-139 °C. It gave positive Libermann-Burchard test with play of colors, characteristic of phytosterols. The phytosterol fraction was not examined further.

**Sitosterol-3-O-β-D-glucoside (2.01, 83 mg)**

It was obtained as colorless crystals from chloroform-methanol, m.p. 290-292 °C. It gave positive Libermann-Burchard test with play of colors, characteristic of steroids. It was identified as sitosterol-3-*O*-*β*-D-glucoside (**2.02**) by direct comparison with authentic sample, isolated from *Justicia diffusa* willd[7] through co-TLC and undepressed mixed melting point.

**3-O-methyl-D-chiroinositol (TL-01, 5.0 g)**

It was obtained as a white crystalline solid from methanol, m.p. 180-182 °C. It was found to be a cyclitol derivative and the details of its structure elucidation have been presented in **chapter-7**.

**Separation of mixture-1 (4.0 g)**

Mixture-1 showed one major spot on silica gel thin layers ($R_f$ 0.40; chloroform: methanol, 8:2). The mixture was chromatographed over silica gel column (150 g) using mixtures of chloroform and methanol, with increasing polarity, for elution. Fractions of 100 mL were collected and monitored over silica gel thin layers. The details of chromatography are noted in table 2.13.

**TABLE 2.13**

**Chromatography of mixture-1**

| Eluant (chloroform: methanol)[a] | Fractions | Compounds | $R_f$ | Yield |
|---|---|---|---|---|
| 98:2 | 1-32 | Oily fractions[b] | -- | -- |
| 95:5 | 33-50 | Intractable gum | -- | -- |
| 93:7 | 51-70 | **TL-02** | 0.40[c] | 170 mg |
| 90:10 | 70-80 | Intractable gum | -- | -- |

[a]Values refer to the ratio of solvents by volume.

[b]Low polar material and moves along with the solvent front on TLC.

[c]Solvent system used for TLC: Chloroform: methanol, 8:2.

**Bergenin (TL-02, 170 mg)**

It was obtained as colorless crystals from aqueous methanol, m.p. 140-142 °C. It was found to be a glucoisocoumarin and its structure elucidation has been presented in **chapter-7**.

**Separation of mixture-2 (4.0 g)**

Mixture-2 (4.0 g) showed one major spot on silica gel thin layers ($R_f$ 0.37; chloroform: methanol, 8:2). The mixture was chromatographed over silica gel column (150 g) using mixtures of chloroform and methanol with increasing polarities, for elution. Fractions of 200 mL were collected and monitored over silica gel thin layers. The details of chromatography are noted in table 2.14.

## TABLE 2.14

**Chromatography of mixture-2**

| Eluant (chloroform: methanol)[a] | Fractions | Compounds/Mixtures | $R_f$ | Yield |
|---|---|---|---|---|
| 95:5 | 1-10 | Oily fractions[b] | -- | -- |
| 90:10 | 11-15 | **TL-02** | 0.40[c] | 10 mg |
| 90:10 | 16-20 | Intractable gum | -- | -- |
| 85:15 | 21-28 | **TL-03** | 0.37[c] | 130 mg |
| 80:20 | 29-35 | Intractable gum | -- | -- |

[a]Values refer to the ratio of solvents by volume.

[b]Low polar material and moves along with the solvent front on TLC.

[c]Solvent system used for TLC: Chloroform: methanol, 8:2.

**Daidzin (TL-03, 130 mg)**

It was obtained as a pale yellow crystalline solid from aqueous methanol, m.p. 232-233 °C. It was found to be a flavonoid and its structure elucidation has been presented in **chapter-7**.

**Separation of mixture-3 (6.0 g)**

Mixture-3 (6.0 g) showed one major spot on silica gel thin layers ($R_f$ 0.35; chloroform: methanol, 8:2). The mixture was chromatographed over silica gel column (180 g) using mixtures of chloroform and methanol with increasing polarities, for elution. Fractions of 200 mL were collected and monitored over silica gel thin layers. The details of chromatography are noted in table 2.15.

## TABLE 2.15

**Chromatography of mixture-3**

| Eluant (chloroform: methanol)[a] | Fractions | Compounds/Mixtures | $R_f$ | Yield |
|---|---|---|---|---|
| 93:7 | 1-10 | Oily fractions[b] | -- | -- |
| 90:10 | 11-24 | **TL-03** | 0.37[c] | 15 mg |
| 85:15 | 25-35 | **TL-04** | 0.35[c] | 45 mg |
| 85:15 | 36-40 | Intractable gum | -- | -- |

[a]Values refer to the ratio of solvents by volume.

[b]Low polar material and moves along with the solvent front on TLC.

[c]Solvent system used for TLC: Chloroform: methanol, 8:2.

**Vitexin (TL-04, 45 mg)**

It was obtained as a yellow amorphous powder from methanol, m.p. 275-277 °C. It was found to be a flavonoid and the details of its structure elucidation have been described in **chapter-7**.

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# STRUCTURE ELUCIDATION OF IRIDOIDS ISOLATED FROM *V. ALTISSIMA*

The details of isolation of eight iridoids (**VA-12–VA-14**, **VA-16-VA-18**, **VA-21** and **VA-22**) have been described in **Chapter-2**. Structure elucidation of these iridoids, belonging to four different groups, namely, mussaenosidic acid (**VA-16**, **VA-12** and **VA-14**), gardoside (**VA-21** and **VA-17**), 8-epiloganic acid (**VA-22** and **VA-18**) derivatives and aucubin (**VA-13**) is presented in the following pages in **section-I** to **section-IV**.

## SECTION-I

### (MUSSAENOSIDIC ACID DERIVATIVES)

## VA-16

It was obtained as a white crystalline solid from methanol, m.p. 161-163 °C, $[\alpha]_D^{25}$ −125° (*c* 0.5, MeOH). Its molecular formula was established as $C_{23}H_{28}O_{12}$ based on elemental analysis and LC-MS data [*m/z* 495 $(M - H)^-$].

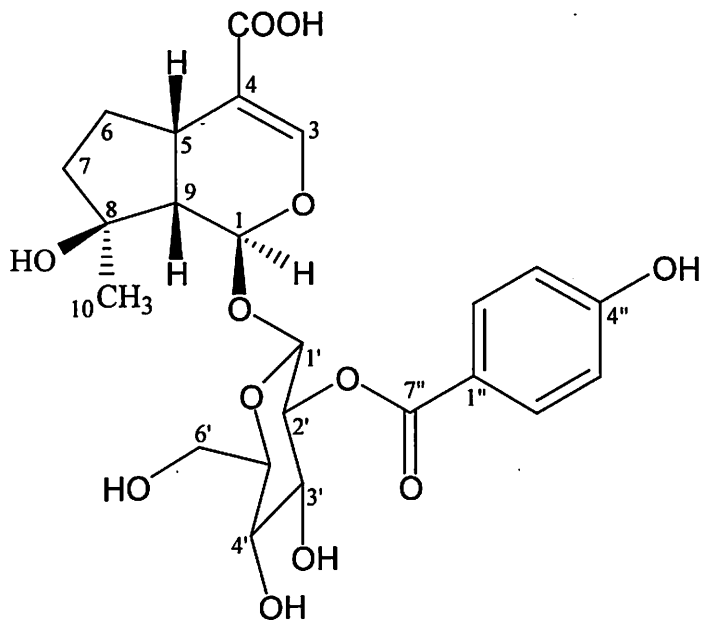
The UV (MeOH) spectrum of VA-16 showed absorption maxima at 255 nm. Its IR (KBr) spectrum showed bands at $\nu_{max}$ 3331 (hydroxyl), 1717 (CO, ester), 1681 (COOH), 1642 (C=C) 1602 and 1508 $cm^{-1}$ (aromatic). The $^1H$ NMR spectral (Fig. 3.01, Table 3.01) data of VA-16 showed the presence of a carboxylic acid (δ 11.55 s), a trisubstituted olefinic proton (δ 7.04, 1H, s, H-3) attributable to β-H of a α,β-unsaturated acid and a doublet at δ 5.30 (1H, d, *J* = 3.6 Hz) assignable to hemiacetal (H-1) proton of an iridoid nucleus.[1]

The $^1H$ NMR spectrum also contained a series of signals between δ 3.23 and 4.85 characteristic of a sugar moiety.[2] The coupling constant observed for the anomeric proton (δ 4.85, d, *J* = 8.1 Hz, H-1′) was consistent with β configuration[3] of the sugar moiety. In addition, the $^1H$ NMR spectrum showed the presence of four aromatic protons constituted by two AB doublets [δ 7.73 (2H, d, *J* = 8.7 Hz) and 6.80 (2H, d, *J* = 8.7 Hz) attributable to a *p*-hydroxybenzoyl unit[4] and a methyl group (δ 1.15, 3H, s, H-10) attached to an oxygen bearing carbon. These spectral data indicated the presence of mussaenosidic acid nucleus[5,6] in VA-16, which was supported further by the $^{13}C$ NMR spectral data.

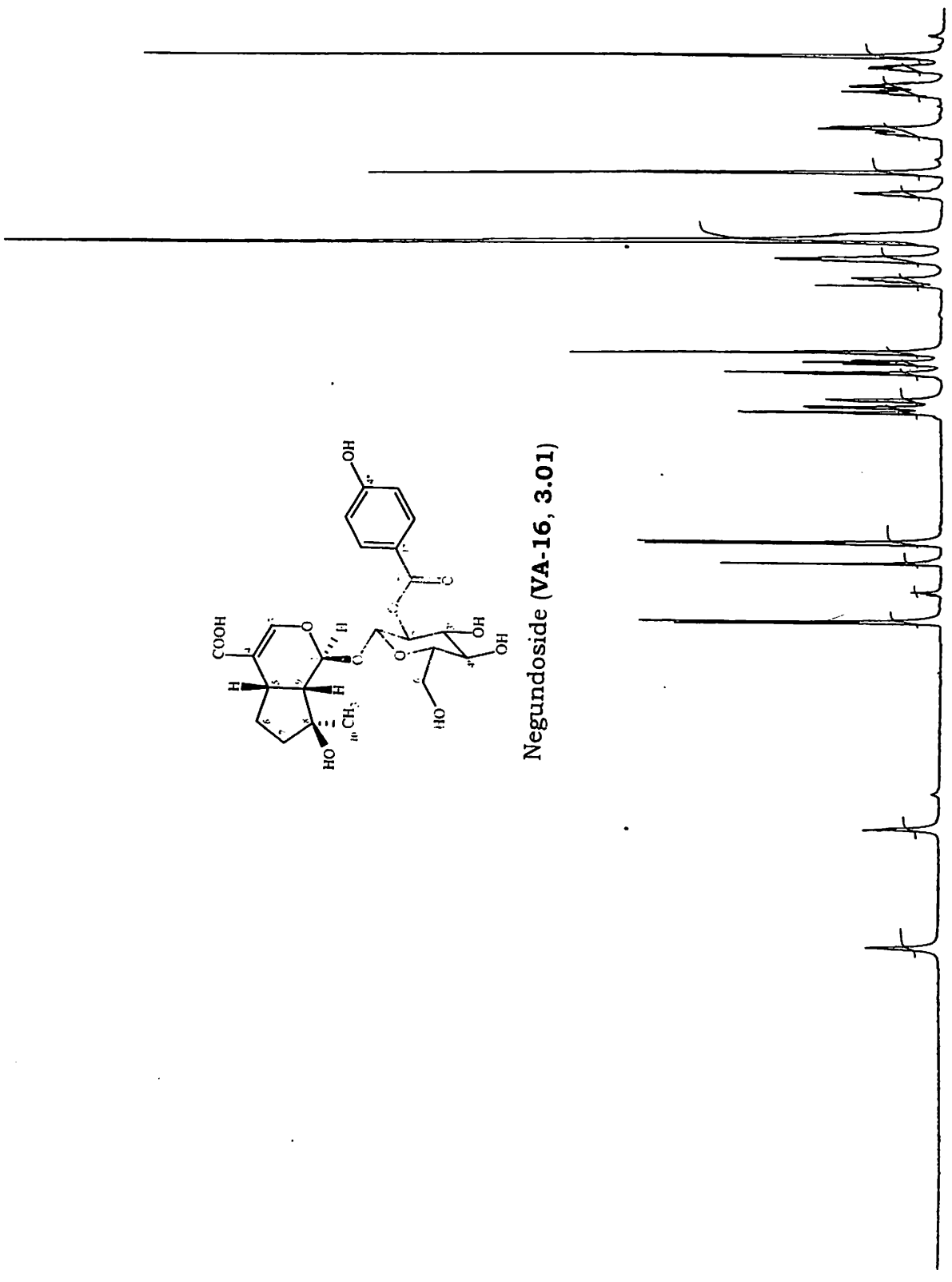
The $^{13}C$ NMR (Fig. 3.02, Table. 3.02) spectrum showed the presence of a carboxylic acid group (δ 169.9, C-11), *p*-hydroxybenzoyl moiety (δc 167.3, 163.2, 132.8, 122.1 and 116.1) and signals at δ 97.7, 75.9, 74.8, 71.7 and 62.7 attributable to sugar moiety. The DEPT (Fig. 3.03) spectrum indicated the presence of an oxygenated quaternary carbon (δ 79.8, C-8), two methylene carbons (δ 41.2 and 30.3), two methine carbons (δ 52.3 and 31.0) and a methyl group (δ 24.3, C-10) assignable to the cyclopentane ring of the mussaenosidic acid.

Literature survey on iridoids of mussaenosidic acid nucleus revealed that the physical, spectral and optical rotation data of VA-16 were in good agreement with those recorded for negundoside[4,7] (2'-*O*-*p*-hydroxybenzoyl mussaenosidic acid (**3.01**), isolated earlier from *Vitex negundo*.[4,7]

Based on the foregoing, the structure of VA-16 was established as negundoside (**3.01**)




**3.01**




Negundoside (**VA-16, 3.01**)

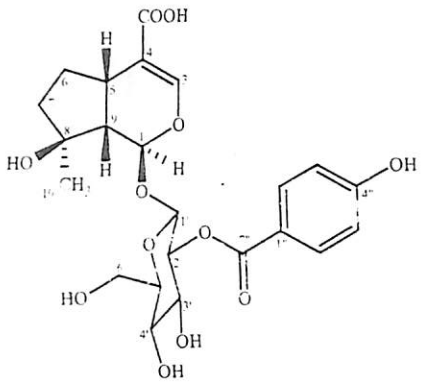
Fig. 3.01: $^1$H NMR spectrum (400 MHz, $d_6$-DMSO) of compound **VA-16 (3.01)**

## TABLE 3.01

**$^1$H NMR spectral data of VA-16 (negundoside, 3.01)**

**(Fig. 3.01, 400 MHz spectrum, $d_6$-DMSO)**

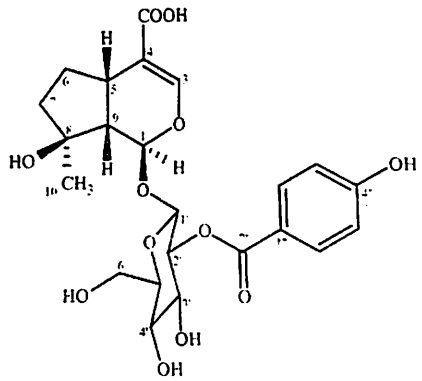
| **Chemical shift (δ)** | **Proton integration** | **Multiplicity (*J* in Hz)** | **Assignment** |
|---|---|---|---|
| 11.55 | 1H | s | COOH |
| 5.30 | 1H | d (3.6) | H-1 |
| 7.04 | 1H | s | H-3 |
| 2.73 | 1H | m | H-5 |
| 2.04 | 1H | m | $H_a$-6 |
| 1.27 | 1H | m | $H_b$-6 |
| 1.44-1.58 | 2H | m | H-7 |
| 1.98 | 1H | dd (9.1, 3.5) | H-9 |
| 1.15 | 3H | s | H-10 |
| 4.85 | 1H | d (8.1) | H-1′ |
| 4.72 | 1H | t (8.3) | H-2′ |
| 3.23 | 1H | m | H-3′ |
| 4.60 | 1H | m | H-4′ |
| 3.75 | 1H | m | H-5′ |
| 3.75 | 1H | m | $H_a$-6′ |
| 3.51 | 1H | m | $H_b$-6′ |
| 7.73 | 2H | d (8.7) | H-2″ and H-6″ |
| 6.80 | 2H | d (8.7) | H-3″ and H-5″ |




Negundoside (**VA-16**, **3.01**)




Fig. 3.02: $^{13}C$ NMR spectrum (75 MHz, $d_4$-MeOH) of compound **VA-16** (**3.01**)




Negundoside (**VA-16**, **3.01**)




Fig. 3.03: DEPT spectrum ($d_4$-MeOH) of compound **VA-16** (**3.01**)

## TABLE 3.02

**$^{13}$C NMR spectral data of VA-16 (negundoside, 3.01)**

**(Fig. 3.02, 75 MHz spectrum, $d_4$-MeOH)**

| Carbon number | Chemical shift (δ) | Carbon number | Chemical Shift (δ) |
|---|---|---|---|
| C-1 | 94.9 | C-2′ | 74.8 |
| C-3 | 151.1 | C-3′ | 75.9 |
| C-4 | 113.6 | C-4′ | 71.7 |
| C-5 | 31.0 | C-5′ | 75.9 |
| C-6 | 30.3 | C-6′ | 62.7 |
| C-7 | 41.2 | C-1″ | 122.1 |
| C-8 | 79.8 | C-2″ and C-6″ | 132.8 |
| C-9 | 52.3 | C-3″ and C-5″ | 116.1 |
| C-10 | 24.3 | C-4″ | 163.2 |
| C-11 | 169.9 | C-7″ | 167.3 |
| C-1′ | 97.7 | | |

## VA-12

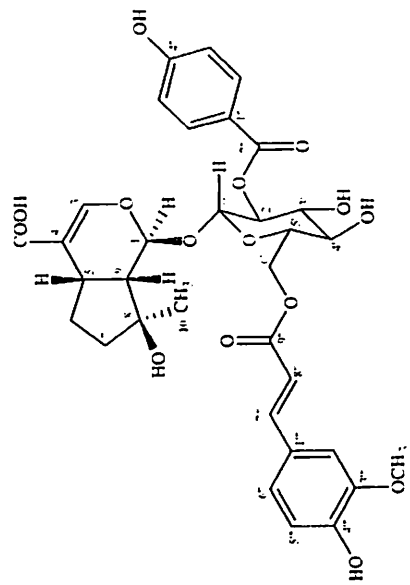
It was obtained as pale-yellow amorphous powder, m.p. 155-156 °C, $[\alpha]_D^{25}$ −74.6° (*c* 0.5, MeOH). The molecular formula of VA-12 was established as $C_{33}H_{36}O_{15}$, based on elemental analysis and LC-MS data [*m/z* 671, $(M - H)^-$].

VA-12 exhibited bands in its UV (MeOH) spectrum at $\lambda_{max}$ 247 and 327 nm. Its IR (KBr) spectrum showed bands at $\nu_{max}$ 3414 (hydroxyl), 1698 (α,β-unsaturated ester), 1633 (C=C), 1601, 1515 (aromatic) and 1271 $cm^{-1}$(C–O). The $^1H$ NMR (Fig. 3.04, Table 3.03) spectral data of VA-12 showed the characteristic signals of an iridoid[1] nucleus [δ 7.11 (1H, d, *J* = 1.0 Hz, H-3) and a doublet at δ 5.31 (1H, *J* = 4.0 Hz, H-1) and δ 1.23 (3H, s, H-10)].

A set of signals [δ 7.85 (2H, d, *J* = 8.5 Hz) and δ 6.83 (2H, d, J = 8.5 Hz)] attributable to *p*-hydroxybenzoyl unit.[4] In addition, the $^1H$ NMR spectrum showed the presence of a *trans*-feruloyl moiety[8] constituted by a methoxyl group (δ 3.88, s, 3H), a 1,2,4-trisubstituted phenyl unit [δ 7.18 (1H, d, *J* = 2.0 Hz), 7.06 (1H, dd, *J* = 8.5, 2.0 Hz) and δ 6.78 (1H, d, *J* = 8.5 Hz)] and two *trans* coupled olefinic protons located at δ 7.64 (1H, d, *J* = 16.0 Hz) and δ 6.40 (1H, d, *J* = 16.0 Hz).

The presence of trans-feruloyl moiety was confirmed further by its $^{13}C$ NMR (Fig. 3.05, Table 3.04) signals at δ 169.0 (C-9‴), 147.2 (C-7‴), 115.2 (C-8‴), 151.3 (C-3‴), 149.3 (C-4‴), 127.6 (C-1‴), 124.2 (C-6‴), 116.5 (C-5‴) and δ 111.7 (C-2‴). These assignments were supported by the DEPT (Fig. 3.06) spectral data and the correlations observed in the HMQC (Fig. 3.07, Table 3.05) spectrum.

The presence of a β-glucopyranose unit was revealed by the observed signals in the $^1H$ NMR (Table 3.03) and $^{13}C$ NMR (Table 3.04) spectral data.



6′-*O*-*trans*-feruloylnegundoside (**VA-12, 3.02**)




Fig. 3.04: $^{1}H$ NMR spectrum (500 MHz, $d_4$-MeOH) of compound **VA-12 (3.02)**

## TABLE 3.03

**$^1$H NMR spectral data of VA-12 (6′-O-*trans*-feruloyl negundoside, 3.02)**

**(Fig. 3.04, 500 MHz spectrum, $d_4$-MeOH)**

| Chemical shift (δ) | Proton integration | Multiplicity (*J* in Hz) | Assignment |
|---|---|---|---|
| 5.31 | 1H | d (4.0) | H-1 |
| 7.11 | 1H | d (1.0) | H-3 |
| 2.93 | 1H | m | H-5 |
| 2.12 | 1H | m | $H_a$-6 |
| 1.33 | 1H | m | $H_b$-6 |
| 1.60 | 2H | m | H-7 |
| 2.12 | 1H | dd (9.0,4.0) | H-9 |
| 1.23 | 3H | s | H-10 |
| 5.01 | 1H | d (8.0) | H-1′ |
| 4.94 | 1H | dd (9.0,8.0) | H-2′ |
| 3.73 | 1H | dd (9.5,9.0) | H-3′ |
| 3.55 | 1H | dd (9.5,9.0) | H-4′ |
| 3.69 | 1H | m | H-5′ |
| 4.56 | 1H | dd (12.0,2.0) | $H_a$-6′ |
| 4.41 | 1H | dd (12.0,5.5) | $H_b$-6′ |
| 7.85 | 2H | d (8.5) | H-2″ and H-6″ |
| 6.83 | 2H | d (8.5) | H-3″ and H-5″ |
| 7.18 | 1H | d (2.0) | H-2‴ |
| 6.78 | 1H | d (8.5) | H-5‴ |
| 7.06 | 1H | dd (8.5,2.0) | H-6‴ |
| 7.64 | 1H | d (16.0) | H-7‴ |
| 6.40 | 1H | d (16.0) | H-8‴ |
| 3.88 | 3H | s | -OMe |

## TABLE 3.03

**$^1$H NMR spectral data of VA-12 (6′-*O*-*trans*-feruloyl negundoside, 3.02)**

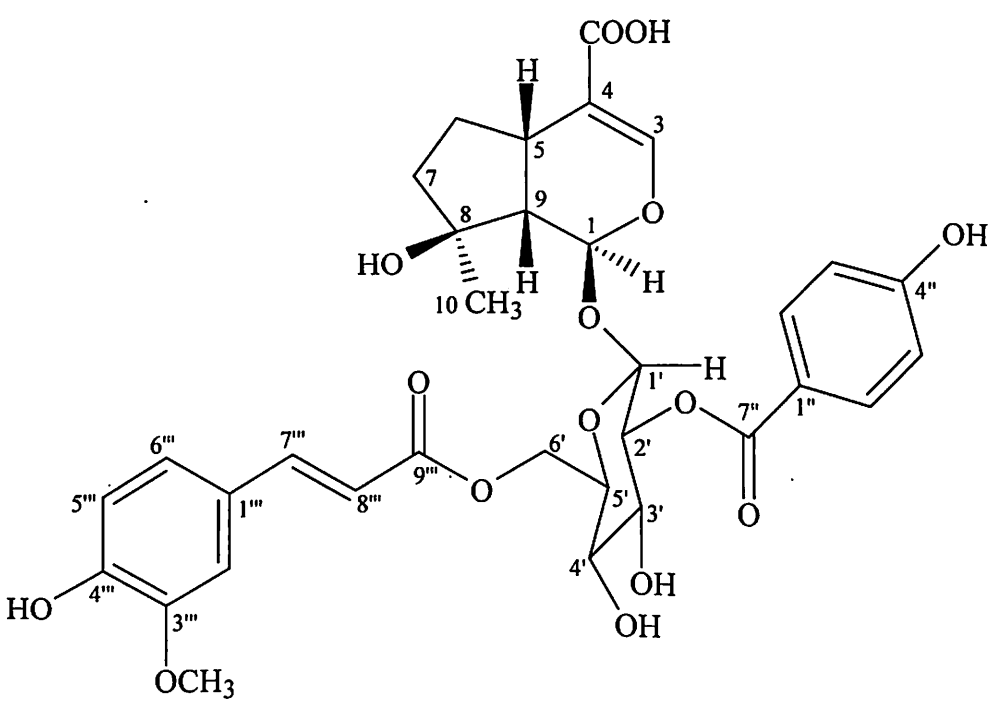
**(Fig. 3.04, 500 MHz spectrum, $d_4$-MeOH)**

| Chemical shift (δ) | Proton integration | Multiplicity (*J* in Hz) | Assignment |
|---|---|---|---|
| 5.31 | 1H | d (4.0) | H-1 |
| 7.11 | 1H | d (1.0) | H-3 |
| 2.93 | 1H | m | H-5 |
| 2.12 | 1H | m | $H_a$-6 |
| 1.33 | 1H | m | $H_b$-6 |
| 1.60 | 2H | m | H-7 |
| 2.12 | 1H | dd (9.0,4.0) | H-9 |
| 1.23 | 3H | s | H-10 |
| 5.01 | 1H | d (8.0) | H-1′ |
| 4.94 | 1H | dd (9.0,8.0) | H-2′ |
| 3.73 | 1H | dd (9.5,9.0) | H-3′ |
| 3.55 | 1H | dd (9.5,9.0) | H-4′ |
| 3.69 | 1H | m | H-5′ |
| 4.56 | 1H | dd (12.0,2.0) | $H_a$-6′ |
| 4.41 | 1H | dd (12.0,5.5) | $H_b$-6′ |
| 7.85 | 2H | d (8.5) | H-2″ and H-6″ |
| 6.83 | 2H | d (8.5) | H-3″ and H-5″ |
| 7.18 | 1H | d (2.0) | H-2‴ |
| 6.78 | 1H | d (8.5) | H-5‴ |
| 7.06 | 1H | dd (8.5,2.0) | H-6‴ |
| 7.64 | 1H | d (16.0) | H-7‴ |
| 6.40 | 1H | d (16.0) | H-8‴ |
| 3.88 | 3H | s | -OMe |

A careful comparison of the above data with those of negundoside (VA-16) revealed that VA-12 contains an additional feruloyl unit. Thus, VA-12 was derived as a feruloyl derivative of negundoside. The observed downfield shift[9] of C-6′ (δ 64.2) and 6′-$H_2$ (δ 4.56-4.41) in VA-12 in comparison with those of negundoside (**3.01**) revealed that the attachment of feruloyl unit is at C-6′ in VA-12. The position of linkage of feruloyl moiety at C-6′ was confirmed by the analysis of the HMBC data. In the HMBC spectrum (Fig. 3.08, Table 3.06) the H-6′ methylene protons (δ 4.56 dd, $J$ = 12.0, 2.0 Hz and 4.41 dd, $J$ = 12.0, 5.5 Hz) of the glucose unit showed correlations with the ester carbonyl (δ 169.0, C-9‴) of feruloyl moiety. The selective HMBC correlations of VA-12 are represented in Fig. 3.09.

The stereochemistry of VA-12 was assumed to be same as that of negundoside (**3.01**), based on the similar chemical shifts and coupling constants observed and the optical rotation data.

Based on the foregoing, VA-12 was derived as 6′-*O-trans* feruloylnegundoside (**3.02**), a new diacylated iridoid glucoside.




**3.02**

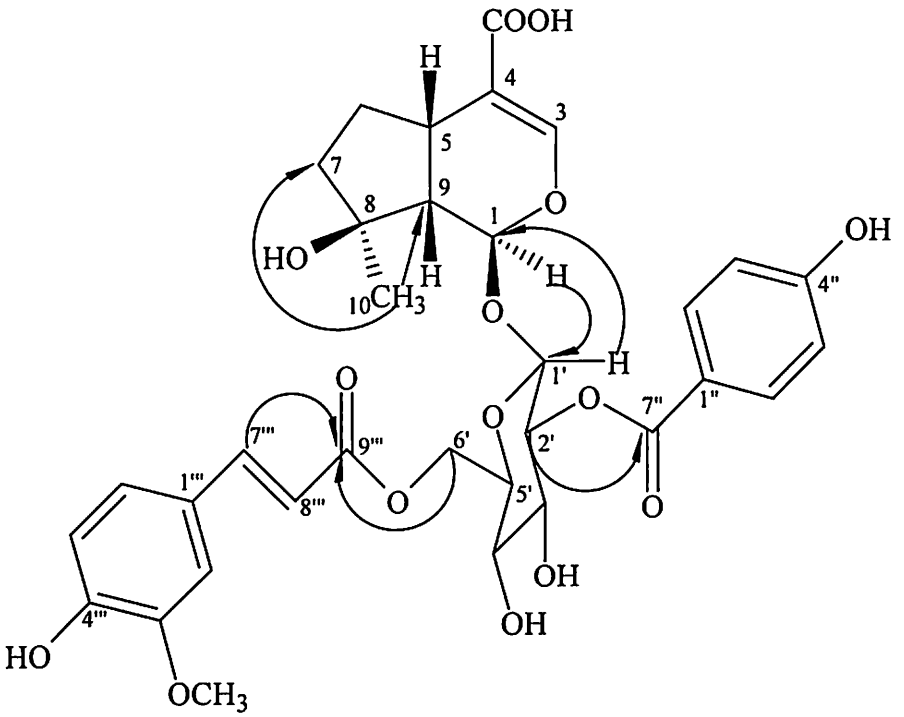
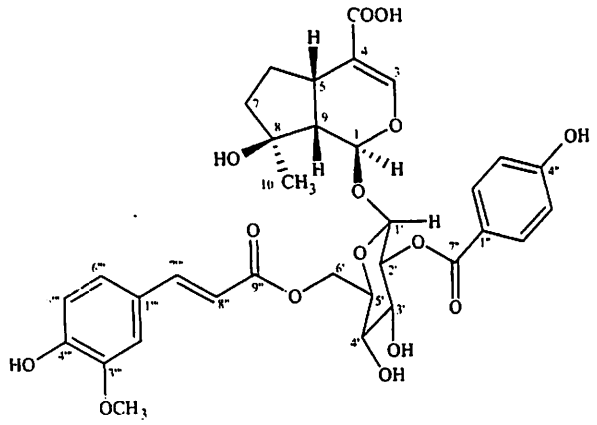

COOH
H
4
3
5
7
9
8
1
O
HO
H
10CH3
H
OH
4"
O
1'
H
O
7"
1"
O
7'''
9'''
6'
2'
1'''
8'''
O
5'
O
OH
OH
4'''
HO
OCH3

**Fig. 3.09**




6′-*O*-*trans*-feruloylnegundoside (**VA-12, 3.02**)

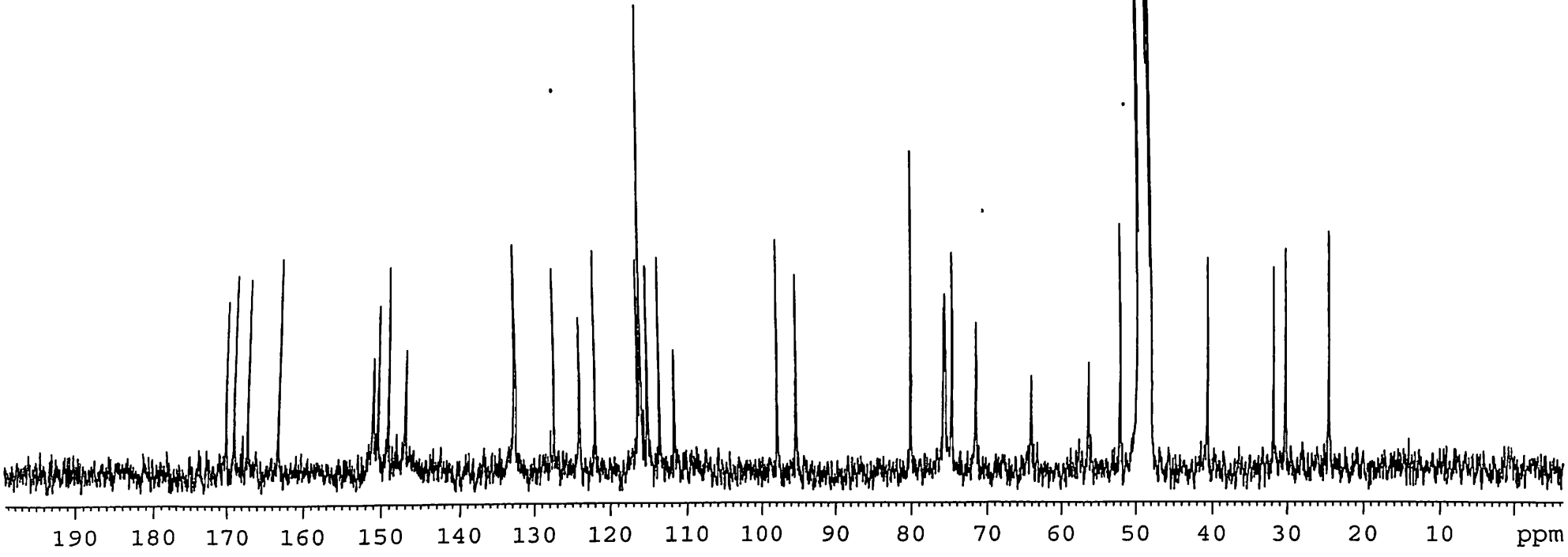


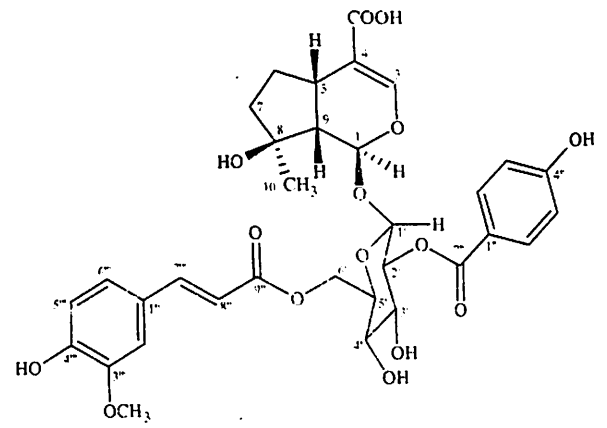

Fig. 3.05: $^{13}C$ NMR spectrum (75 MHz, $d_4$-MeOH) of compound **VA-12 (3.02)**

## TABLE 3.04

**$^{13}$C NMR spectral data of VA-12 (6′-*O*-*trans*-feruloyl negundoside, 3.02)**

**(Fig. 3.05, 75 MHz spectrum, $d_4$-MeOH)**

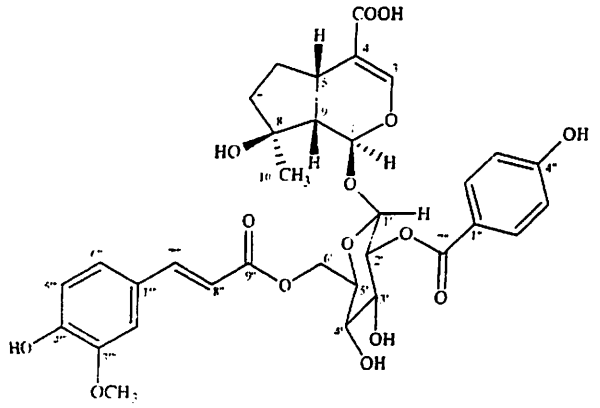
| Carbon number | Chemical shift (δ) | Carbon number | Chemical shift (δ) |
|---|---|---|---|
| C-1 | 95.4 | C-1″ | 122.2 |
| C-3 | 150.6 | C-2″ and C-6″ | 132.9 |
| C-4 | 113.6 | C-3″ and C-5″ | 116.1 |
| C-5 | 31.8 | C-4″ | 163.3 |
| C-6 | 30.3 | C-7″ | 167.3 |
| C-7 | 40.7 | C-1‴ | 127.6 |
| C-8 | 80.2 | C-2‴ | 111.7 |
| C-9 | 52.2 | C-3‴ | 151.3 |
| C-10 | 24.6 | C-4‴ | 149.3 |
| C-11 | 170.0 | C-5‴ | 116.5 |
| C-1′ | 97.9 | C-6‴ | 124.2 |
| C-2′ | 74.8 | C-7‴ | 147.2 |
| C-3′ | 75.8 | C-8‴ | 115.2 |
| C-4′ | 71.7 | C-9‴ | 169.0 |
| C-5′ | 75.8 | -OMe | 56.4 |
| C-6′ | 64.2 | | |




6'-*O*-*trans*-feruloylnegundoside (**VA-12**, **3.02**)




Fig. 3.06: DEPT spectrum ($d_4$-MeOH) of compound **VA-12** (**3.02**)




6'-O-*trans*-feruloylnegundoside (**VA-12, 3.02**)

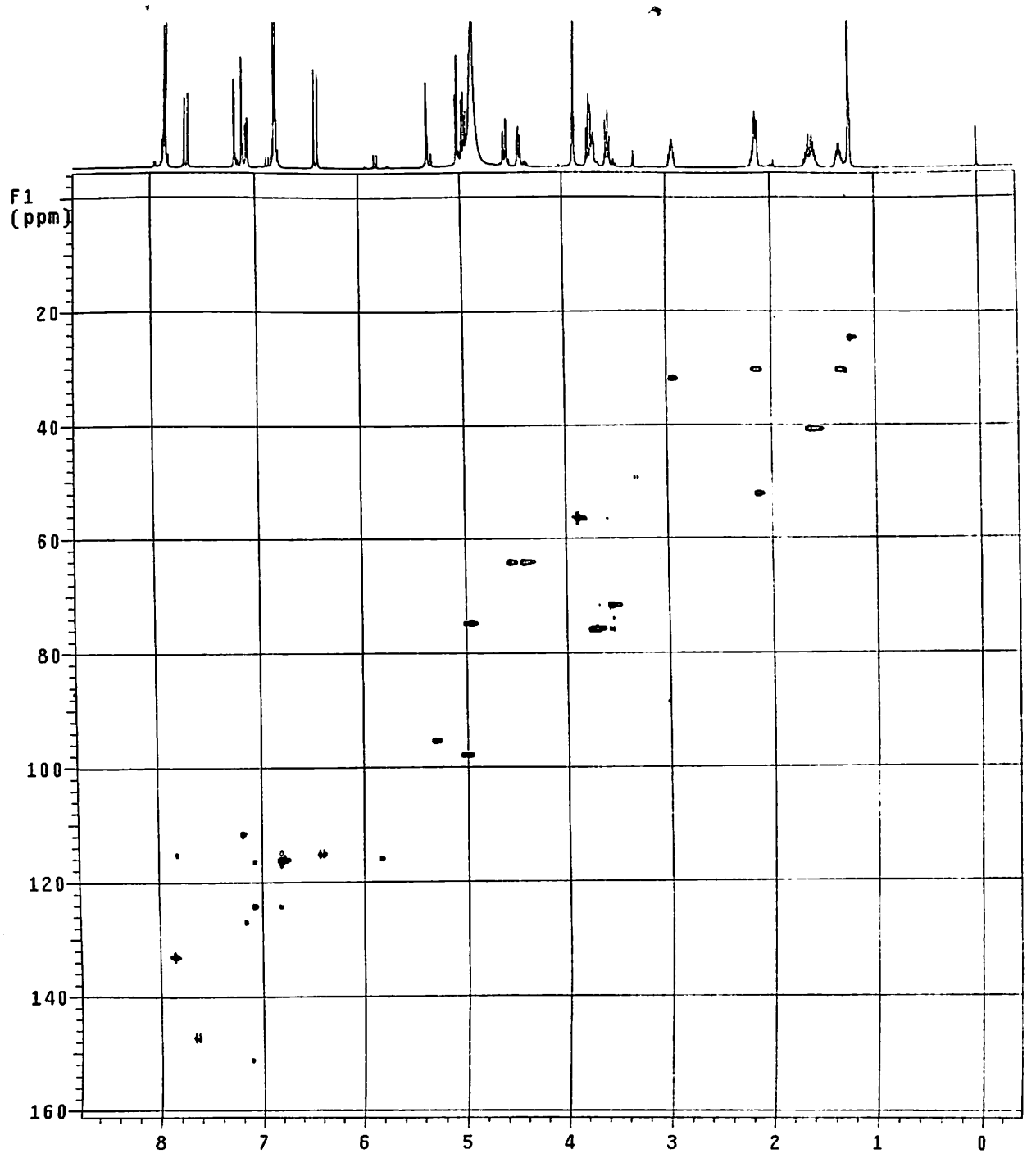


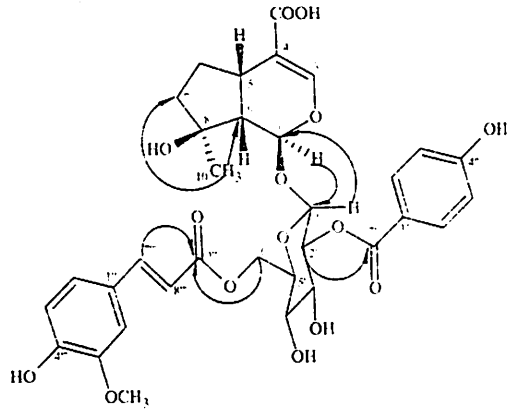

Fig. 3.07: HMQC spectrum (500 MHz, $d_4$-MeOH) of compound **VA-12 (3.02)**

## TABLE 3.05

**HMQC spectral data of VA-12 (6′-*O*-*trans*-feruloylnegundoside, 3.02)**

**(Fig. 3.07, solvent: $d_4$-MeOH)**

| **Proton chemical shift (δ)** | **Correlated carbon chemical shift (δ)** | **Assignment** |
|---|---|---|
| 5.31 (H-1) | 95.4 | C-1 |
| 7.11 (H-3) | 150.6 | C-3 |
| 2.93 (H-5) | 31.8 | C-5 |
| 2.12 and 1.33 | 30.3 | C-6 |
| ($H_a$-6 and $H_b$-6) | | |
| 1.60 (H-7) | 40.7 | C-7 |
| 2.12 (H-9) | 52.2 | C-9 |
| 1.23 (H-10) | 24.6 | C-10 |
| 5.01 (H-1′) | 97.9 | C-1′ |
| 4.94 (H-2′) | 74.8 | C-2′ |
| 3.73 (H-3′) | 75.8 | C-3′ |
| 3.55 (H-4′) | 71.7 | C-4′ |
| 3.69 (H-5′) | 75.8 | C-5′ |
| 4.56 and 4.41 | 64.2 | C-6′ |
| ($H_a$-6′ and $H_b$-6′) | | |
| 7.85 (H-2″, H-6″) | 132.9 | C-2″, C-6″ |
| 6.83 (H-3″, H-5″) | 116.1 | C-3″, C-5″ |
| 7.18 (H-2‴) | 111.7 | C-2‴ |
| 6.78(H-5‴) | 116.5 | C-5‴ |
| 7.06 (H-6‴) | 124.2 | C-6‴ |
| 7.64 (H-7‴) | 147.2 | C-7‴ |
| 6.40 (H-8‴) | 115.2 | C-8‴ |
| 3.88 (-OMe) | 56.4 | -OMe |




6'-*O*-*trans*-feruloylnegundoside (**VA-12**, **3.02**)

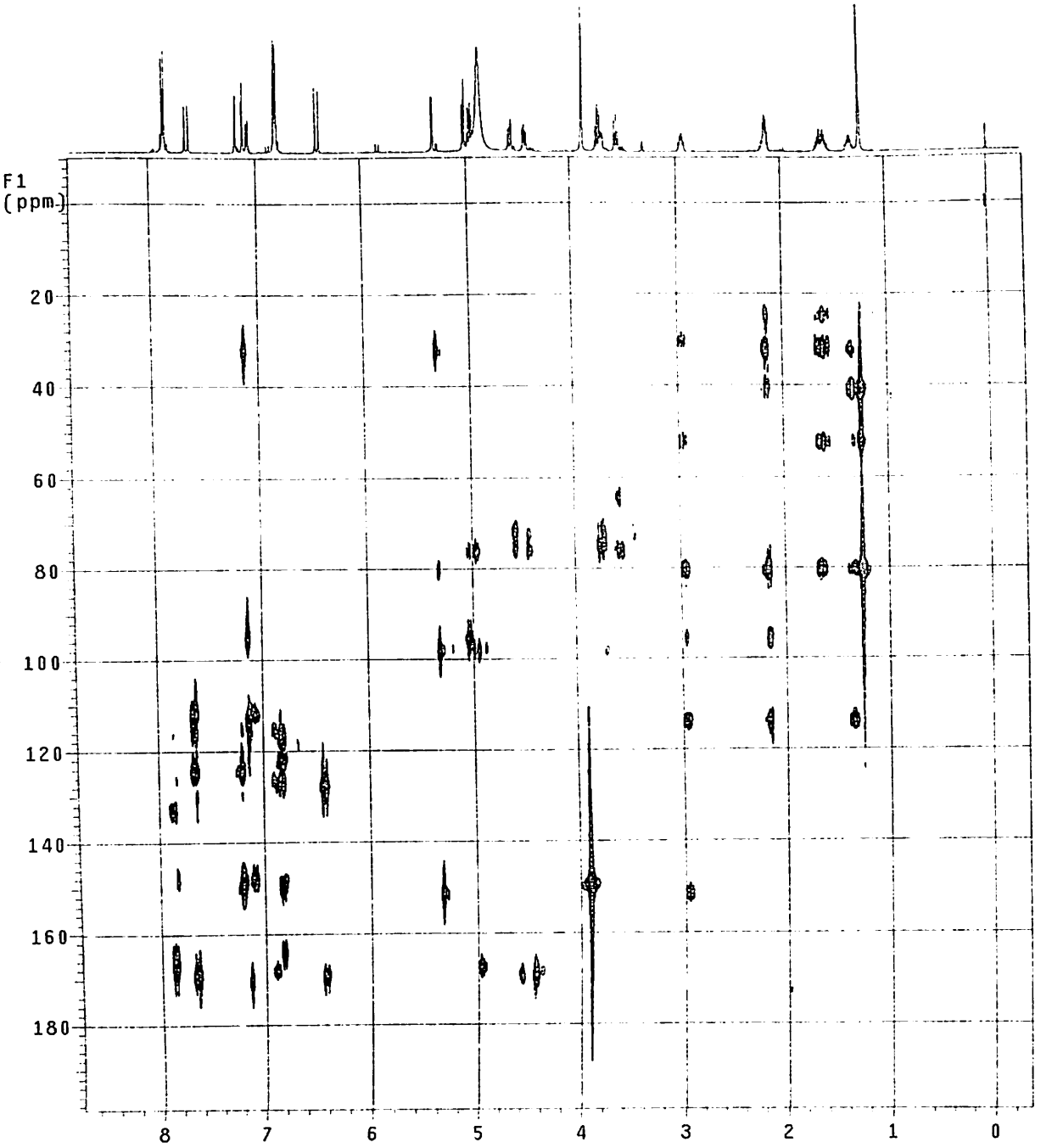
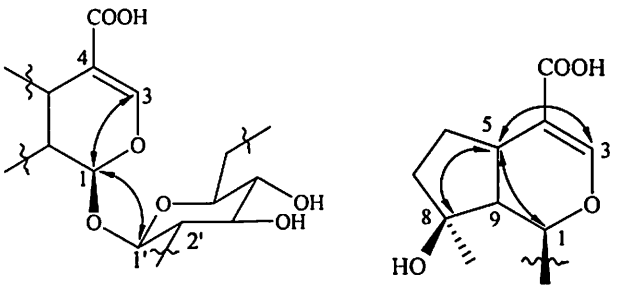
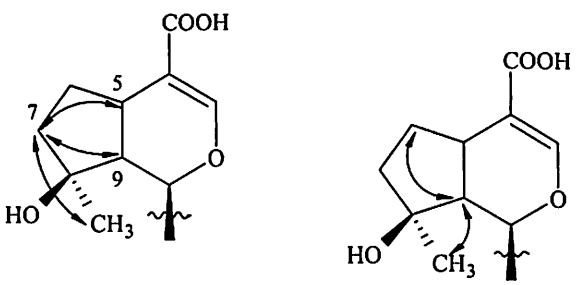
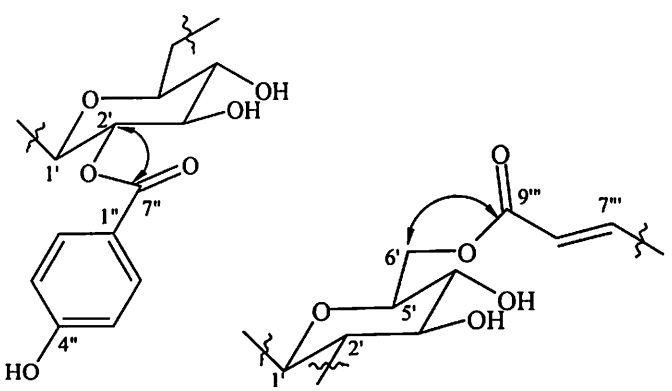
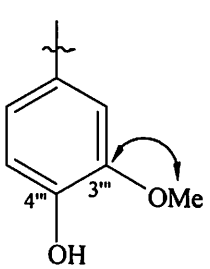



Fig. 3.08: HMBC spectrum (500 MHz, $d_4$-MeOH) of compound **VA-12** (**3.02**)

# TABLE 3.06

**HMBC spectral data of VA-12 (6′-*O*-*trans*-feruloylnegundoside, 3.02)**

**(Fig. 3.08, solvent: $d_4$-MeOH)**

| Chemical shift (δ) | Correlated carbons | Structural units derived |
|---|---|---|
| 5.31 (H-1) | C-1′, C-3 |  |
| 2.93 (H-5) | C-1, C-3, C-8 | |
| 1.60 (H-7) | C-5, C-9, C-10 |  |
| 2.12 (H-9) | C-10, C-6 | |
| 4.94 (H-2′) | C-7″ |  |
| 4.56-4.41 (H-6′) | C-9‴ | |
| 3.88 (OMe) | C-3‴ |  |

## VA-14

It was obtained as pale-yellow amorphous powder, m.p. 168-169 °C, $[\alpha]_D^{25}$ -109.3° (*c* 0.5, MeOH). Its molecular formula was deduced as $C_{32}H_{34}O_{15}$, based on elemental analysis and LC-MS [*m/z* 657, $(M - H)^-$] data.

The spectral characteristics of VA-14 are: UV (MeOH) $\lambda_{max}$: 249 and 331 nm; IR (KBr) $\nu_{max}$: 3398 (hydroxyl), 1695 (α,β-unsaturated ester), 1633 (C=C), 1604 and 1517 $cm^{-1}$(aromatic). The $^1H$ NMR (Fig. 3.10, Table 3.07) spectrum showed the signals, indicative of an iridoid nucleus[1] [δ 5.31 (1H, d, *J* = 4.0 Hz, H-1), 7.11 (1H, d, *J* = 0.5 Hz, H-3) and δ 1.23 (3H, s, H-10)] and a *p*-hydroxybenzoyl unit[4] [δ 7.85 (2H, d, *J* = 8.5 Hz) and δ 6.80 (2H, d, *J* = 8.5 Hz)]. The $^1H$ NMR spectrum also showed a series of signals between δ 3.54 and 5.01 attributable to a sugar moiety.[2] The configuration of glucose was established as β based on the coupling constant of anomeric proton (δ 5.01 d, *J* = 8.0 Hz, H-1′).

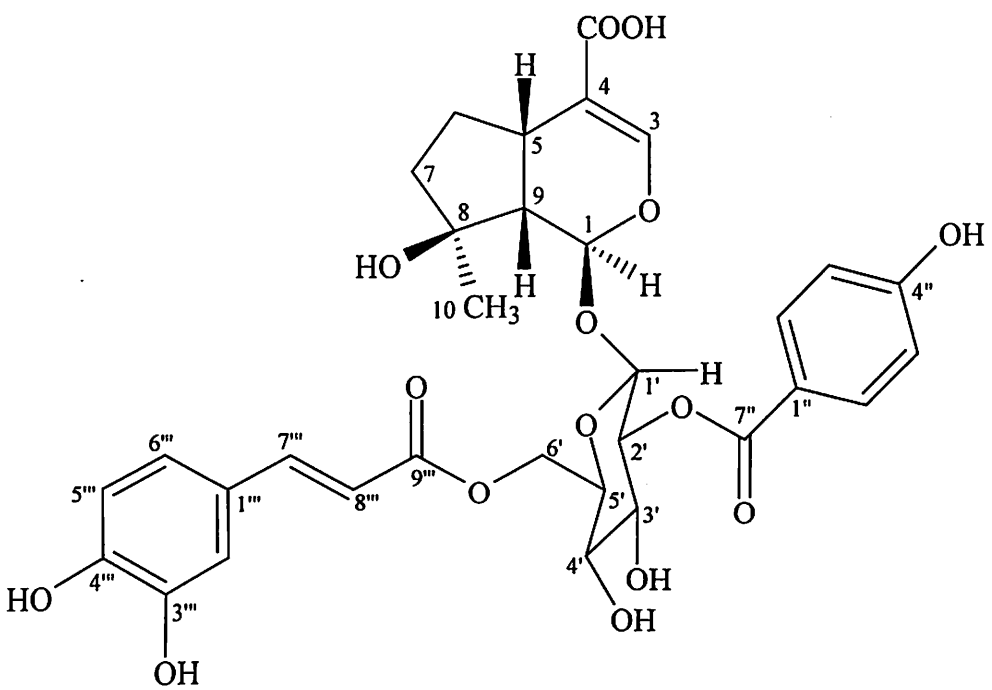
In addition, the $^1H$ NMR spectrum showed the presence of a *trans*-caffeoyl unit[10] constituted by a 1,2,4-trisubstituted phenyl unit [δ 7.05 (1H, d, *J* = 2 Hz), 6.95 (1H, dd, *J* = 8.5, 2.0 Hz) and δ 6.78 (1H, d, *J* = 8.5 Hz)] and a pair of *trans* coupled olefinic protons at δ 7.58 (1H, d, *J* = 16.0 Hz) and δ 6.30 (1H, d, *J* = 16.0 Hz).

The $^{13}C$ NMR (Fig. 3.11, Table 3.08) spectrum of VA-14 showed the presence of a carboxylic acid (δ 170.1), two ester carbonyl carbons (δ 169.0, C-9‴ and δ 167.3, C-7‴), seven quaternary carbons (δ 163.4, 149.6, 146.8 127.6, 122.1, 113.6 and 80.2), three methylene carbons (δ 64.1, 40.8, 30.3), and a methyl group (δ 24.5, C-10) as revealed by the DEPT (Fig. 3.12) and HMQC (Fig. 3.13, Table 3.09) spectra.

A perusal of the above spectral data in comparison with VA-12 revealed that the feruloyl unit of VA-12 is replaced by a caffeoyl unit in

VA-14, which was evident by the absence of methoxyl group in the $^{1}H$ and $^{13}C$ NMR spectra of VA-14 and the difference of 14 mass units in comparison with VA-12. The linkage of caffeoyl moiety with the glucose unit at C-6′ was confirmed by the observed downfield shift[9] of C-6′ ($\delta_C$ 64.1) and as expected, in the HMBC (Fig. 3.14, Table. 3.10) spectrum, H-6′ methylene protons [δ 4.55 (1H, dd, $J$ = 12.0, 2.0 Hz) and δ 4.40 (1H, dd, $J$ = 12.0, 6.0 Hz)] of sugar showed correlations with the ester carbonyl ($\delta_C$ 169.0, C-9‴) of caffeoyl moiety. The selective HMBC correlations of VA-14 are represented in Fig. 3.15.

From the foregoing, the structure of VA-14 was established as 6′-*O*-*trans*-caffeoylnegundoside, **3.03**), a new diacylated iridoid glucoside.




**3.03**

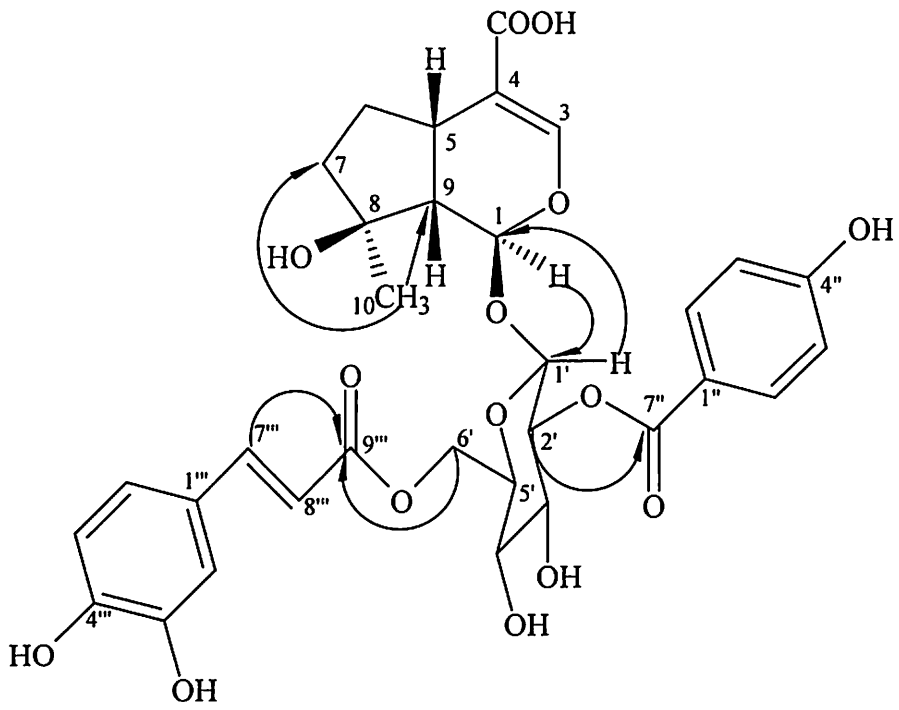

COOH
H
4
3
5
7
9
8
1
O
HO
H
H
10CH3
O
OH
4"
1'
H
O
7"
1"
O
7'''
9'''
6'
2'
1'''
8'''
O
5'
O
O
OH
OH
4'''
HO
OH

**Fig. 3.15**

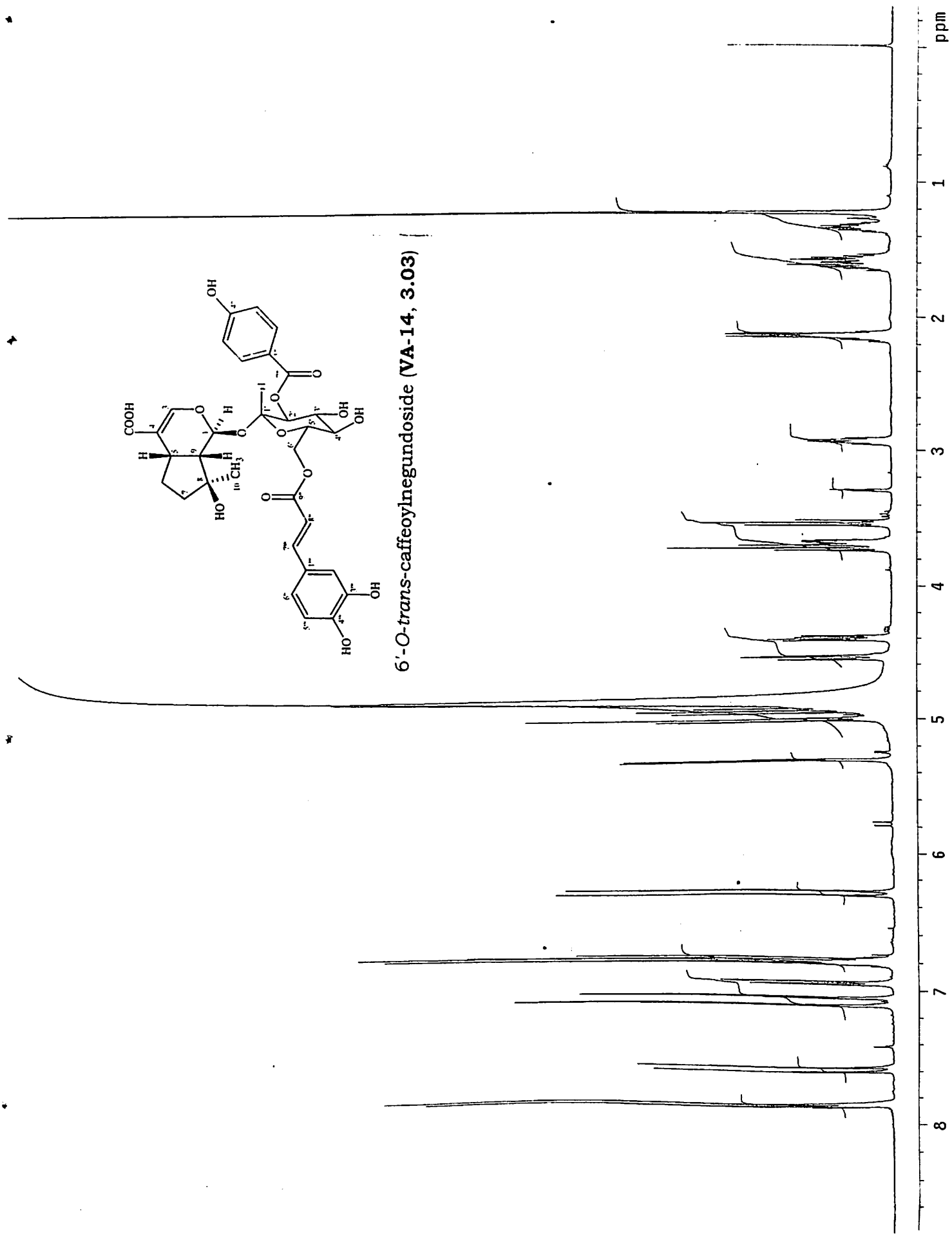


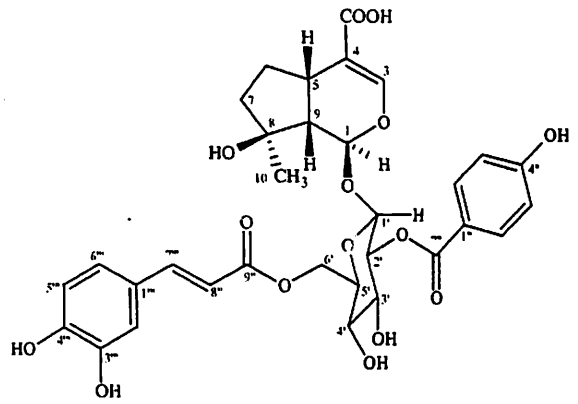

Fig. 3.10: $^1$H NMR spectrum (500 MHz, $d_4$-MeOH) of compound **VA-14 (3.03)**

## TABLE 3.07

**$^1$H NMR spectral data of VA-14 (6′-*O*-*trans*-caffeoyl negundoside, 3.03)**

**(Fig. 3.10, 500 MHz spectrum, $d_4$-MeOH)**

| Chemical shift (δ) | Proton integration | Multiplicity (*J* in Hz) | Assignment |
|---|---|---|---|
| 5.31 | 1H | d (4.0) | H-1 |
| 7.11 | 1H | d (0.5) | H-3 |
| 2.93 | 1H | m | H-5 |
| 2.14 | 1H | m | $H_a$-6 |
| 1.34 | 1H | m | $H_b$-6 |
| 1.63 | 2H | m | H-7 |
| 2.14 | 1H | dd (9.0,4.0) | H-9 |
| 1.23 | 3H | s | H-10 |
| 5.01 | 1H | d (8.0) | H-1′ |
| 4.95 | 1H | dd (9.0,8.0) | H-2′ |
| 3.73 | 1H | dd (9.5,9.0) | H-3′ |
| 3.54 | 1H | dd (9.5,9.0) | H-4′ |
| 3.68 | 1H | m | H-5′ |
| 4.55 | 1H | dd (12.0,2.0) | $H_a$-6′ |
| 4.40 | 1H | dd (12.0,6.0) | $H_b$-6′ |
| 7.85 | 2H | d (8.5) | H-2″ and H-6″ |
| 6.80 | 2H | d (8.5) | H-3″ and H-5″ |
| 7.05 | 1H | d (2.0) | H-2‴ |
| 6.78 | 1H | d (8.5) | H-5‴ |
| 6.95 | 1H | dd (8.5,2.0) | H-6‴ |
| 7.58 | 1H | d (16.0) | H-7‴ |
| 6.30 | 1H | d (16.0) | H-8‴ |




6′-*O*-*trans*-caffeoylnegundoside (**VA-14**, **3.03**)




Fig. 3.11: $^{13}C$ NMR spectrum (75 MHz, $d_4$-MeOH) of compound **VA-14** (**3.03**)

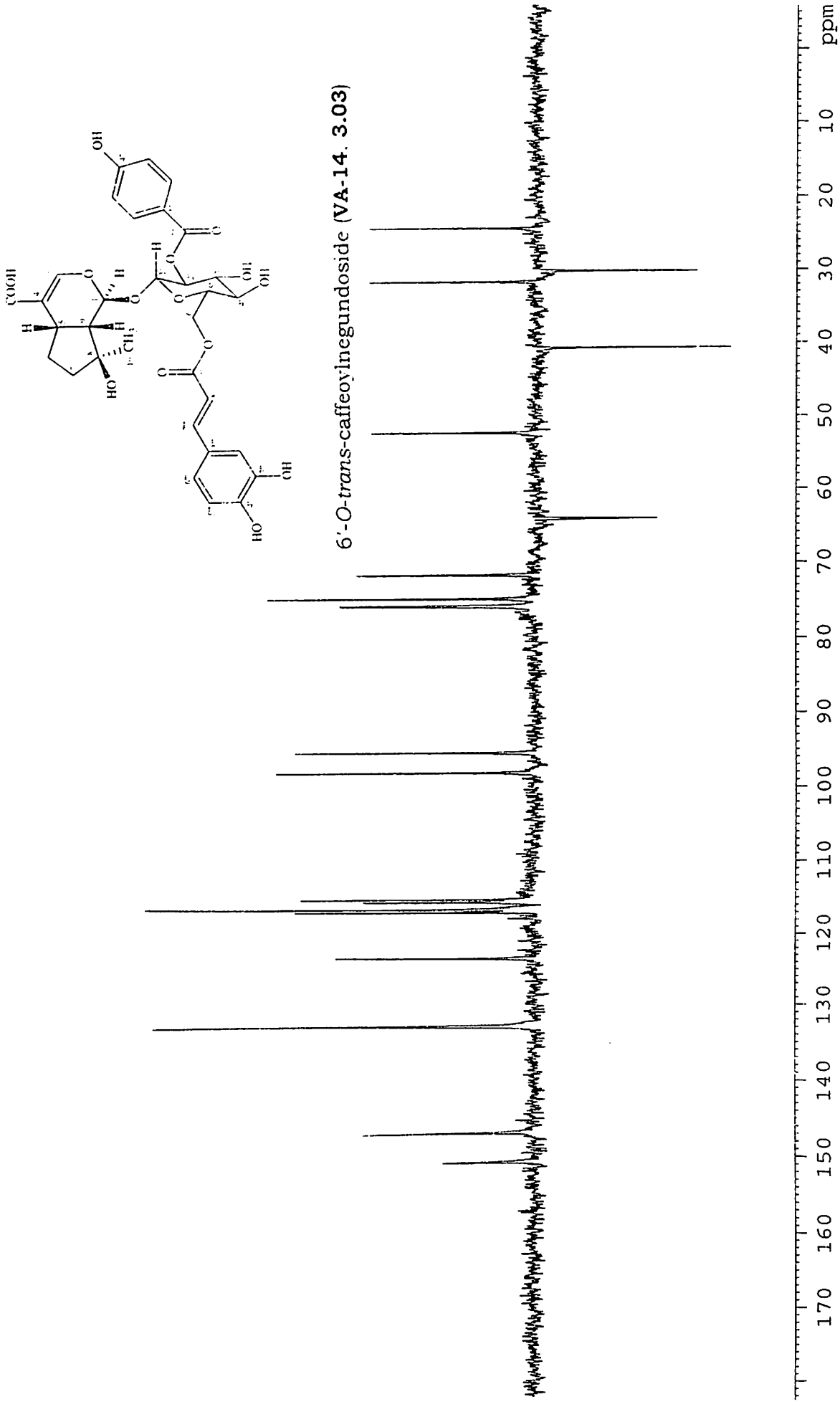


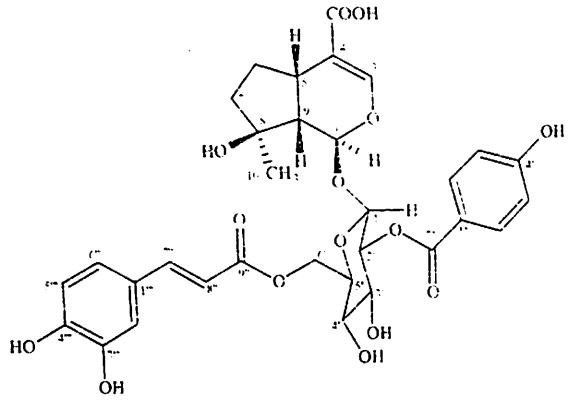

Fig. 3.12: DEPT spectrum ($d_4$-MeOH) of compound **VA-14 (3.03)**

## TABLE 3.08

**$^{13}C$ NMR spectral data of VA-14 (6′-*O*-*trans*-caffeoyl negundoside, 3.03)**

**(Fig. 3.11, 75 MHz spectrum, $d_4$-MeOH)**

| Carbon number | Chemical shift (δ) | Carbon number | Chemical shift (δ) |
|---|---|---|---|
| C-1 | 95.3 | C-1″ | 122.1 |
| C-3 | 151.3 | C-2″ and C-6″ | 132.9 |
| C-4 | 113.6 | C-3″ and C-5″ | 116.1 |
| C-5 | 31.8 | C-4″ | 163.4 |
| C-6 | 30.3 | C-7″ | 167.3 |
| C-7 | 40.8 | C-1‴ | 127.6 |
| C-8 | 80.2 | C-2‴ | 115.1 |
| C-9 | 52.2 | C-3‴ | 146.8 |
| C-10 | 24.5 | C-4‴ | 149.6 |
| C-11 | 170.1 | C-5‴ | 116.5 |
| C-1′ | 97.9 | C-6‴ | 123.1 |
| C-2′ | 74.8 | C-7‴ | 147.3 |
| C-3′ | 75.9 | C-8‴ | 114.7 |
| C-4′ | 71.7 | C-9‴ | 169.0 |
| C-5′ | 75.9 | | |
| C-6′ | 64.1 | | |




6'-*O*-*trans*-caffeoylnegundoside (**VA-14, 3.03**)

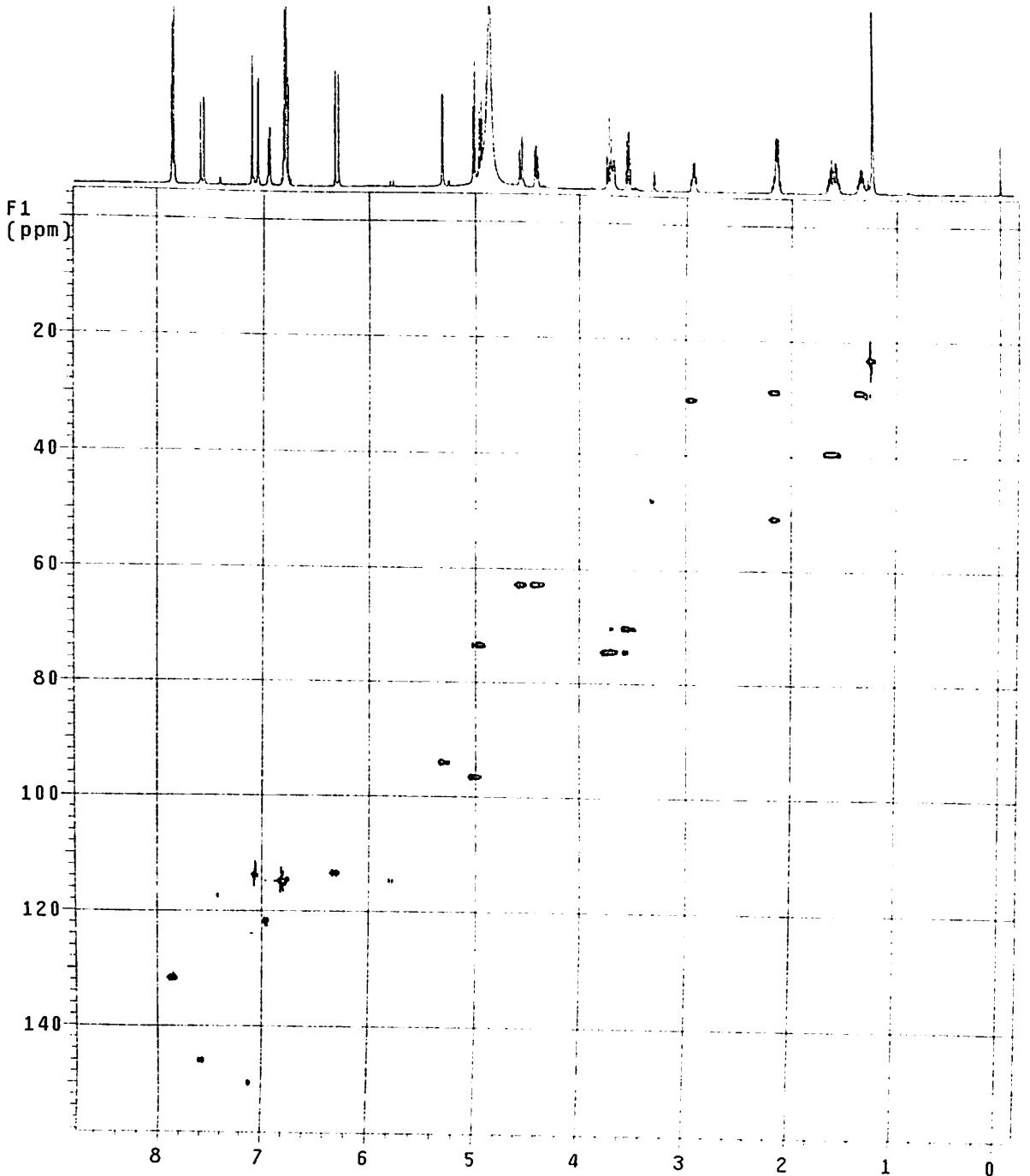



Fig. 3.13: HMQC spectrum (500 MHz, $d_4$-MeOH) of compound **VA-14 (3.03)**

## TABLE 3.09

**HMQC spectral data of VA-14 (6′-*O*-*trans*-caffeoylnegundoside, 3.03)**

**(Fig. 3.13, solvent: $d_4$-MeOH)**

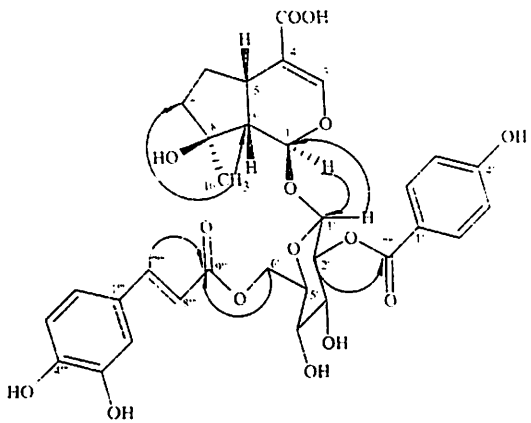
| **Proton chemical shift (δ)** | **Correlated carbon chemical shift (δ)** | **Assignment** |
|---|---|---|
| 5.31 (H-1) | 95.3 | C-1 |
| 7.11 (H-3) | 151.3 | C-3 |
| 2.93 (H-5) | 31.8 | C-5 |
| 2.14 and 1.34 ($H_a$-6 and $H_b$-6) | 30.3 | C-6 |
| 1.63 (H-7) | 40.8 | C-7 |
| 2.14 (H-9) | 52.2 | C-9 |
| 1.23 (H-10) | 24.5 | C-10 |
| 5.01 (H-1′) | 97.9 | C-1′ |
| 4.95 (H-2′) | 74.8 | C-2′ |
| 3.73 (H-3′) | 75.9 | C-3′ |
| 3.54 (H-4′) | 71.7 | C-4′ |
| 3.68 (H-5′) | 75.9 | C-5′ |
| 4.55 and 4.40 ($H_a$-6′ and $H_b$-6′) | 64.1 | C-6′ |
| 7.85 (H-2″, H-6″) | 132.9 | C-2″, C-6″ |
| 6.80 (H-3″, H-5″) | 116.1 | C-3″, C-5″ |
| 7.05 (H-2‴) | 115.1 | C-2‴ |
| 6.78(H-5‴) | 116.5 | C-5‴ |
| 6.95 (H-6‴) | 123.1 | C-6‴ |
| 7.58 (H-7‴) | 147.3 | C-7‴ |
| 6.30 (H-8‴) | 114.7 | C-8‴ |

## TABLE 3.09

**HMQC spectral data of VA-14 (6′-*O*-*trans*-caffeoylnegundoside, 3.03)**

**(Fig. 3.13, solvent: $d_4$-MeOH)**

| **Proton chemical shift (δ)** | **Correlated carbon chemical shift (δ)** | **Assignment** |
|---|---|---|
| 5.31 (H-1) | 95.3 | C-1 |
| 7.11 (H-3) | 151.3 | C-3 |
| 2.93 (H-5) | 31.8 | C-5 |
| 2.14 and 1.34 ($H_a$-6 and $H_b$-6) | 30.3 | C-6 |
| 1.63 (H-7) | 40.8 | C-7 |
| 2.14 (H-9) | 52.2 | C-9 |
| 1.23 (H-10) | 24.5 | C-10 |
| 5.01 (H-1′) | 97.9 | C-1′ |
| 4.95 (H-2′) | 74.8 | C-2′ |
| 3.73 (H-3′) | 75.9 | C-3′ |
| 3.54 (H-4′) | 71.7 | C-4′ |
| 3.68 (H-5′) | 75.9 | C-5′ |
| 4.55 and 4.40 ($H_a$-6′ and $H_b$-6′) | 64.1 | C-6′ |
| 7.85 (H-2″, H-6″) | 132.9 | C-2″, C-6″ |
| 6.80 (H-3″, H-5″) | 116.1 | C-3″, C-5″ |
| 7.05 (H-2‴) | 115.1 | C-2‴ |
| 6.78(H-5‴) | 116.5 | C-5‴ |
| 6.95 (H-6‴) | 123.1 | C-6‴ |
| 7.58 (H-7‴) | 147.3 | C-7‴ |
| 6.30 (H-8‴) | 114.7 | C-8‴ |




6'-*O*-*trans*-caffeoylnegundoside (**VA-14, 3.03**)

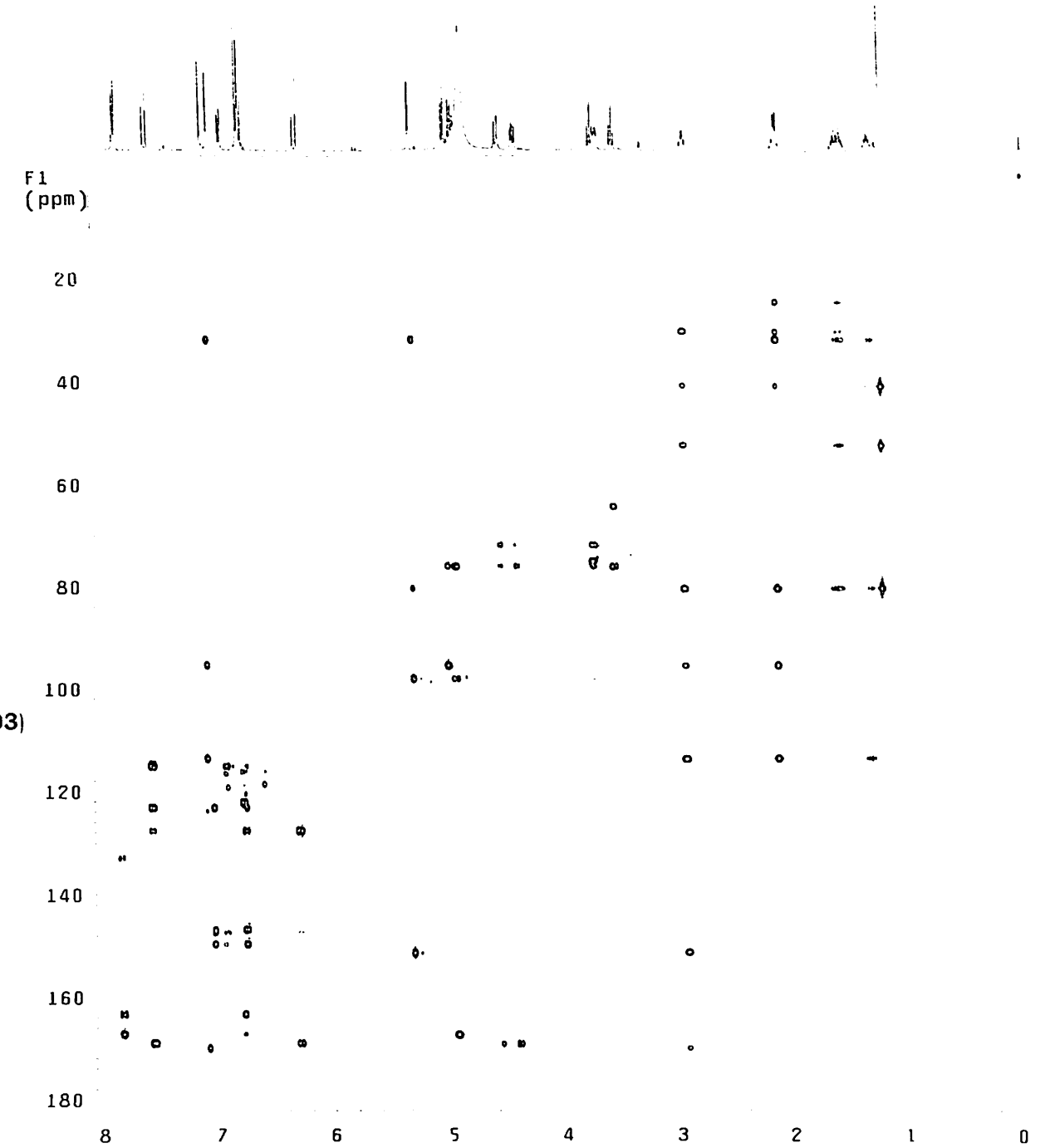
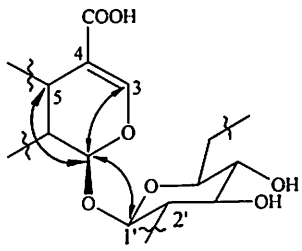
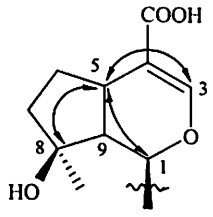
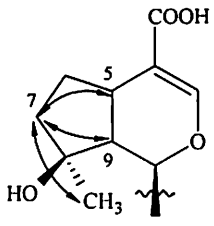
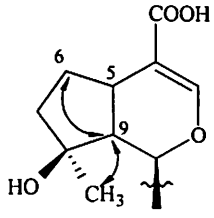
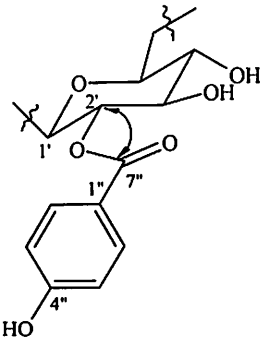
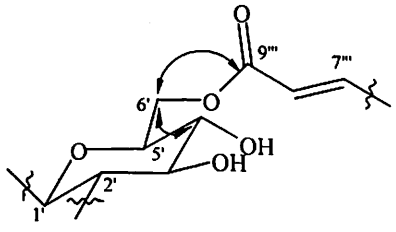
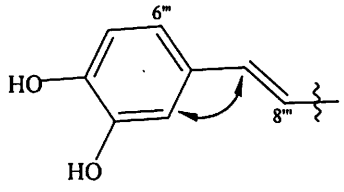



Fig. 3.14: HMBC spectrum (500 MHz, $d_4$-MeOH) of compound **VA-14 (3.03)**

# TABLE 3.10

**HMBC spectral data of VA-14 (6′-*O*-*trans*-caffeoylnegundoside, 3.03)**

**(Fig. 3.14, solvent: $d_4$-MeOH)**

| Chemical shift (δ) | Correlated carbons | Structural units derived |
|---|---|---|
| 5.31 (H-1) | C-1′, C-3, C-5, C-8 |  |
| 2.93 (H-5) | C-1, C-3-, C-8 |  |
| 1.63 (H-7) | C-5, C-9, C-10 |  |
| 2.14 (H-9) | C-6, C-10 |  |
| 4.95 (H-2′) | C-7″ |  |
| 4.55,4.40 (H-6′) | C-9‴, C-4′ |  |
| 7.58 (H-7‴) | C-2‴ |  |

## SECTION-II

### (GARDOSIDE DERIVATIVES)

## VA-21

It was obtained as colorless amorphous powder, m.p. 215-216 °C, $[\alpha]_D^{25}$ −41.3° (*c* 0.5, MeOH). Its molecular formula, $C_{23}H_{26}O_{12}$, was deduced from elemental analysis and LC-MS [*m/z* 493 $(M - H)^-$] data.

The UV spectrum (MeOH) of VA-21 showed absorption maxima at 255 nm. Its IR (KBr) spectrum showed bands at $\nu_{max}$ 3368 (hydroxyl), 1703 (CO, ester), 1636 (C=C), 1603 and 1512 $cm^{-1}$(aromatic). The $^1H$ NMR (Fig. 3.16, Table 3.11) spectrum showed the presence of a hemiacetal proton (δ 5.35, 1H, d, *J* = 3.5 Hz, H-1) and a trisubstituted olefinic proton at δ 7.03 (1H, br s, H-3) indicative of an iridoid[1] nucleus and a *p*-hydroxybenzoyl moiety[4] [δ 7.84 (2H, br s) and δ 6.80 (2H, br s)]. The spectrum also contained a series of signals between δ 3.41 and 4.97 assignable to a sugar moiety.[2]

The coupling constant of the anomeric proton (δ 4.97, 1H, d, *J* = 8.5, H-1′) was consistent with the β configuration of the sugar unit.

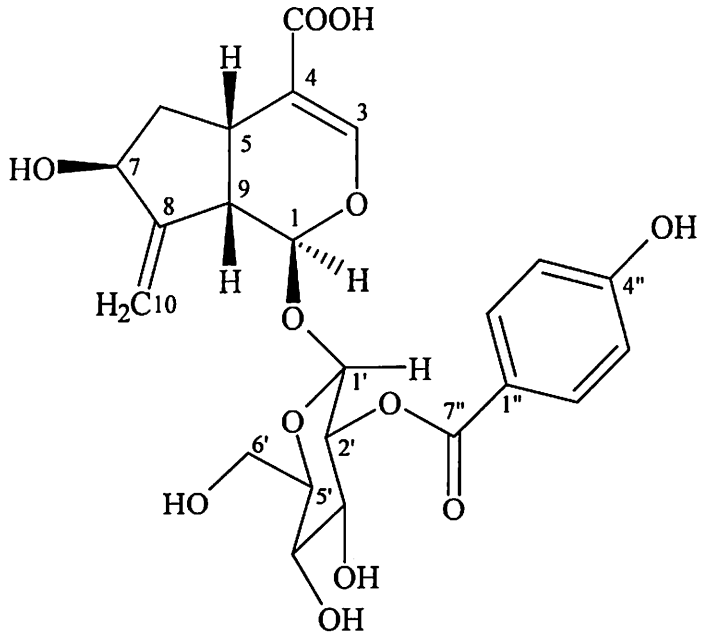
In addition, the $^1H$ NMR spectrum showed the presence of an exocyclic methylene[11] protons (δ 5.29, 2H, brs, H-10) and a signal at δ 4.30 (1H, br s, H-7) attributable to an allylic hydroxymethine proton. These, assignments were supported further by the $^{13}C$ NMR signal at δ 73.9 (C-7), 112.5 (C-10) and δ 153.2 (C-8), DEPT (Fig. 3.17) spectral data and the $^1H$-$^1H$ COSY (Fig. 3.18, Table 3.12) correlations.

The $^{13}C$ NMR spectrum (Fig. 3.19, Table 3.13) showed the presence of a *p*-hydroxybenzoyl unit (δc 167.5, 163.4, 132.9, 122.3 and

δ 116.2) and signals at δ 98.3, 78.5, 76.3, 75.1, 71.8 and 62.7 attributable to a glucopyranose moiety.

A comparison of the above data with those of negundoside (**3.01**) revealed that VA-21 differs from negundoside (**3.01**) only in the nature of cyclopentane ring of the iridoid skeleton. The absence of a tertiary methyl signal (δ 1.23, 3H, s) present in negundoside and the presence of an $\Delta^{8(10)}$ exocyclic methylene group (δ 5.29, 2H, brs) and a hydroxymethine proton (δ 4.30, brs) indicated the presence of gardoside nucleus[12,13] in VA-21.

Based on the foregoing, VA-21 was deduced as 2'-*O*-*p*-hydroxybenzoylgardoside (**3.04**), a new acylated iridoid glucoside.




**3.04**

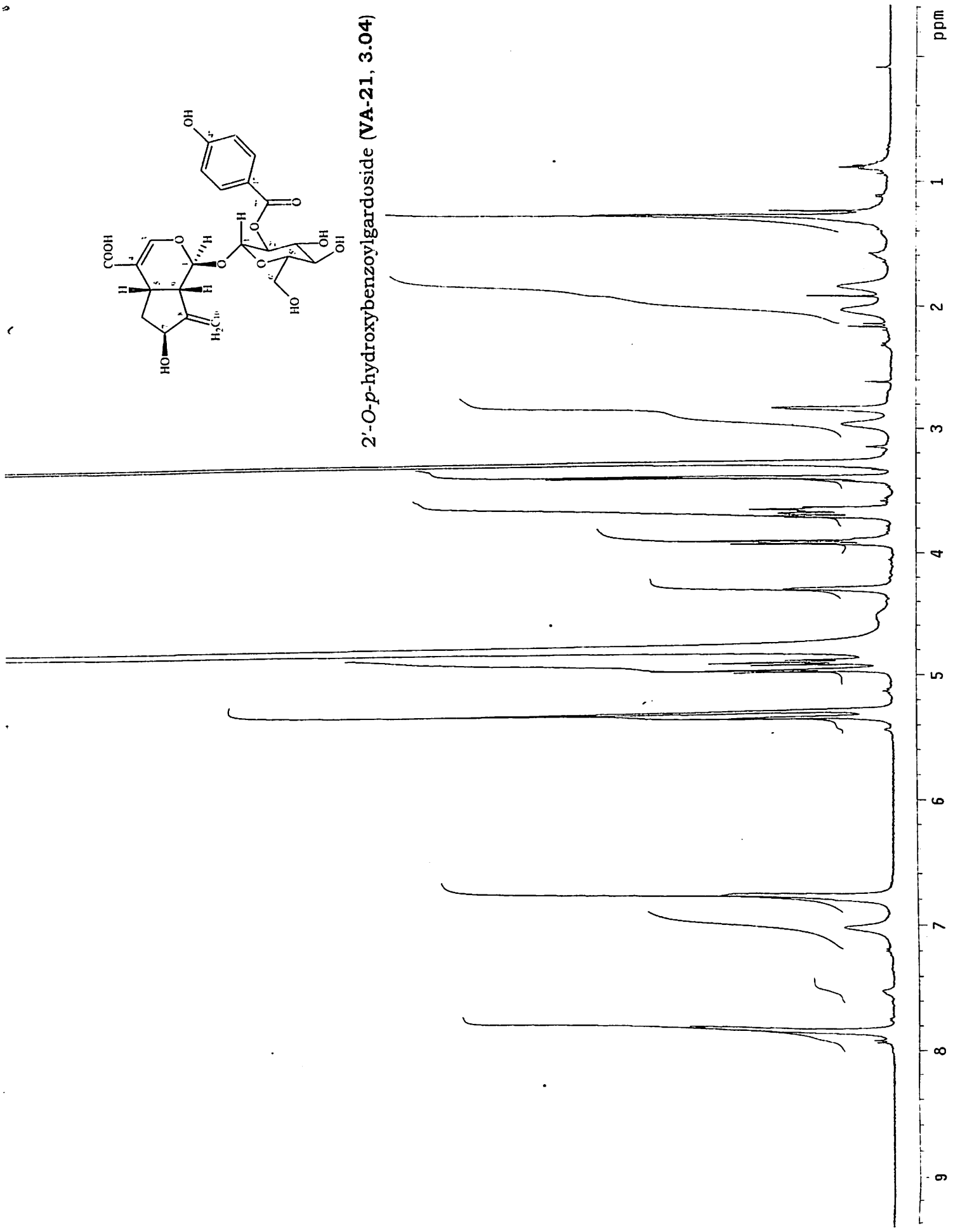


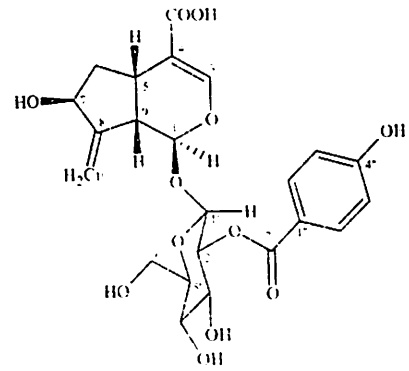

Fig. 3.16: $^1$H NMR spectrum (500 MHz, $d_4$-MeOH) of compound **VA-21 (3.04)**

## TABLE 3.11

**$^1$H NMR spectral data of VA-21 (2′-*O*-*p*-hydroxybenzoyl gardoside, 3.04)**

**(Fig. 3.16, 500 MHz spectrum, $d_4$-MeOH)**

| **Chemical shift (δ)** | **Proton integration** | **Multiplicity (*J* in Hz)** | **Assignment** |
|---|---|---|---|
| 5.35 | 1H | d (3.5) | H-1 |
| 7.03 | 1H | br s | H-3 |
| 2.97 | 1H | br s | H-5 |
| 2.03 | 1H | m | $H_a$-6 |
| 1.85 | 1H | m | $H_b$-6 |
| 4.30 | 1H | br s | H-7 |
| 2.84 | 1H | br s | H-9 |
| 5.29 | 2H | br s | H-10 |
| 4.97 | 1H | d (8.5) | H-1′ |
| 4.90 | 1H | dd (9.5,8.0) | H-2′ |
| 3.71 | 1H | m | H-3′ |
| 3.41 | 1H | m | H-4′ |
| 3.69 | 1H | m | H-5′ |
| 3.93 | 1H | d (12.0) | $H_a$-6′ |
| 3.66 | 1H | m | $H_b$-6′ |
| 7.84 | 2H | br s | H-2″ and H-6″ |
| 6.80 | 2H | br s | H-3″ and H-5″ |




2'-*O*-*p*-hydroxybenzoylgardoside (**VA-21**, **3.04**)

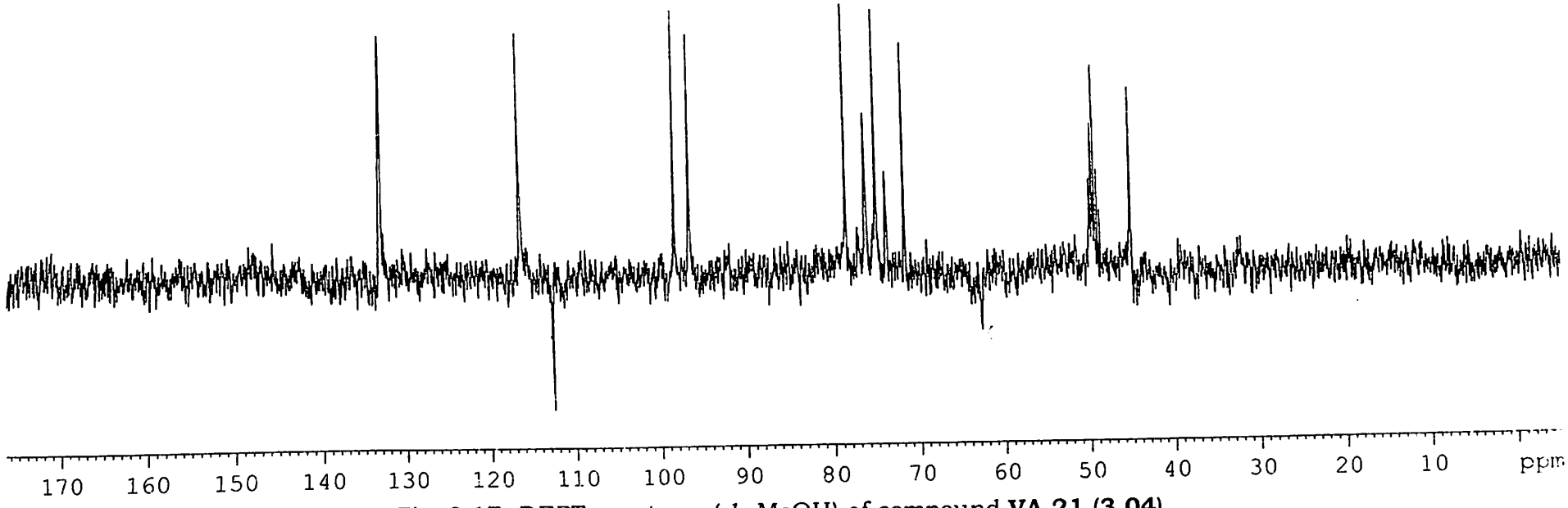


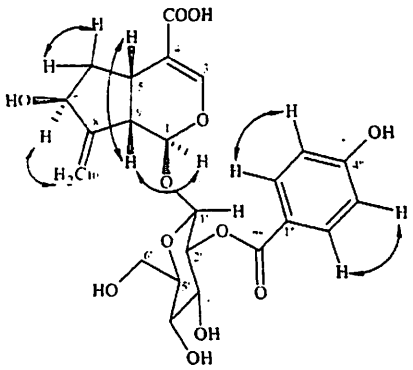

Fig. 3.17: DEPT spectrum ($d_4$-MeOH) of compound **VA-21 (3.04)**

## TABLE 3.12

**$^1$H-$^1$H COSY spectral data of VA-21 (2′-*O*-*p*-hydroxybenzoyl gardoside, 3.04)**

**(Fig. 3.18, solvent: $d_4$-MeOH)**

| Chemical shifts of coupled protons | Type of coupling | Assignment |
|---|---|---|
| 3.93 and 3.66 | geminal | $H_a$-6′ and $H_b$-6′ |
| 2.03 and 1.85 | geminal | $H_a$-6 and $H_b$-6 |
| 4.30 and 5.29 | allylic | H-7 and H-10 |
| 2.97 and 2.84 | vicinal | H-5 and H-9 |
| 5.35 and 2.84 | vicinal | H-1 and H-9 |
| 7.84 and 6.80 | ortho | H-2″ and H-3″ |




2'-*O*-*p*-hydroxybenzoylgardoside (**VA-21**, **3.04**)

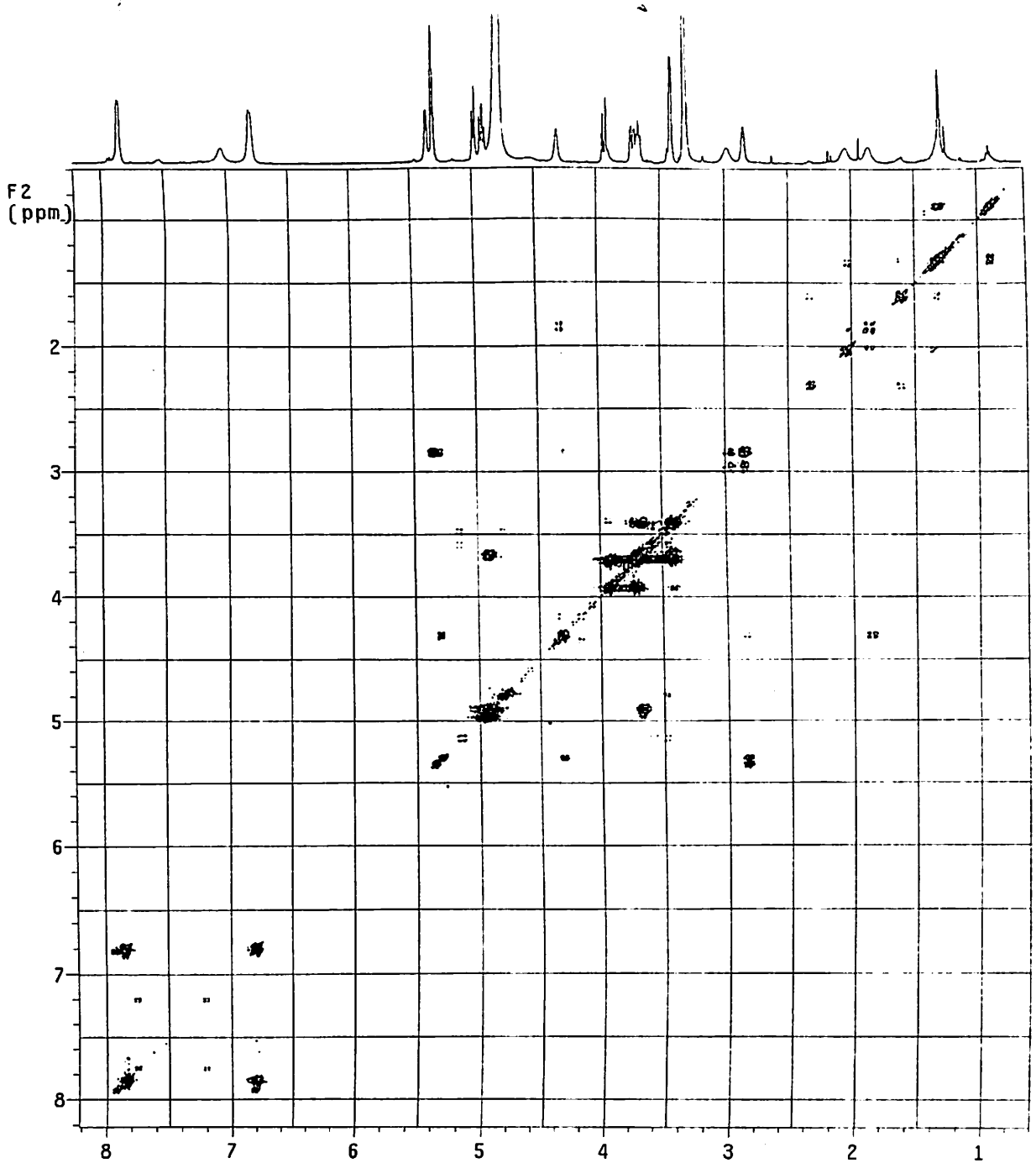
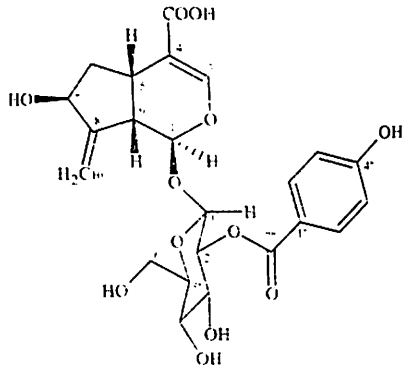



Fig. 3.18: $^{1}H$-$^{1}H$ COSY spectrum (500 MHz, $d_4$-MeOH) of compound **VA-21** (**3.04**)




2'-*O*-*p*-hydroxybenzoylgardoside (**VA-21**, **3.04**)




Fig. 3.19: $^{13}C$ NMR spectrum (75 MHz, $d_4$-MeOH) of compound **VA-21** (**3.04**)

## TABLE 3.13

**$^{13}$C NMR spectral data of VA-21 (2′-*O*-*p*-hydroxybenzoyl gardoside, 3.04)**

**(Fig. 3.19, 75 MHz spectrum, $d_4$-MeOH)**

| Carbon number | Chemical shift (δ) | Carbon number | Chemical shift (δ) |
|---|---|---|---|
| C-1 | 96.5 | C-2′ | 75.1 |
| C-3 | 151.0 | C-3′ | 76.3 |
| C-4 | [a] | C-4′ | 71.8 |
| C-5 | 30.7 | C-5′ | 78.5 |
| C-6 | 40.5 | C-6′ | 62.7 |
| C-7 | 73.9 | C-1″ | 122.3 |
| C-8 | 153.2 | C-2″ and C-6″ | 132.9 |
| C-9 | 45.2 | C-3″ and C-5″ | 116.2 |
| C-10 | 112.5 | C-4″ | 163.4 |
| C-11 | [a] | C-7″ | 167.5 |
| C-1′ | 98.3 | | |

[a]signals not observed

## VA-17

It was obtained as pale-yellow amorphous powder, m.p. 191-192 °C, $[\alpha]_D^{25}$ −32.6° (*c* 0.5, MeOH). The molecular formula of VA-17 was established as $C_{32}H_{32}O_{15}$ based on elemental analysis and LC-MS [*m/z* 655 $(M - H)^-$] data.

VA-17 exhibited bands in its UV (MeOH) spectrum at $\lambda_{max}$ 250 and 330 nm. Its IR (KBr) spectrum showed bands at $\nu_{max}$ 3406 (hydroxyl), 1698 (α,β-unsaturated ester), 1633 (C=C), 1604 and 1516 $cm^{-1}$(aromatic). The $^1H$ NMR spectrum (Fig. 3.20, Table 3.14) showed the presence of hemiacetal proton (δ 5.24, 1H, d, *J* = 4.5 Hz, H-1), a trisubstituted olefinic proton (δ 7.09, 1H, br s, H-3), two exocyclic methylene protons (δ 5.25, br s and δ 5.22 br s, H-10) and an allylic hydroxymethine proton at δ 4.26 (1H, br s, H-7) assignable to a gardoside nucleus.[12,13]

The $^1H$ NMR spectrum also contained two AB doublets at δ 7.85 (2H, d, *J* = 8.5 Hz) and δ 6.80 (2H, d, *J* = 8.5 Hz) attributable to *p*-hydroxybenzoyl unit[4] and a series of signals between δ 3.51 and 4.99 attributable to a sugar moiety.[2] The configuration of sugar was established as β based on the coupling constant of anomeric proton (δ 4.99, d, *J* = 8.0 Hz, H-1′).

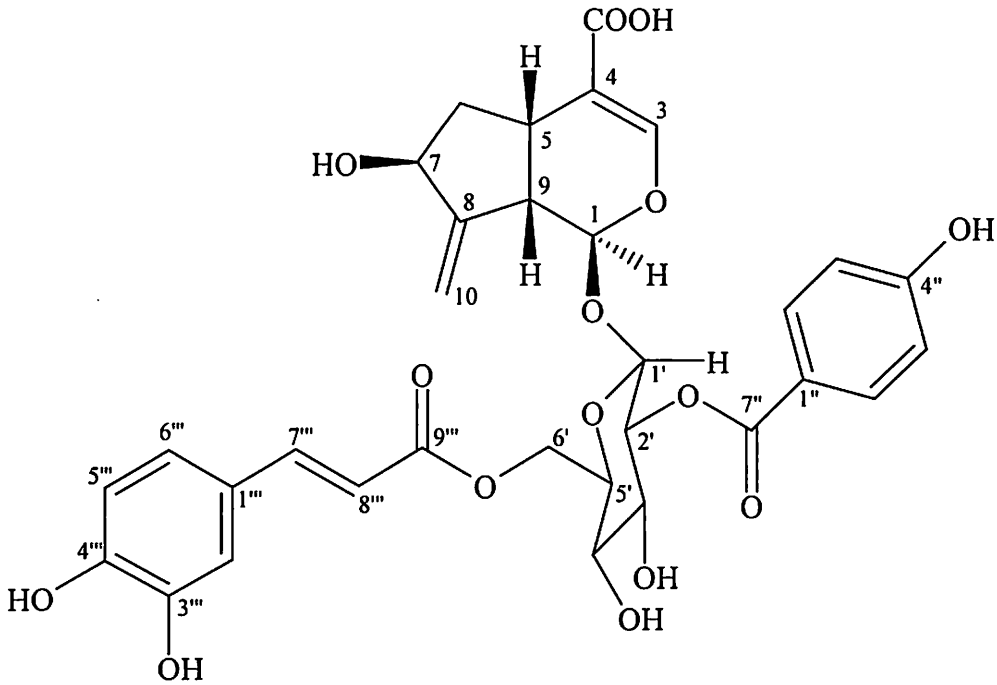
In addition, the $^1H$ NMR spectrum showed the presence of a *trans*-caffeoyl unit[10] constituted by 1,2,4-trisubstituted phenyl unit [δ 7.05 (1H, d, *J* = 2.0 Hz), 6.95 (1H, dd, *J* = 8.5, 2.0 Hz) and δ 6.78 (1H, d, *J* = 8.5 Hz)] and a pair of trans coupled olefinic protons at δ 7.58 (1H, d, *J* = 16.0 Hz) and δ 6.30 (1H, d, *J* = 16.0 Hz).

The $^{13}C$ NMR (Fig. 3.21, Table 3.15) spectrum of VA-17 showed the presence of two ester carbonyl carbons (δ 169.0, C-9‴ and 167.4, C-7″) two olefinic carbons (δ 152.7, C-8 and δ 112.7, C-10) and a

oxymethine carbon (δ 73.8, C-7) as revealed by the DEPT (Fig. 3.22) and HMQC (Fig. 3.23, Table 3.16) spectra.

A perusal of the above spectral data in comparison with VA-21 revealed that the spectral data of VA-17 closely resembled those of VA-21, except for the additional signals of a caffeoyl moiety present in VA-17. Thus VA-17 was derived as a caffeoyl derivative of VA-21. The downfield shift of C-6′ (64.3) and 6′-$H_2$ (δ 4.53-4.42) in VA-17 in comparison with those of VA-21 ($\delta_C$ 62.7 and $\delta_H$ 3.93-3.66) indicated the attachment of caffeoyl unit at C-6′ in VA-17. The position of linkage of caffeoyl unit at C-6′ was confirmed by the analysis of the HMBC data. In the HMBC spectrum (Fig. 3.24, Table 3.17) the H-6′ methylne protons (δ 4.53, dd, $J$ = 12.0, 2.0 Hz and δ 4.42, dd, $J$ = 12.0, 6.0 Hz) of the glucose unit showed correlations with the ester carbonyl ($\delta_C$ 169.0, C-9‴) of caffeoyl unit. The selective HMBC correlations of VA-17 are represented in Fig. 3.25.

Based on the foregoing, the structure of VA-17 was derived as 2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoylgardoside (**3.05**), a new diacylated iridoid glucoside.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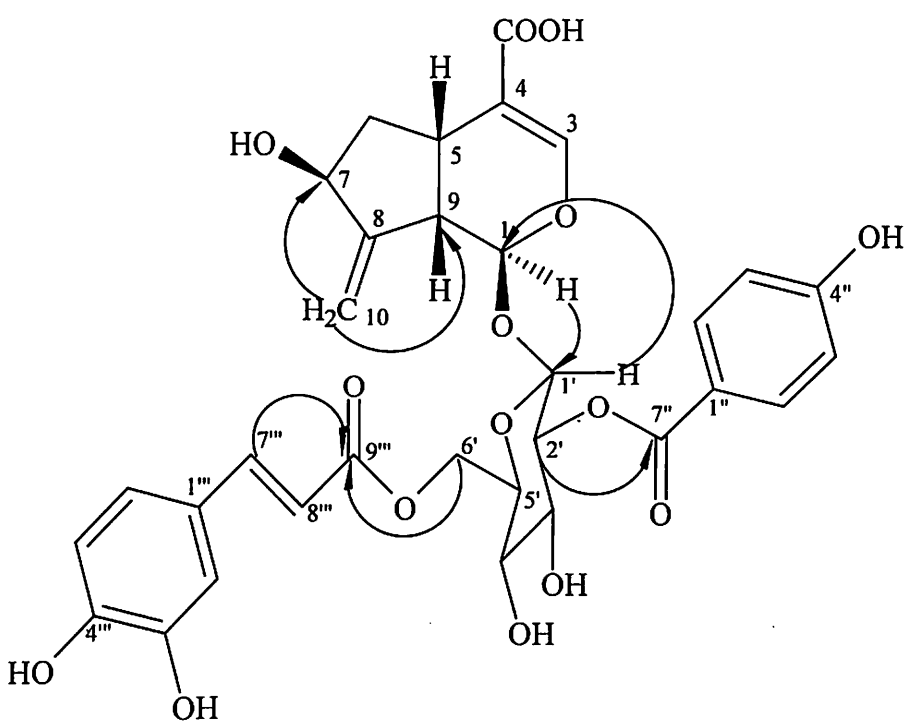
3
5
HO
7
9
8
1
O
OH
4"
10
H
H
O
1'
H
O
7"
1"
O
6'
2'
6'''
7'''
9'''
O
5'''
1'''
8'''
5'
4'''
HO
3'''
OH
OH
OH

**3.05**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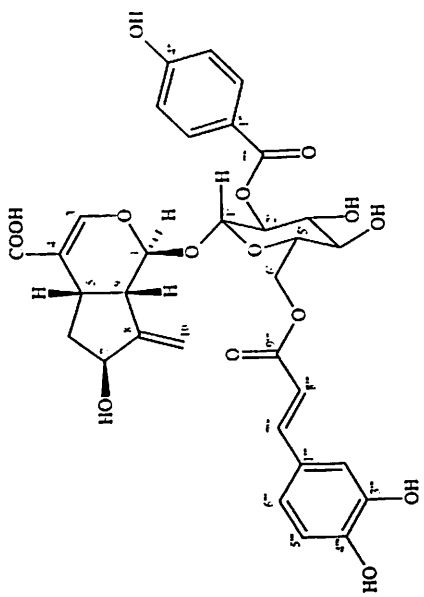
3
HO
5
7
9
8
O
OH
4"
H2C 10
H
H
O
1'
H
O
7"
1"
O
7'''
9'''
6'
2'
O
1'''
O
5'
8'''
OH
4'''
HO
OH
OH

**Fig. 3.25**



2'-*O*-*p*-hydroxybenzoyl-6'-*O*-*trans*-caffeoylgardoside (**VA-17**, **3.05**)



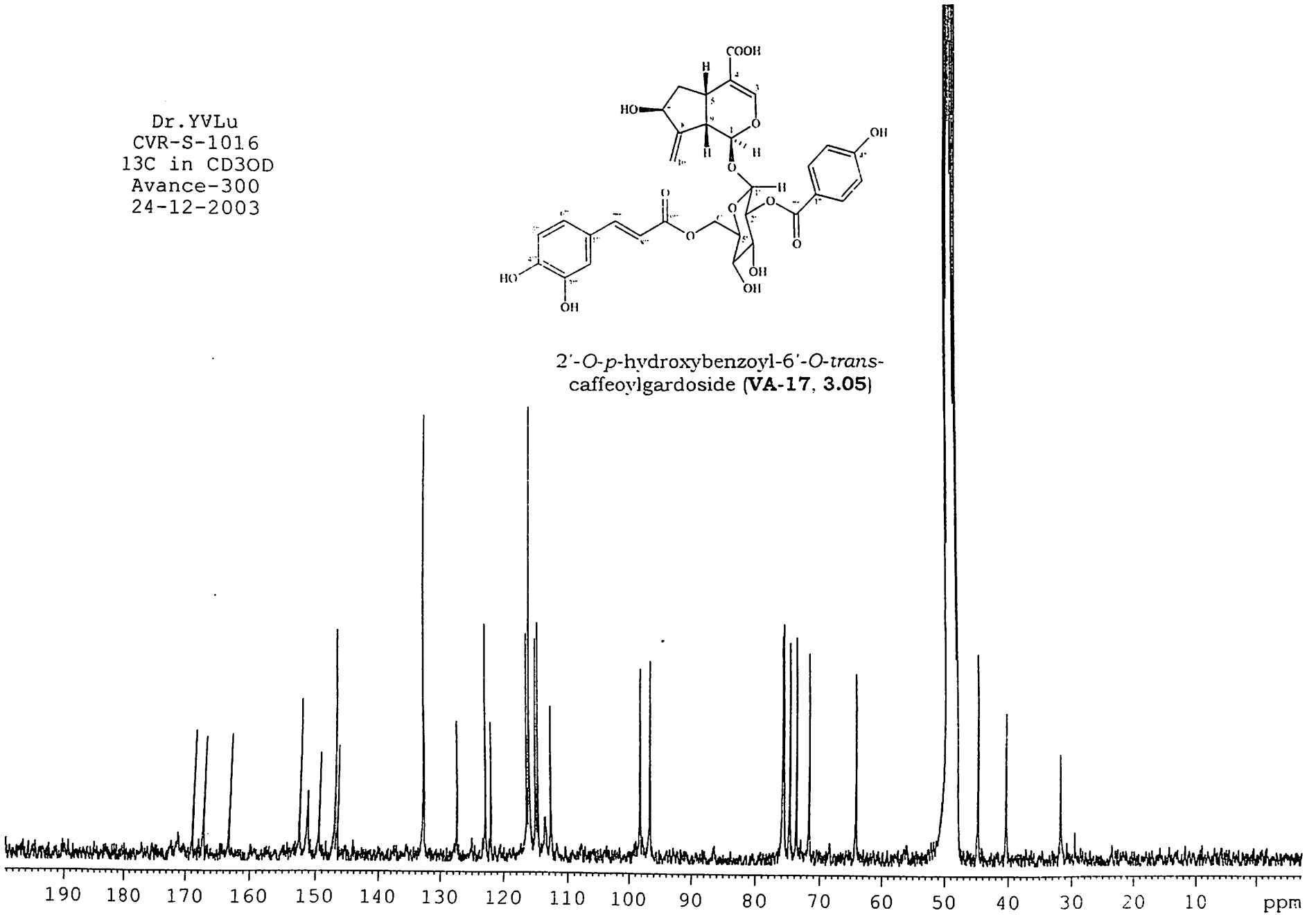

Fig. 3.20: $^1$H NMR spectrum (500 MHz, $d_4$-MeOH) of compound **VA-17** (**3.05**)

## TABLE 3.14

**$^1$H NMR spectral data of VA-17 (2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoylgardoside, 3.05)**

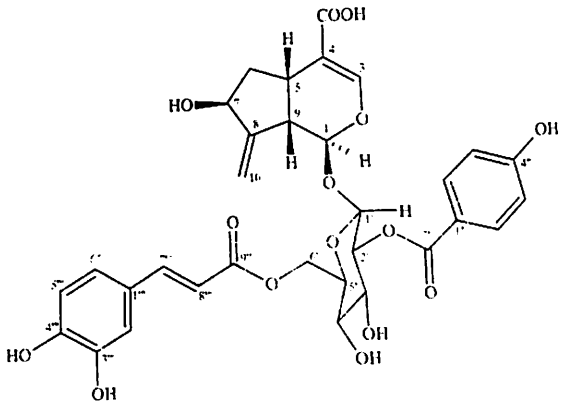
**(Fig. 3.20, 500 MHz spectrum, $d_4$-MeOH)**

| Chemical shift (δ) | Proton integration | Multiplicity (*J* in Hz) | Assignment |
|---|---|---|---|
| 5.24 | 1H | d (4.5) | H-1 |
| 7.09 | 1H | br s | H-3 |
| 2.95 | 1H | m | H-5 |
| 1.94 | 1H | m | $H_a$-6 |
| 1.83 | 1H | m | $H_b$-6 |
| 4.26 | 1H | br s | H-7 |
| 2.86 | 1H | br s | H-9 |
| 5.25-5.22 | 2H | br s | H-10 |
| 4.99 | 1H | d (8.0) | H-1′ |
| 4.95 | 1H | dd (9.0,8.0) | H-2′ |
| 3.71 | 1H | dd (9.0,9.0) | H-3′ |
| 3.51 | 1H | dd (9.5,9.0) | H-4′ |
| 3.67 | 1H | m | H-5′ |
| 4.53 | 1H | dd (12.0,2.0) | $H_a$-6′ |
| 4.42 | 1H | dd (12.0,6.0) | $H_b$-6′ |
| 7.85 | 2H | d (8.5) | H-2″ and H-6″ |
| 6.80 | 2H | d (8.5) | H-3″ and H-5″ |
| 7.05 | 1H | d (2.0) | H-2‴ |
| 6.78 | 1H | d (8.5) | H-5‴ |
| 6.95 | 1H | dd (8.5,2.0) | H-6‴ |
| 7.58 | 1H | d (16.0) | H-7‴ |
| 6.30 | 1H | d (16.0) | H-8‴ |




2'-*O*-*p*-hydroxybenzoyl-6'-*O*-*trans*-caffeoylgardoside (**VA-17**, **3.05**)

Fig. 3.21: $^{13}$C NMR spectrum (75 MHz, $d_4$-MeOH) of compound **VA-17** (**3.05**)




2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoylgardoside (**VA-17**, **3.05**)



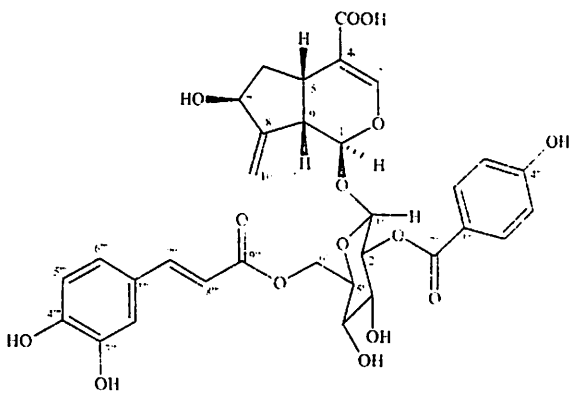

Fig. 3.22: DEPT spectrum ($d_4$-MeOH) of compound **VA-17** (**3.05**)

## TABLE 3.15

**$^{13}$C NMR spectral data of VA-17 (2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoylgardoside, 3.05)**

**(Fig. 3.21, 75 MHz spectrum, $d_4$-MeOH)**

| Carbon number | Chemical shift (δ) | Carbon number | Chemical shift (δ) |
|---|---|---|---|
| C-1 | 96.8 | C-1″ | 122.2 |
| C-3 | 151.5 | C-2″ and C-6″ | 132.9 |
| C-4 | 113.0 | C-3″ and C-5″ | 116.2 |
| C-5 | 31.7 | C-4″ | 163.4 |
| C-6 | 40.4 | C-7″ | 167.4 |
| C-7 | 73.8 | C-1‴ | 127.6 |
| C-8 | 152.7 | C-2‴ | 115.2 |
| C-9 | 44.8 | C-3‴ | 146.8 |
| C-10 | 112.7 | C-4‴ | 149.7 |
| C-11 | 170.1 | C-5‴ | 116.5 |
| C-1′ | 98.4 | C-6‴ | 123.1 |
| C-2′ | 74.9 | C-7‴ | 147.3 |
| C-3′ | 75.8 | C-8‴ | 114.8 |
| C-4′ | 71.8 | C-9‴ | 169.0 |
| C-5′ | 75.9 | | |
| C-6′ | 64.3 | | |




2'-O-*p*-hydroxybenzoyl-6'-O-*trans*-caffeoylgardoside (**VA-17**, **3.05**)

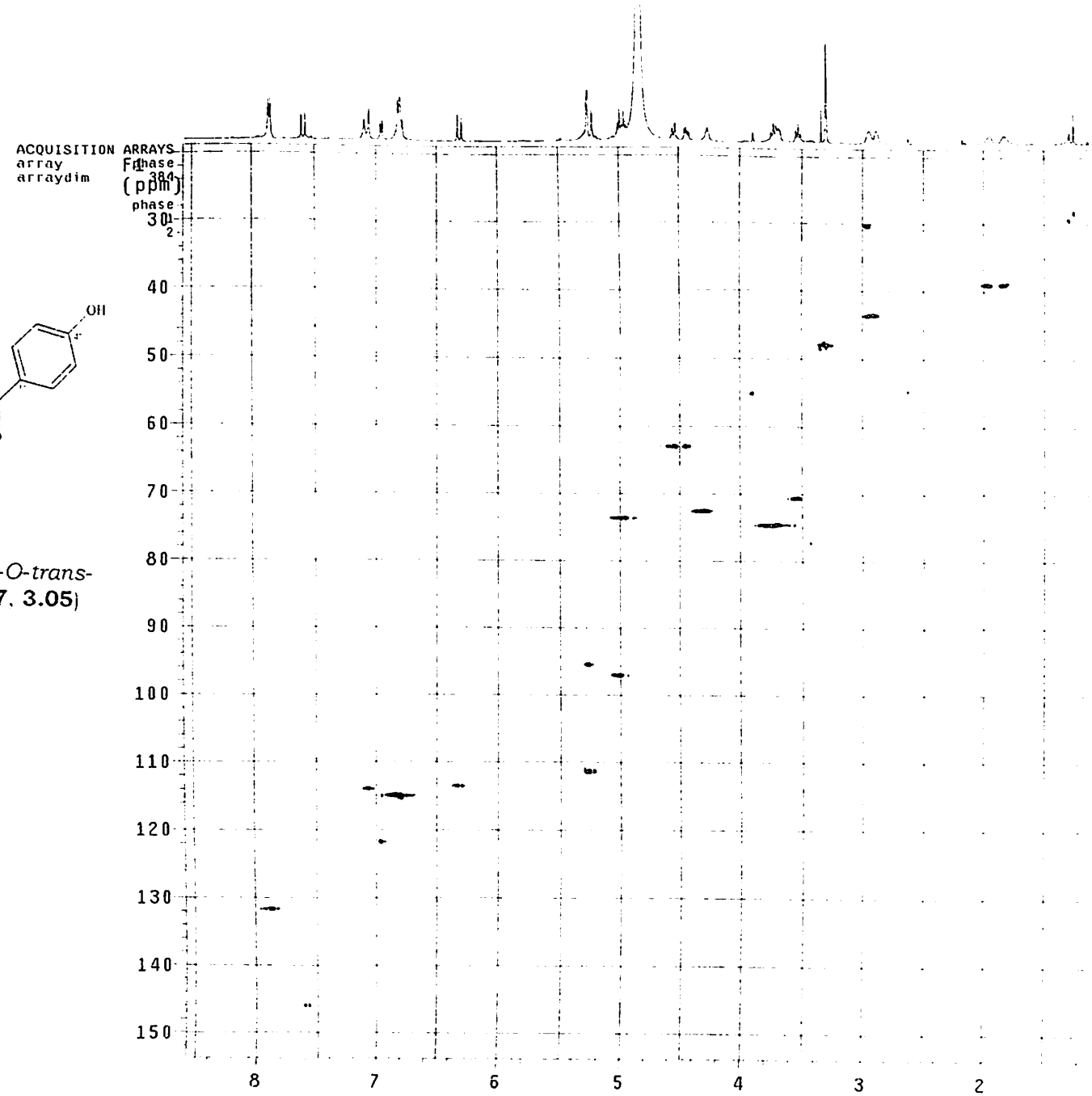
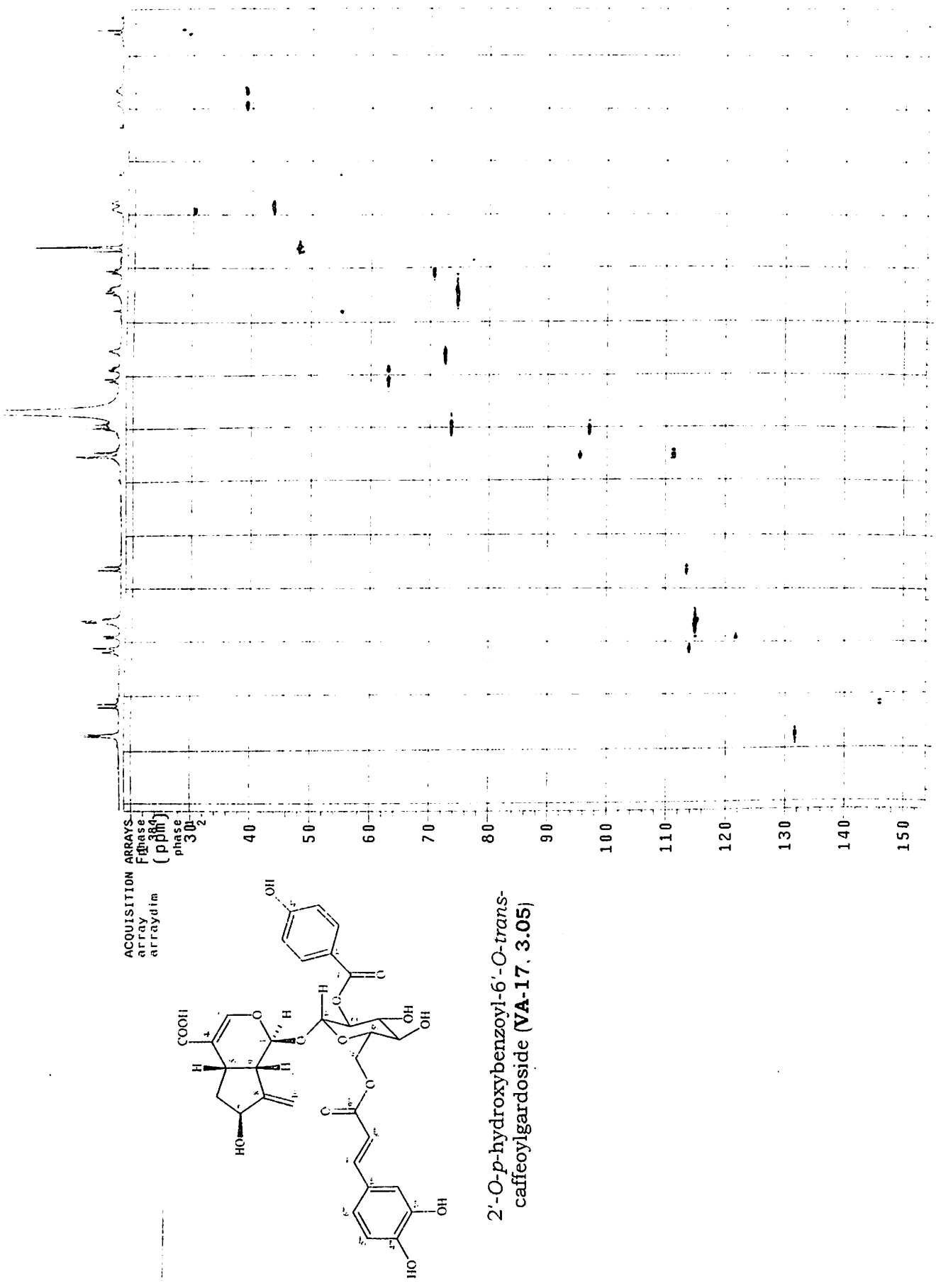



Fig. 3.23: HMQC spectrum (500 MHz, $d_4$-MeOH) of compound **VA-17** (**3.05**)




2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoylgardoside (**VA-17**, **3.05**)

Fig. 3.23: HMQC spectrum (500 MHz, $d_4$-MeOH) of compound **VA-17 (3.05)**

## TABLE 3.16

**HMQC spectral data of VA-17 (2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoylgardoside, 3.05)**

**(Fig. 3.23, solvent: $d_4$-MeOH)**

| **Proton chemical shift (δ)** | **Correlated carbon chemical shift (δ)** | **Assignment** |
|---|---|---|
| 5.24 (H-1) | 96.8 | C-1 |
| 7.09 (H-3) | 151.5 | C-3 |
| 2.95 (H-5) | 31.7 | C-5 |
| 1.94 and 1.83 | 40.4 | C-6 |
| ($H_a$-6 and $H_b$-6) | | |
| 4.26 (H-7) | 73.8 | C-7 |
| 2.86 (H-9) | 44.8 | C-9 |
| 5.25 and 5.22 (H-10) | 112.7 | C-10 |
| 4.99 (H-1′) | 98.4 | C-1′ |
| 4.95 (H-2′) | 74.9 | C-2′ |
| 3.71 (H-3′) | 75.8 | C-3′ |
| 3.51 (H-4′) | 71.8 | C-4′ |
| 3.67 (H-5′) | 75.9 | C-5′ |
| 4.53 and 4.42 | 64.3 | C-6′ |
| ($H_a$-6′ and $H_b$-6′) | | |
| 7.85 (H-2″, H-6″) | 132.9 | C-2″, C-6″ |
| 6.80 (H-3″, H-5″) | 116.2 | C-3″, C-5″ |
| 7.05 (H-2‴) | 115.2 | C-2‴ |
| 6.78(H-5‴) | 116.5 | C-5‴ |
| 6.95 (H-6‴) | 123.1 | C-6‴ |
| 7.58 (H-7‴) | 147.3 | C-7‴ |
| 6.30 (H-8‴) | 114.8 | C-8‴ |

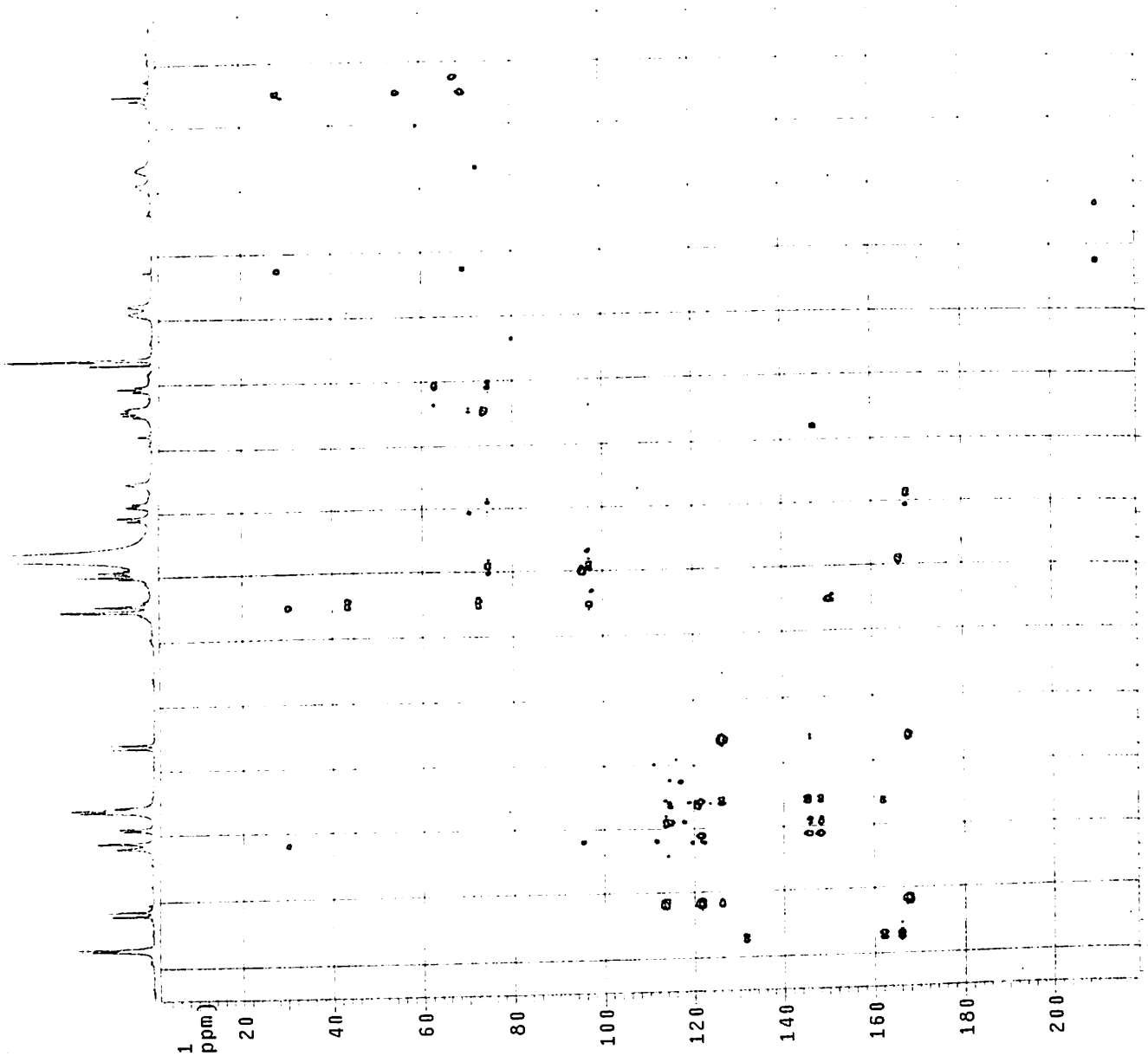
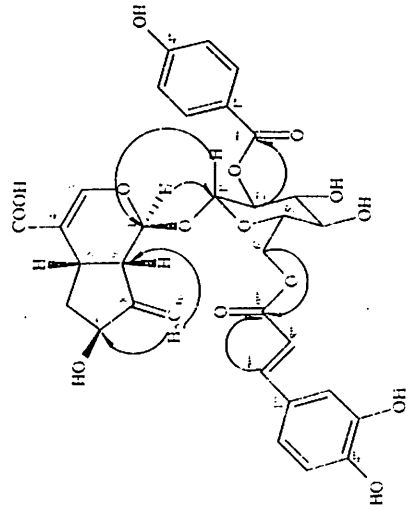



Fig. 3.24: HMBC spectrum (500 MHz, $d_4$-MeOH) of compound **VA-17 (3.05)**

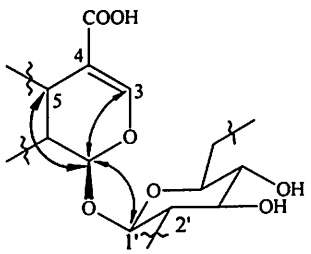
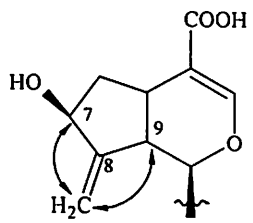
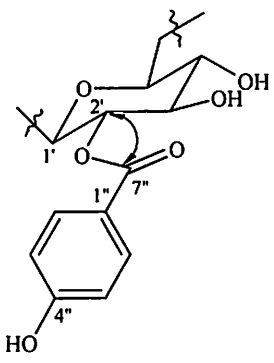
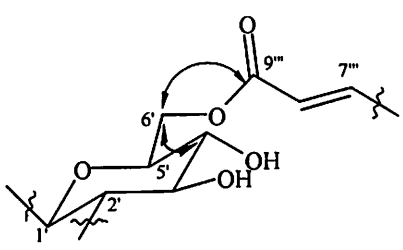
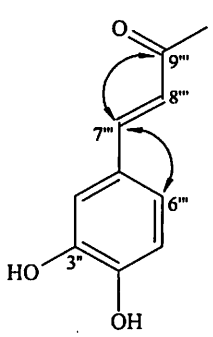



2'-*O*-*p*-hydroxybenzoyl-6'-*O*-*trans*-caffeoylgardoside (**VA-17, 3.05**)

# TABLE 3.17

**HMBC spectral data of VA-17 (2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoylgardoside, 3.05)**

**(Fig. 3.24, solvent: $d_4$-MeOH)**

| Chemical shift (δ) | Correlated carbons | Structural units derived |
|---|---|---|
| 5.24 (H-1) | C-1′, C-3, C-5 |  |
| 5.25-5.22 (H-10) | C-7, C-9 |  |
| 4.95 (H-2′) | C-7″ |  |
| 4.53-4.42 (H-6′) | C-9‴, C-4′ |  |
| 7.58 (H-7‴) | C-9‴, C-6‴ |  |

## SECTION-III

### (8-EPILOGANIC ACID DERIVATIVES)

## VA-22

It was obtained as a colorless amorphous powder, m.p 219-220 °C, $[\alpha]_D^{25}$ −119.9° (*c* 0.75, MeOH). Its molecular formula was established as $C_{23}H_{28}O_{12}$ based on elemental analysis and LC-MS data [*m/z* 495 $(M-H)^-$].

The UV (MeOH) spectrum of VA-22 showed absorption maxima at 255 nm. Its IR (KBr) spectrum showed bands at $\nu_{max}$ 3405 (hydroxyl), 1703 (CO, ester), 1648 (C=C), 1609 and 1516 $cm^{-1}$(aromatic). The $^1H$ NMR spectral (Fig. 3.26, Table. 3.18) data showed the presence of a hemiacetal proton (δ 5.40, br s, H-1) and a trisubstituted olefinic proton (δ 7.03, br s, H-3) indicative of an iridoid nucleus[1] and a *p*-hydroxybenzoyl moiety[4] [δ 7.83 (2H, br s) and δ 6.80 (2H, br s)]. The spectrum also contained a series of signals between δ 3.39 and 4.95 assignable to a sugar moiety.[2] The coupling constant of the anomeric proton (δ 4.95, 1H, d, *J* = 8.0, H-1′) was consistent with the β configuration of the sugar unit.

In addition, the $^1H$ NMR spectrum showed the presence of a secondary methyl signal at δ 1.00 (3H, d, *J* = 7.0 Hz, H-10), a hydroxymethine proton (δ 3.72, 1H, br s, H-7), and a methine proton signal at δ 2.04 (1H, d, *J* = 5.5 Hz, H-8). These, assignments were supported further by the $^{13}C$ NMR signals at δ 14.4 (C-10), δ 44.8 (C-8) and δ 79.4 (C-7). In the $^1H$-$^1H$ COSY spectrum (Fig. 3.27, Table 3.19) correlations were observed between H-8 and H-10 protons.

The $^{13}C$ NMR spectrum (Fig. 3.28, Table 3.20) showed the presence of a *p*-hydroxybenzoyl unit (δc 167.4, 163.3, 132.9, 122.3 and

## SECTION-III

### (8-EPILOGANIC ACID DERIVATIVES)

## VA-22

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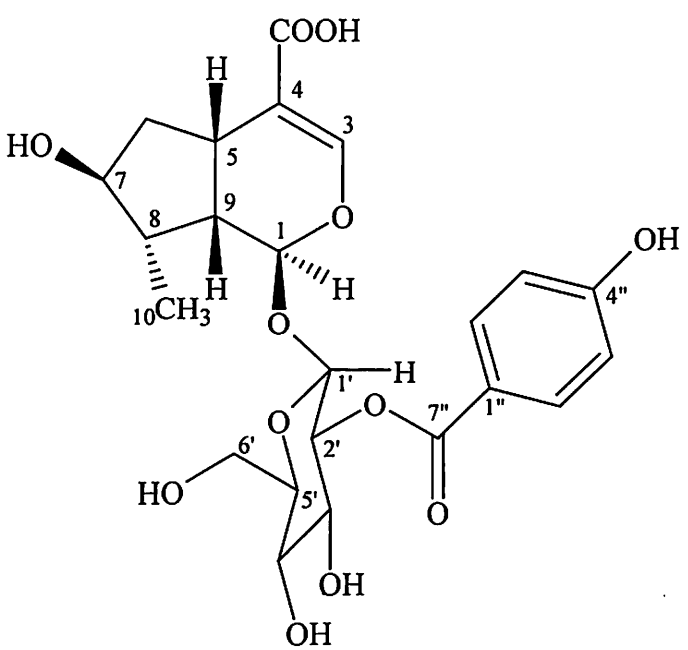
In addition, the $^1H$ NMR spectrum showed the presence of a secondary methyl signal at δ 1.00 (3H, d, *J* = 7.0 Hz, H-10), a hydroxymethine proton (δ 3.72, 1H, br s, H-7), and a methine proton signal at δ 2.04 (1H, d, *J* = 5.5 Hz, H-8). These, assignments were supported further by the $^{13}C$ NMR signals at δ 14.4 (C-10), δ 44.8 (C-8) and δ 79.4 (C-7). In the $^1H$-$^1H$ COSY spectrum (Fig. 3.27, Table 3.19) correlations were observed between H-8 and H-10 protons.

The $^{13}C$ NMR spectrum (Fig. 3.28, Table 3.20) showed the presence of a *p*-hydroxybenzoyl unit (δc 167.4, 163.3, 132.9, 122.3 and

δ 116.2) and signals at δ 98.0, 78.5, 76.3, 75.0, 71.8 and 62.8 attributable to a glucopyranose moiety. These assignments were supported by the DEPT spectral (Fig. 3.29) data.

A comparison of the above data with those of VA-21 revealed that VA-22 differs from **3.04** only in the nature of cyclopentane ring of the iridoid skeleton. The absence of an $\Delta^{8(10)}$ exocyclic methylene group (δ 5.29, 2H, brs) present in **3.04** and the presence of a secondary methyl (δ 1.00, d, $J$ = 7.0 Hz, H-10) and a methine proton (δ 2.04, d, $J$ = 5.5 Hz) indicated the presence of 8-epiloganic acid skeleton[14,15] in VA-22.

Based on the forgoing, VA-22 was deduced as 2'-*O*-*p*-hydroxybenzoyl-8-epiloganic acid (**3.06**), a new monoacylated iridoid glucoside.




**3.06**

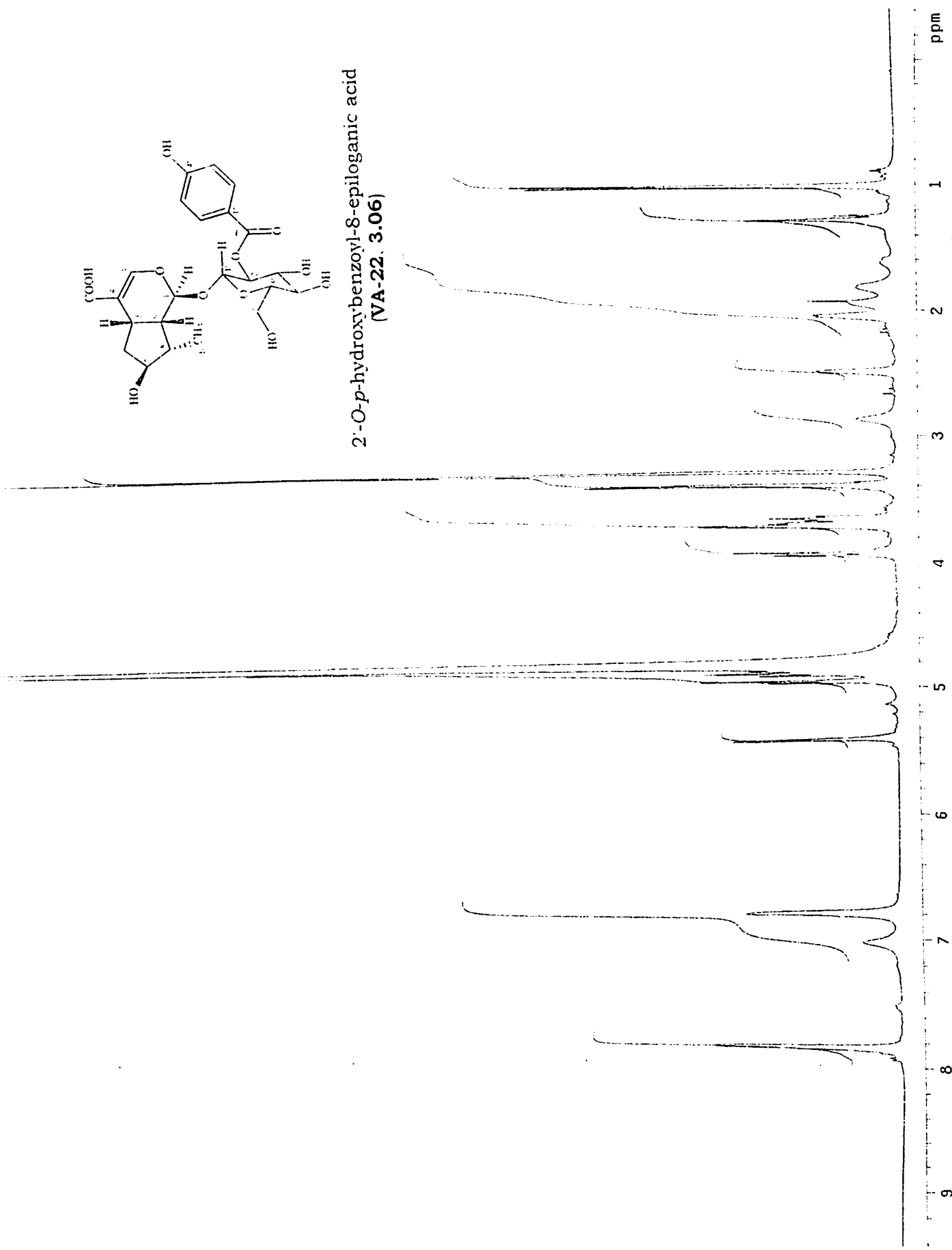



Fig. 3.26: $^1$H NMR spectrum (500 MHz, $d_4$-MeOH) of compound **VA-22 (3.06)**

## TABLE 3.18

**$^1$H NMR spectral data of VA-22 (2′-*O*-*p*-hydroxybenzoyl-8-epiloganic acid, 3.06)**

**(Fig. 3.26, 500 MHz spectrum, $d_4$-MeOH)**

| **Chemical shift (δ)** | **Proton integration** | **Multiplicity (*J* in Hz)** | **Assignment** |
|---|---|---|---|
| 5.40 | 1H | br s | H-1 |
| 7.03 | 1H | br s | H-3 |
| 2.86 | 1H | br s | H-5 |
| 1.93 | 1H | br s | $H_a$-6 |
| 1.82 | 1H | br s | $H_b$-6 |
| 3.72 | 1H | br s | H-7 |
| 2.04 | 1H | d (5.5) | H-8 |
| 2.48 | 1H | br s | H-9 |
| 1.00 | 3H | d (7.0) | H-10 |
| 4.95 | 1H | d (8.0) | H-1′ |
| 4.88 | 1H | dd (9.0,8.0) | H-2′ |
| 3.68 | 1H | m | H-3′ |
| 3.39 | 1H | m | H-4′ |
| 3.67 | 1H | m | H-5′ |
| 3.93 | 1H | d (12.0) | $H_a$-6′ |
| 3.65 | 1H | m | $H_b$-6′ |
| 7.83 | 2H | br s | H-2″ and H-6″ |
| 6.80 | 2H | br s | H-3″ and H-5″ |

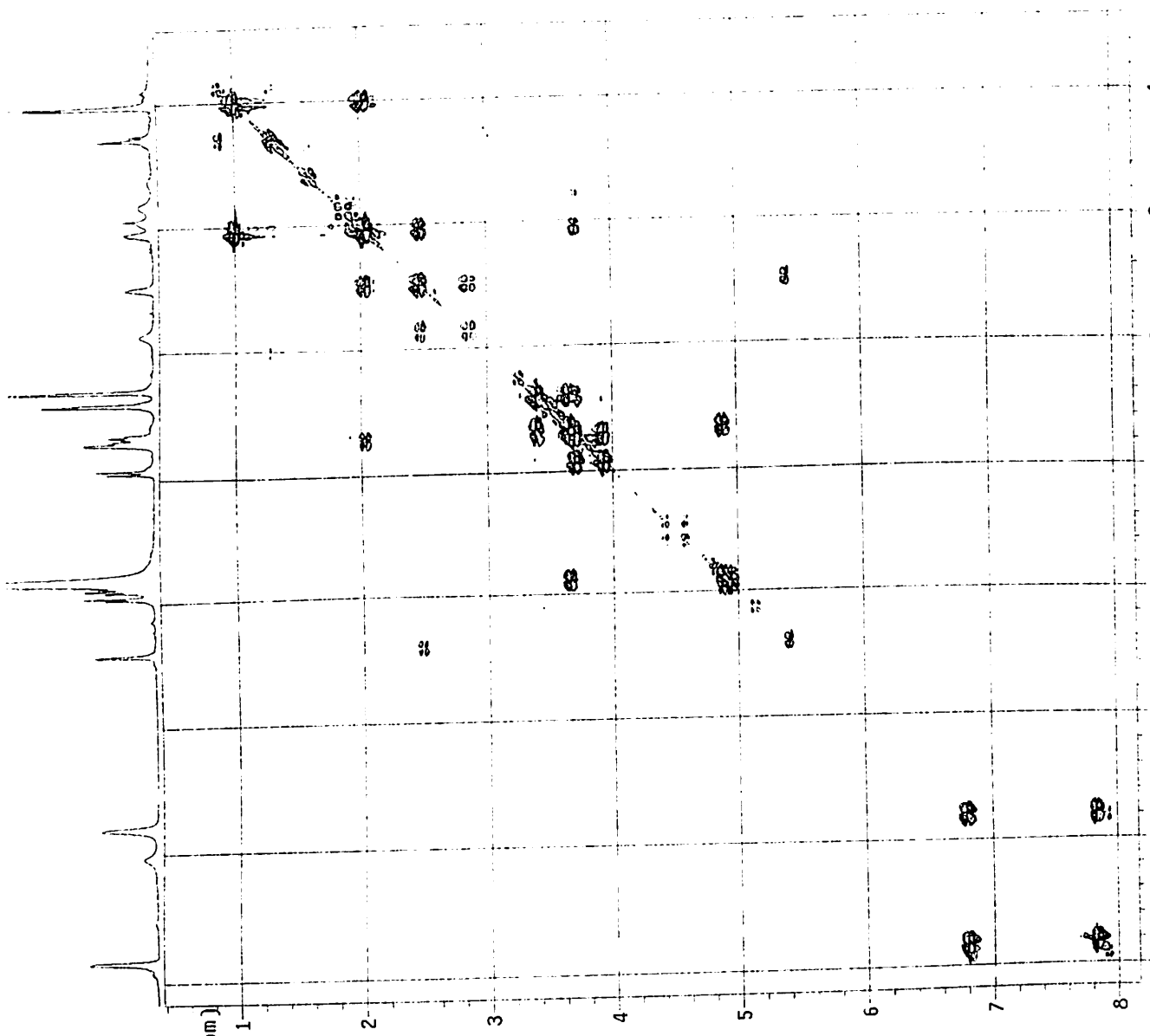
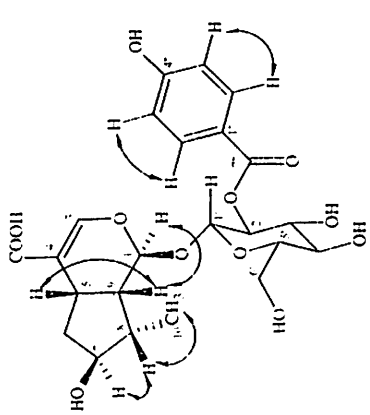



Fig. 3.27: $^{1}$H-$^{1}$H COSY spectrum (500 MHz, $d_4$-MeOH) of compound **VA-22 (3.06)**



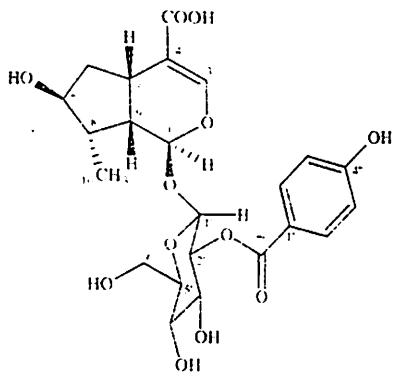

2'-*O*-*p*-hydroxybenzoyl-8-epiloganic acid
**(VA-22, 3.06)**

## TABLE 3.19

**$^1$H-$^1$H COSY spectral data of VA-22 (2′-*O*-*p*-hydroxybenzoyl-8-epiloganic acid, 3.06)**

**(Fig. 3.27, solvent: $d_4$-MeOH)**

| Chemical shifts of coupled protons | Type of coupling | Assignment |
|---|---|---|
| 2.04 and 1.00 | vicinal | H-8 and H-10 |
| 2.48 and 2.04 | vicinal | H-9 and H-8 |
| 3.72 and 2.04 | allylic | H-7 and H-8 |
| 2.86 and 2.48 | vicinal | H-5 and H-9 |
| 5.40 and 2.48 | vicinal | H-1 and H-9 |
| 7.83 and 6.80 | ortho | H-2″ and H-3″ |




2′-*O*-*p*-hydroxybenzoyl-8-epiloganic acid
**(VA-22, 3.06)**




Fig. 3.28: $^{13}$C NMR spectrum (75 MHz, $d_4$-MeOH) of compound **VA-22 (3.06)**

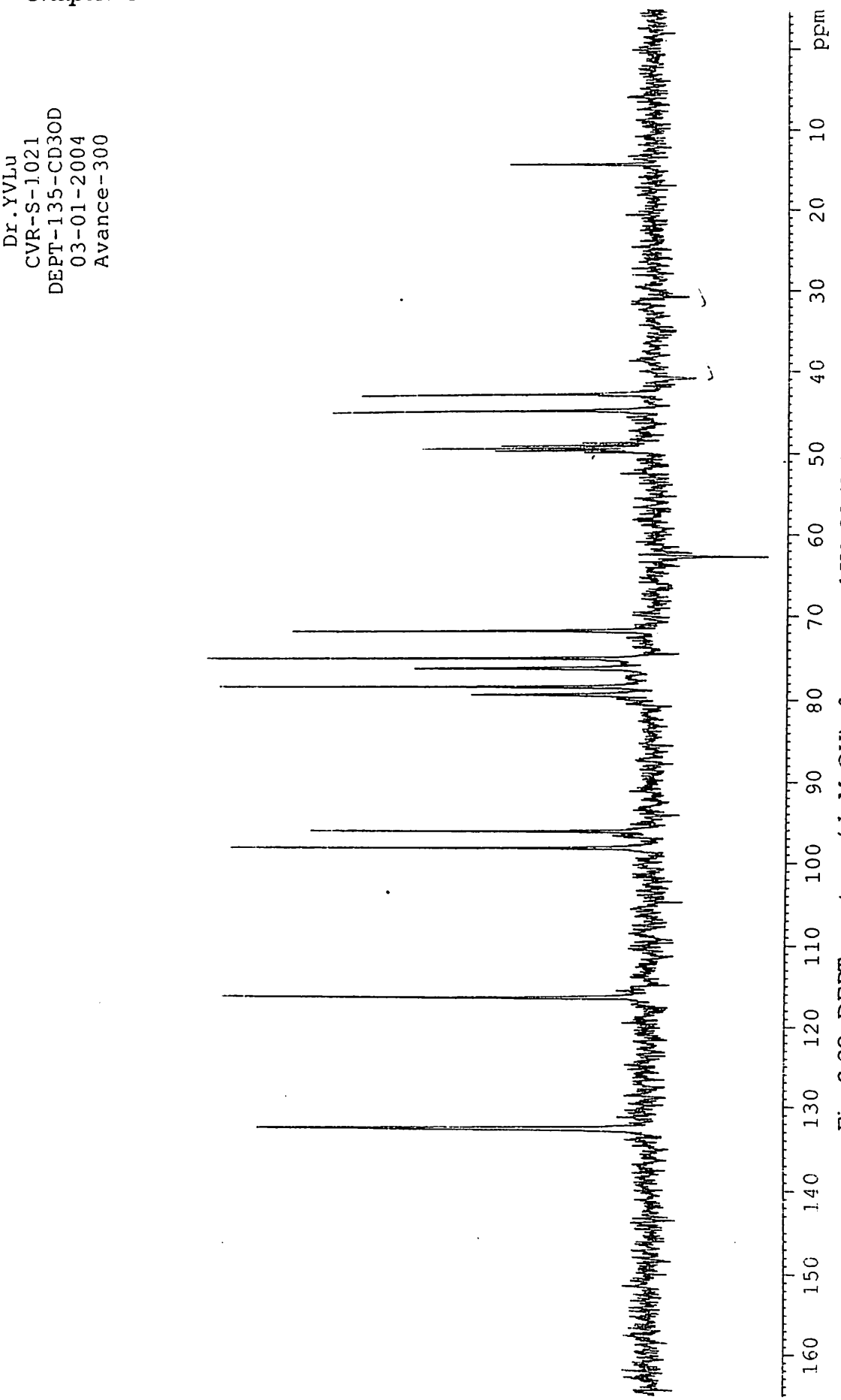



Fig. 3.29: DEPT spectrum ($d_4$-MeOH) of compound **VA-22 (3.06)**

## TABLE 3.20

**$^{13}$C NMR spectral data of VA-22 (2′-*O*-p-hydroxybenzoyl-8-epiloganic acid, 3.06)**
**(Fig. 3.28, 75 MHz spectrum, $d_4$-MeOH)**

| Carbon number | Chemical shift (δ) | Carbon number | Chemical shift (δ) |
|---|---|---|---|
| C-1 | 96.1 | C-2′ | 75.0 |
| C-3 | 151.0 | C-3′ | 76.3 |
| C-4 | [a] | C-4′ | 71.8 |
| C-5 | 31.3 | C-5′ | 78.5 |
| C-6 | 40.9 | C-6′ | 62.8 |
| C-7 | 79.4 | C-1″ | 122.3 |
| C-8 | 44.8 | C-2″ and C-6″ | 132.9 |
| C-9 | 42.8 | C-3″ and C-5″ | 116.2 |
| C-10 | 14.4 | C-4″ | 163.3 |
| C-11 | [a] | C-7″ | 167.4 |
| C-1′ | 98.0 | | |

[a]signals not observed

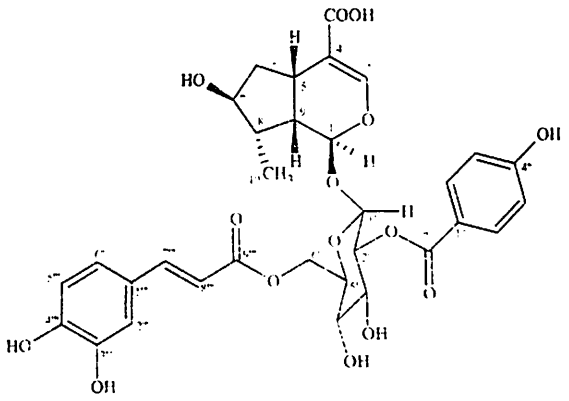
## VA-18

It was obtained as pale-yellow amorphous powder, m.p. 158-160 °C, $[\alpha]_D^{25}$ −88.6° (*c* 0.5, MeOH). The molecular formula of VA-18 was established as $C_{32}H_{34}O_{15}$based on elemental analysis and LC-MS [*m/z* 657 $(M - H)^-$] data.

VA-18 exhibited bands in its UV (MeOH) spectrum at $\lambda_{max}$ 249 and 330 nm. Its IR (KBr) spectrum showed bands at $\nu_{max}$ 3400 (hydroxyl), 1698 (α,β-unsaturated ester), 1604 and 1517 $cm^{-1}$(aromatic). The $^1H$ NMR spectrum (Fig. 3.30, Table. 3.21) showed the presence of hemiacetal proton (δ 5.29, 1H, d, *J* = 4.0 Hz, H-1), a trisubstituted olefinic proton (δ 7.09, 1H, brs, H-3), a secondary methyl signal at δ 0.96 (3H, d, *J* = 7.5 Hz, H-10), a hydroxymethine proton (δ 3.70, 1H, m, H-7) and a methine proton signal at δ 2.02 (1H, dd, *J* = 14.0, 7.0 Hz, H-8) indicative of 8-epiloganic acid nucleus[14,15] in VA-18.

The $^1H$ NMR spectrum also contained two AB doublets at δ 7.84 (2H, d, *J* = 8.5 Hz) and δ 6.81 (2H, d, *J* = 8.5 Hz) attributable to *p*-hydroxybenzoyl unit[4] and a series of signals between δ 3.52 and 4.99 attributable to a sugar moiety.[2] The configuration of sugar was established as β based on the coupling constant of anomeric proton (δ 4.99, d, *J* = 8.0 Hz, H-1′).

In addition, the $^1H$ NMR spectrum showed the presence of a *trans*-caffeoyl unit[10] constituted by 1,2,4-trisubstituted phenyl unit [δ 7.05 (1H, s,), 6.95 (1H, dd, *J* = 8.5, 1.5 Hz) and δ 6.77 (1H, d, *J* = 8.5 Hz)] and a pair of trans coupled olefinic protons at δ 7.59 (1H, d, *J* = 15.5 Hz) and δ 6.31 (1H, d, *J* = 15.5 Hz). These, assignments were supported by the correlations observed in $^1H$-$^1H$ COSY spectrum (Fig. 3.31, Table 3.22)




2'-*O*-*p*-hydroxybenzoyl-6'-*O*-*trans*-caffeoyl-8-epiloganic acid
(**VA-18**, **3.07**)




Fig. 3.30: $^1$H NMR spectrum (500 MHz, $d_4$-MeOH) of compound **VA-18** (**3.07**)

## TABLE 3.21

**$^1$H NMR spectral data of VA-18 (2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoyl-8-epiloganic acid, 3.07)**

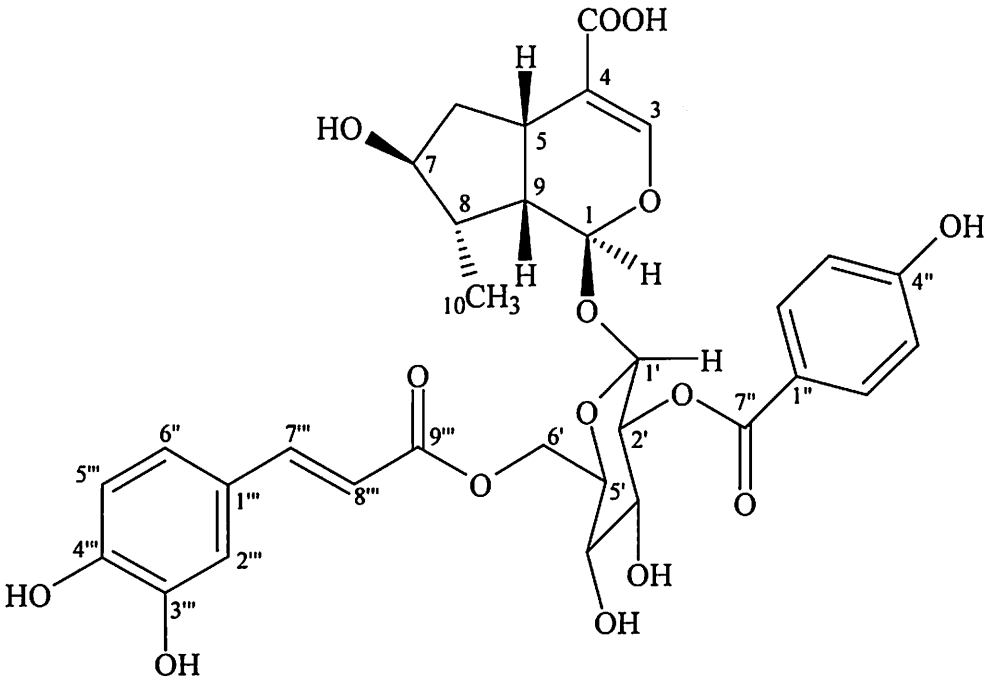
**(Fig. 3.30, 500 MHz spectrum, $d_4$-MeOH)**

| Chemical shift (δ) | Proton integration | Multiplicity (*J* in Hz) | Assignment |
|---|---|---|---|
| 5.29 | 1H | d (4.0) | H-1 |
| 7.09 | 1H | br s | H-3 |
| 2.83 | 1H | m | H-5 |
| 1.92 | 1H | m | $H_a$-6 |
| 1.74 | 1H | m | $H_b$-6 |
| 3.70 | 1H | m | H-7 |
| 2.02 | 1H | dd (14.0, 7.0) | H-8 |
| 2.45 | 1H | m | H-9 |
| 0.96 | 3H | d (7.5) | H-10 |
| 4.99 | 1H | d (8.0) | H-1′ |
| 4.94 | 1H | dd (9.5,8.0) | H-2′ |
| 3.69 | 1H | m | H-3′ |
| 3.52 | 1H | dd (9.5,9.0) | H-4′ |
| 3.67 | 1H | m | H-5′ |
| 4.54 | 1H | dd (12.0,2.0) | $H_a$-6′ |
| 4.42 | 1H | dd (12.0,6.0) | $H_b$-6′ |
| 7.84 | 2H | d (8.5) | H-2″ and H-6″ |
| 6.81 | 2H | d (8.5) | H-3″ and H-5″ |
| 7.05 | 1H | s | H-2‴ |
| 6.77 | 1H | d (8.5) | H-5‴ |
| 6.95 | 1H | dd (8.5,1.5) | H-6‴ |
| 7.59 | 1H | d (15.5) | H-7‴ |
| 6.31 | 1H | d (15.5) | H-8‴ |

The $^{13}C$ NMR (Fig. 3.32, Table 3.23) spectrum of VA-18 showed the presence of two ester carbonyl carbons (δ 169.0, C-9‴ and 167.4, C-7″), a secondary methyl carbon (δ 14.3, C-10), an oxymethine carbon (δ 79.3, C-7) and a methine carbon (δ 44.7, C-8). The assignments were supported by the DEPT (Fig. 3.33) spectral data and the correlations observed in the HMQC (Fig. 3.34, Table. 3.24) spectrum.

A perusal of the above spectral data in comparison with 2′-*O*-*p*-hydroxybenzoyl-8-epiloganic acid (VA-22) revealed that the spectral data of VA-18 closely resembled those of VA-22, except for the additional signals of a caffeoyl moiety present in VA-18. Thus VA-18 was derived as a caffeoyl derivative of 2′-*O*-*p*-hydroxybenzoyl-8-epiloganic acid (VA-22). The downfield shift of C-6′ (64.2) and 6′-$H_2$ (δ 4.54-4.42) in VA-18 in comparison with those of VA-22 ($\delta_C$ 62.8 and $\delta_H$ 3.93-3.65) indicated the attachment of caffeoyl unit at C-6′ in VA-18. The position of linkage of caffeoyl unit at C-6′ was confirmed by the analysis of the HMBC data. In the HMBC spectrum (Fig. 3.35, Table. 3.25) the H-6′ methylne protons (δ 4.54, dd, $J$ = 12.0, 2.0 Hz and δ 4.42, dd, $J$ = 12.0, 6.0 Hz) of the glucose unit showed correlations with the ester carbonyl ($\delta_C$ 169.0, C-9‴) of caffeoyl unit. The selective HMBC correlations of VA-18 are represented in Fig. 3.36.

Based on the foregoing, the structure of VA-18 was derived as 2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoyl-8-epiloganic acid (**3.07**), a new diacylated iridoid glucoside.


COOH
H
4
3
HO
5
7
9
8
1
O
OH
4"
H
H
10CH3
O
1'
H
O
7"
1"
O
6"
7'''
9'''
6'
5'''
O
2'
1'''
8'''
5'
O
4'''
2'''
HO
OH
3'''
OH
OH

**3.07**

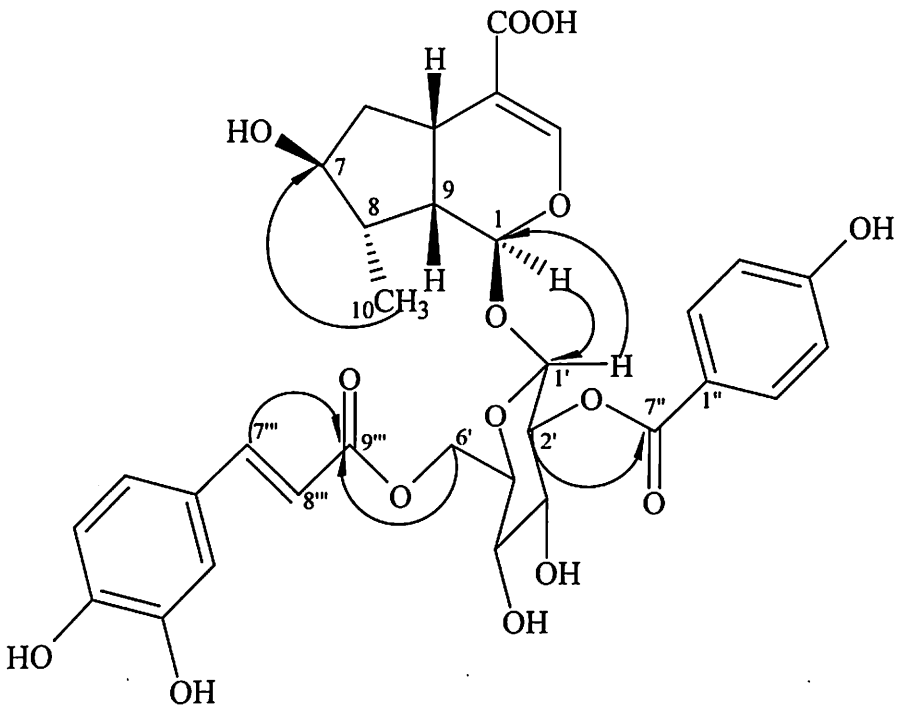
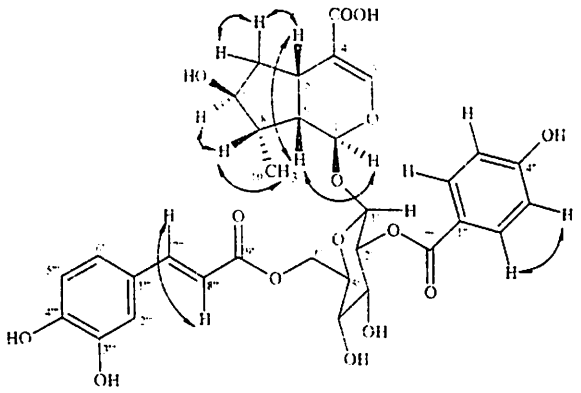

COOH
H
HO
7
8
9
1
O
OH
H
H
10CH3
O
1'
H
O
7"
1"
O
7'''
9'''
6'
2'
O
8'''
O
O
OH
OH
HO
OH

**Fig. 3.36**



2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoyl-8-epiloganic acid
(**VA-18**, **3.07**)

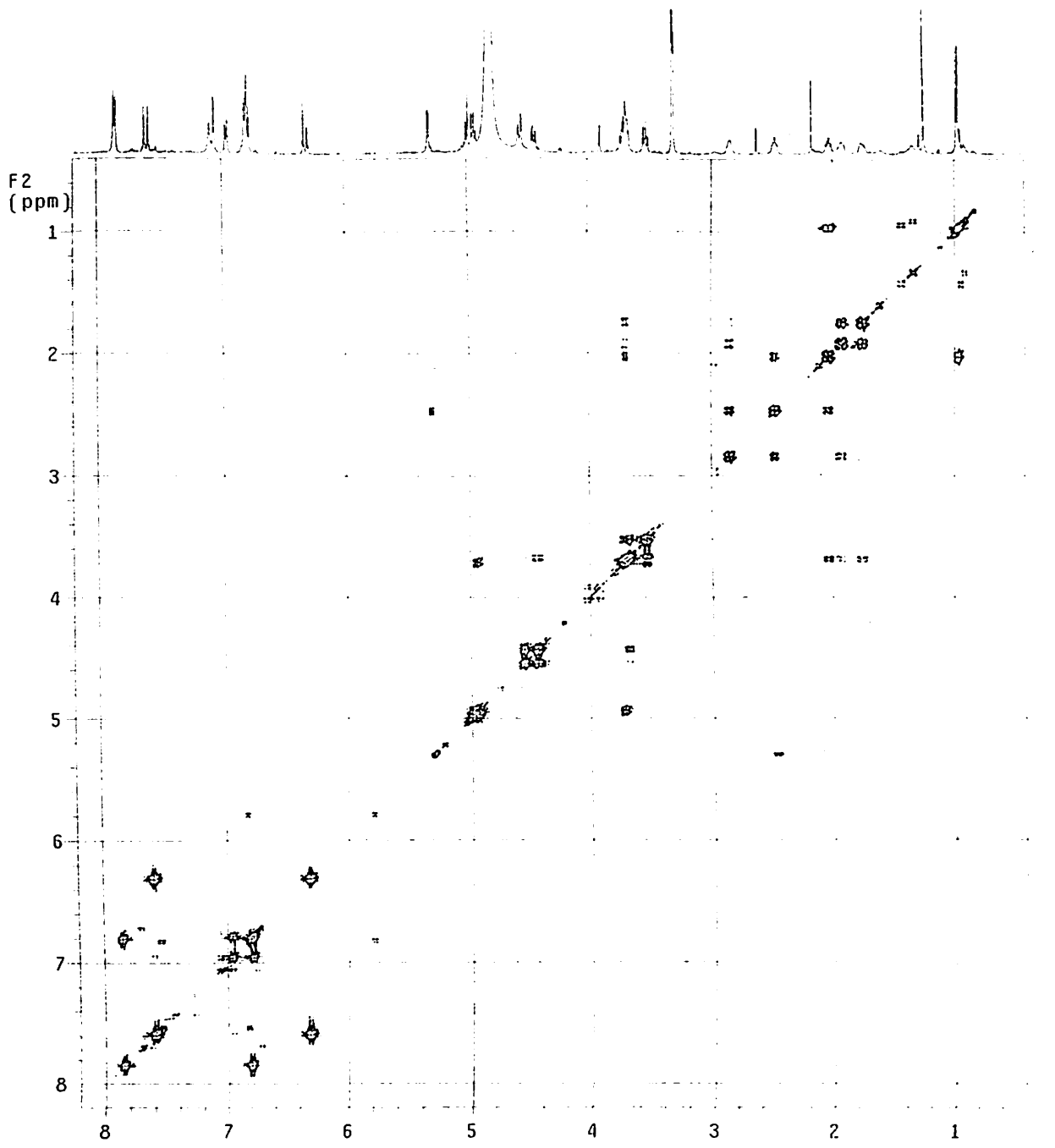


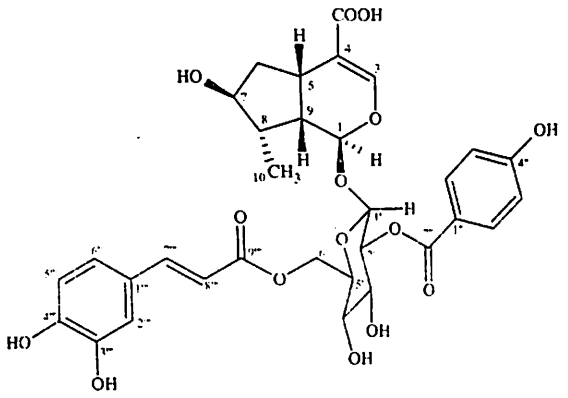

Fig. 3.31: $^{1}$H-$^{1}$H COSY spectrum (500 MHz, $d_4$-MeOH) of compound **VA-18** (**3.07**)

## TABLE 3.22

**$^1$H-$^1$H COSY spectral data of VA-18 (2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoyl-8-epiloganic acid. 3.07)**

**(Fig. 3.31, solvent: $d_6$-DMSO)**

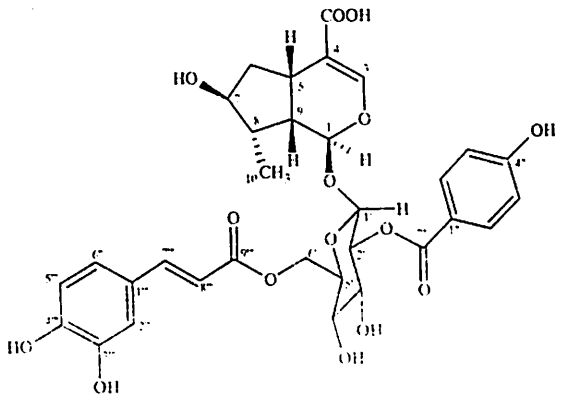
| Chemical shifts of coupled protons | Type of coupling | Assignment |
|---|---|---|
| 2.02 and 0.96 | vicinal | H-8 and H-10 |
| 1.92 and 1.74 | geminal | $H_a$-6 and $H_b$-6 |
| 3.70 and 2.02 | vicinal | H-7 and H-8 |
| 2.83 and 2.45 | vicinal | H-5 and H-9 |
| 2.83 and 1.92 | vicinal | H-5 and H-6 |
| 5.29 and 2.45 | vicinal | H-1 and H-9 |
| 4.94 and 3.69 | vicinal | H-2′ and H-3′ |
| 7.59 and 6.31 | vicinal | H-7‴ and H-8‴ |
| 7.85 and 6.83 | ortho | H-2″ and H-3″ |




2'-*O*-*p*-hydroxybenzoyl-6'-*O*-*trans*-caffeoyl-8-epiloganic acid
**(VA-18, 3.07)**




Fig. 3.32: $^{13}C$ NMR spectrum (75 MHz, $d_4$-MeOH) of compound **VA-18 (3.07)**




2'-*O*-*p*-hydroxybenzoyl-6'-*O*-*trans*-caffeoyl-8-epiloganic acid
**(VA-18, 3.07)**

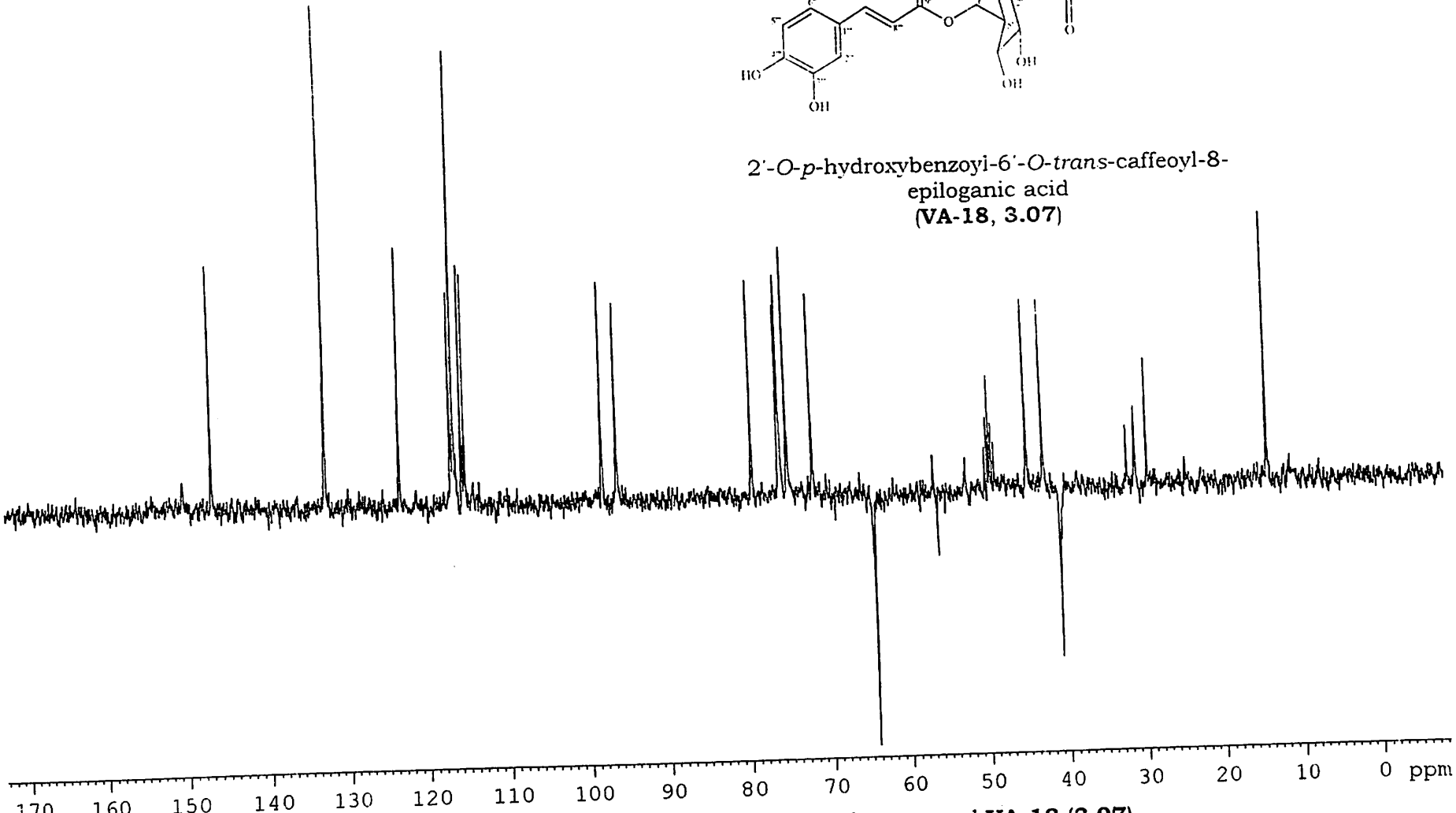
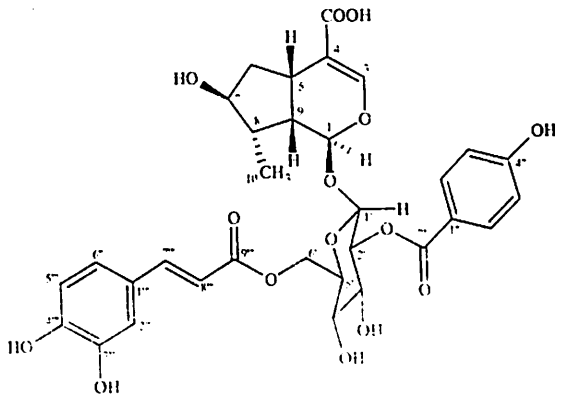



Fig. 3.33: DEPT spectrum ($d_4$-MeOH) of compound **VA-18 (3.07)**




2'-*O*-*p*-hydroxybenzoyl-6'-*O*-*trans*-caffeoyl-8-epiloganic acid
**(VA-18, 3.07)**

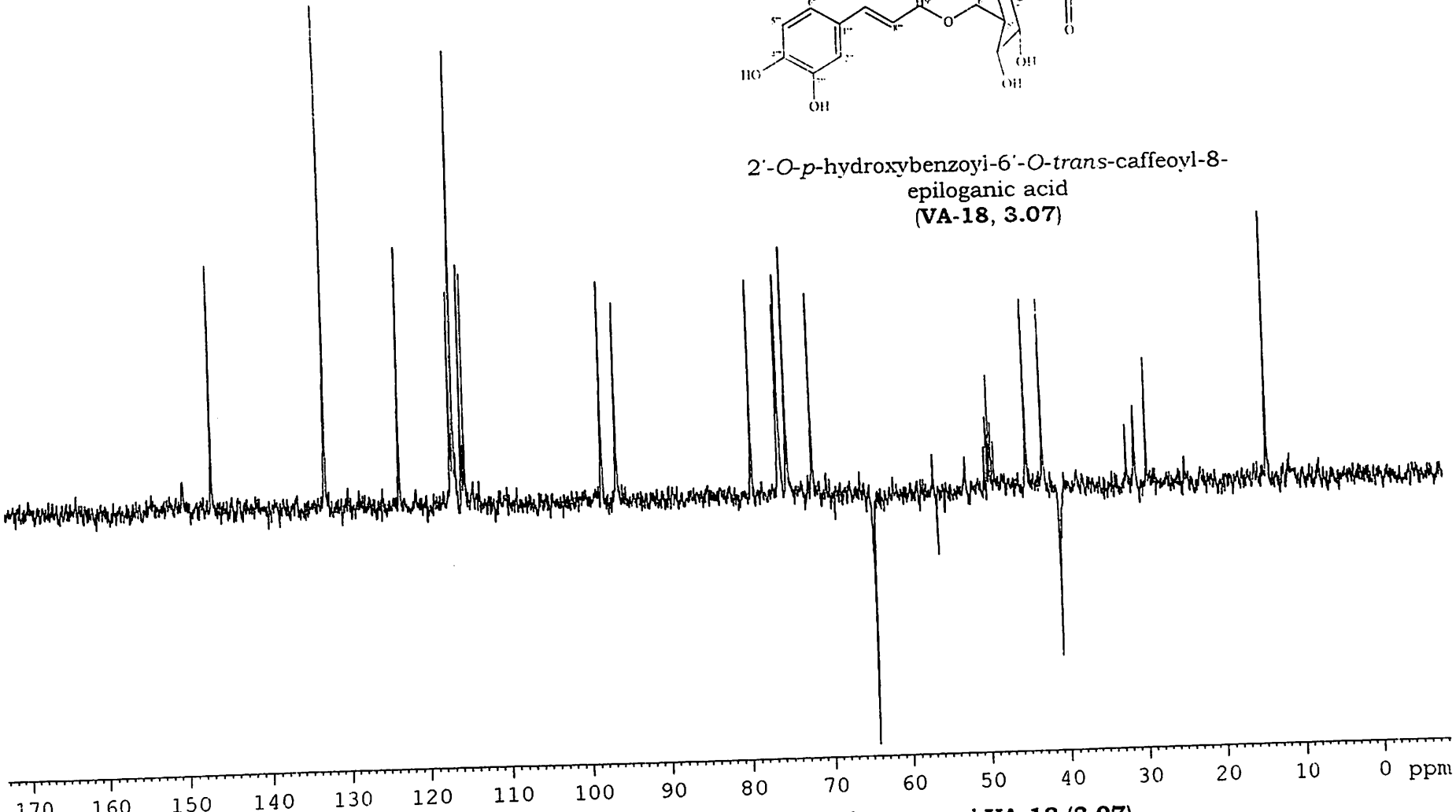



Fig. 3.33: DEPT spectrum ($d_4$-MeOH) of compound **VA-18 (3.07)**

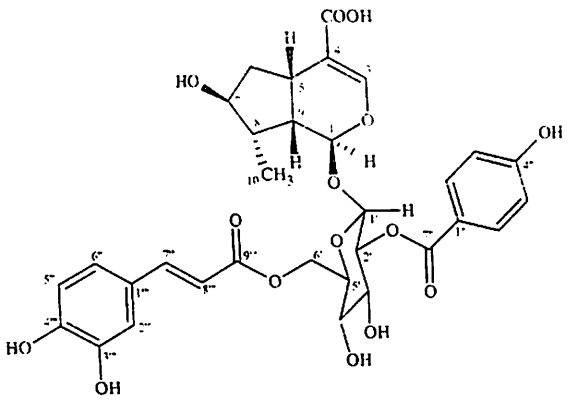
## TABLE 3.23

**$^{13}$C NMR spectral data of VA-18 (2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoyl-8-epiloganic acid, 3.07)**

**(Fig. 3.32, 75 MHz spectrum, $d_4$-MeOH)**

| Carbon number | Chemical shift (δ) | Carbon number | Chemical shift (δ) |
|---|---|---|---|
| C-1 | 96.1 | C-1″ | 122.2 |
| C-3 | 151.0 | C-2″ and C-6″ | 132.9 |
| C-4 | 113.8 | C-3″ and C-5″ | 116.2 |
| C-5 | 31.6 | C-4″ | 163.3 |
| C-6 | 40.8 | C-7″ | 167.4 |
| C-7 | 79.3 | C-1‴ | 127.7 |
| C-8 | 44.7 | C-2‴ | 115.2 |
| C-9 | 42.7 | C-3‴ | 146.8 |
| C-10 | 14.3 | C-4‴ | 149.6 |
| C-11 | [a] | C-5‴ | 116.6 |
| C-1′ | 97.9 | C-6‴ | 123.1 |
| C-2′ | 74.9 | C-7‴ | 147.3 |
| C-3′ | 75.8 | C-8‴ | 114.8 |
| C-4′ | 71.9 | C-9‴ | 169.0 |
| C-5′ | 76.0 | | |
| C-6′ | 64.2 | | |

[a]signal not observed




2'-*O*-*p*-hydroxybenzoyl-6'-*O*-*trans*-caffeoyl-8-epiloganic acid
(**VA-18**, **3.07**)

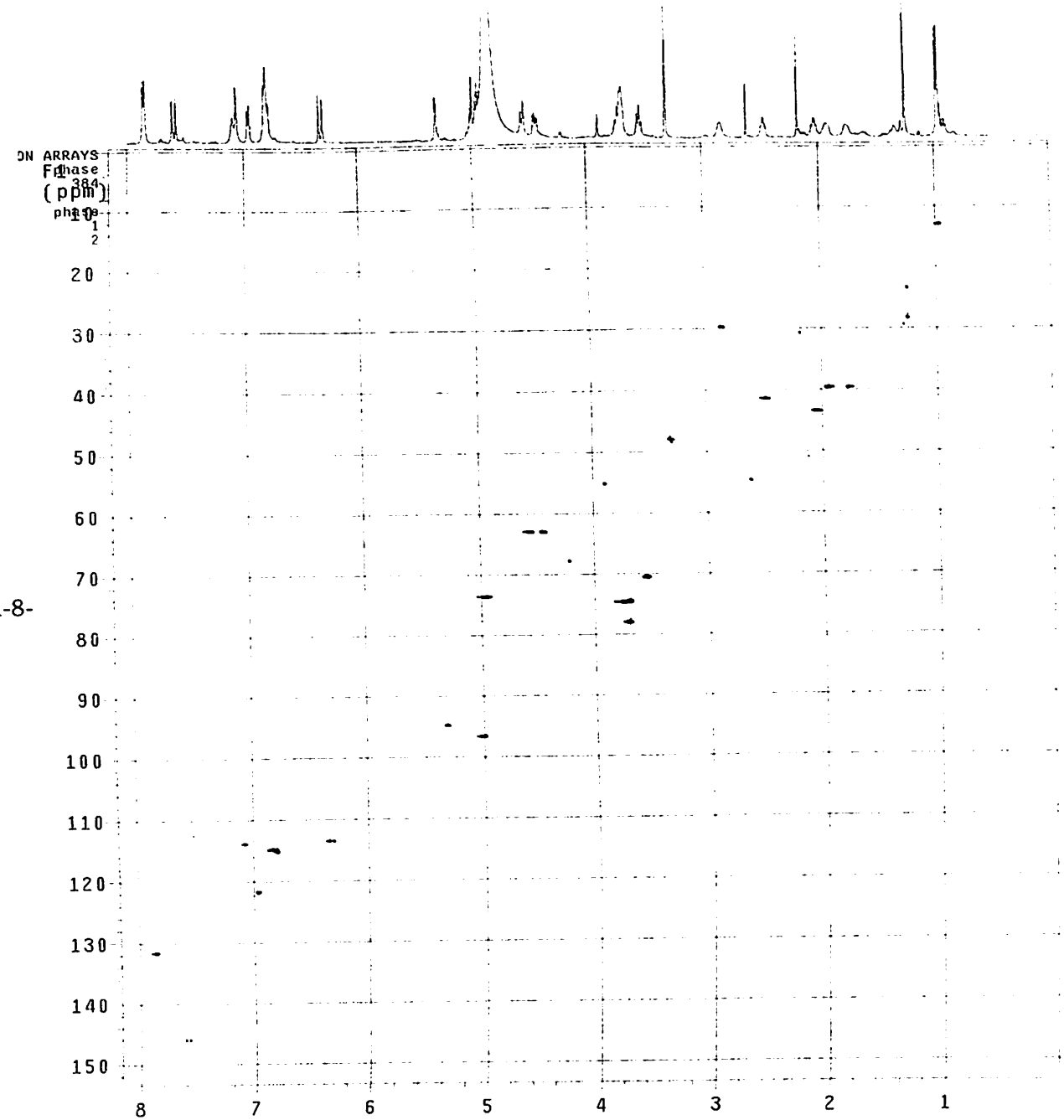


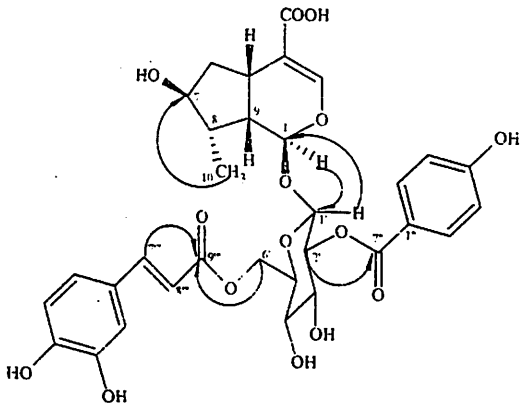

Fig. 3.34: HMQC spectrum (500 MHz, $d_4$-MeOH) of compound **VA-18** (**3.07**)

## TABLE 3.24

**HMQC spectral data of VA-18 (2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoyl-8-epiloganic acid, 3.07)**

**(Fig. 3.34, solvent: $d_4$-MeOH)**

| **Proton chemical shift (δ)** | **Correlated carbon chemical shift (δ)** | **Assignment** |
|---|---|---|
| 5.29 (H-1) | 96.1 | C-1 |
| 7.09 (H-3) | 151.0 | C-3 |
| 2.83 (H-5) | 31.6 | C-5 |
| 1.92 and 1.74 | 40.8 | C-6 |
| ($H_a$-6 and $H_b$-6) | | |
| 3.70 (H-7) | 79.3 | C-7 |
| 2.02 (H-8) | 44.7 | C-8 |
| 2.45 (H-9) | 42.7 | C-9 |
| 0.96 (H-10) | 14.3 | C-10 |
| 4.99 (H-1′) | 97.9 | C-1′ |
| 4.94 (H-2′) | 74.9 | C-2′ |
| 3.69 (H-3′) | 75.8 | C-3′ |
| 3.52 (H-4′) | 71.9 | C-4′ |
| 3.67 (H-5′) | 76.0 | C-5′ |
| 4.54 and 4.42 | 64.2 | C-6′ |
| ($H_a$-6′ and $H_b$-6′) | | |
| 7.84 (H-2″, H-6″) | 132.9 | C-2″, C-6″ |
| 6.81 (H-3″, H-5″) | 116.2 | C-3″, C-5″ |
| 7.05 (H-2‴) | 115.2 | C-2‴ |
| 6.77(H-5‴) | 116.6 | C-5‴ |
| 6.95 (H-6‴) | 123.1 | C-6‴ |
| 7.59 (H-7‴) | 147.3 | C-7‴ |
| 6.31 (H-8‴) | 114.8 | C-8‴ |




2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoyl-8-epiloganic acid
**(VA-18, 3.07)**

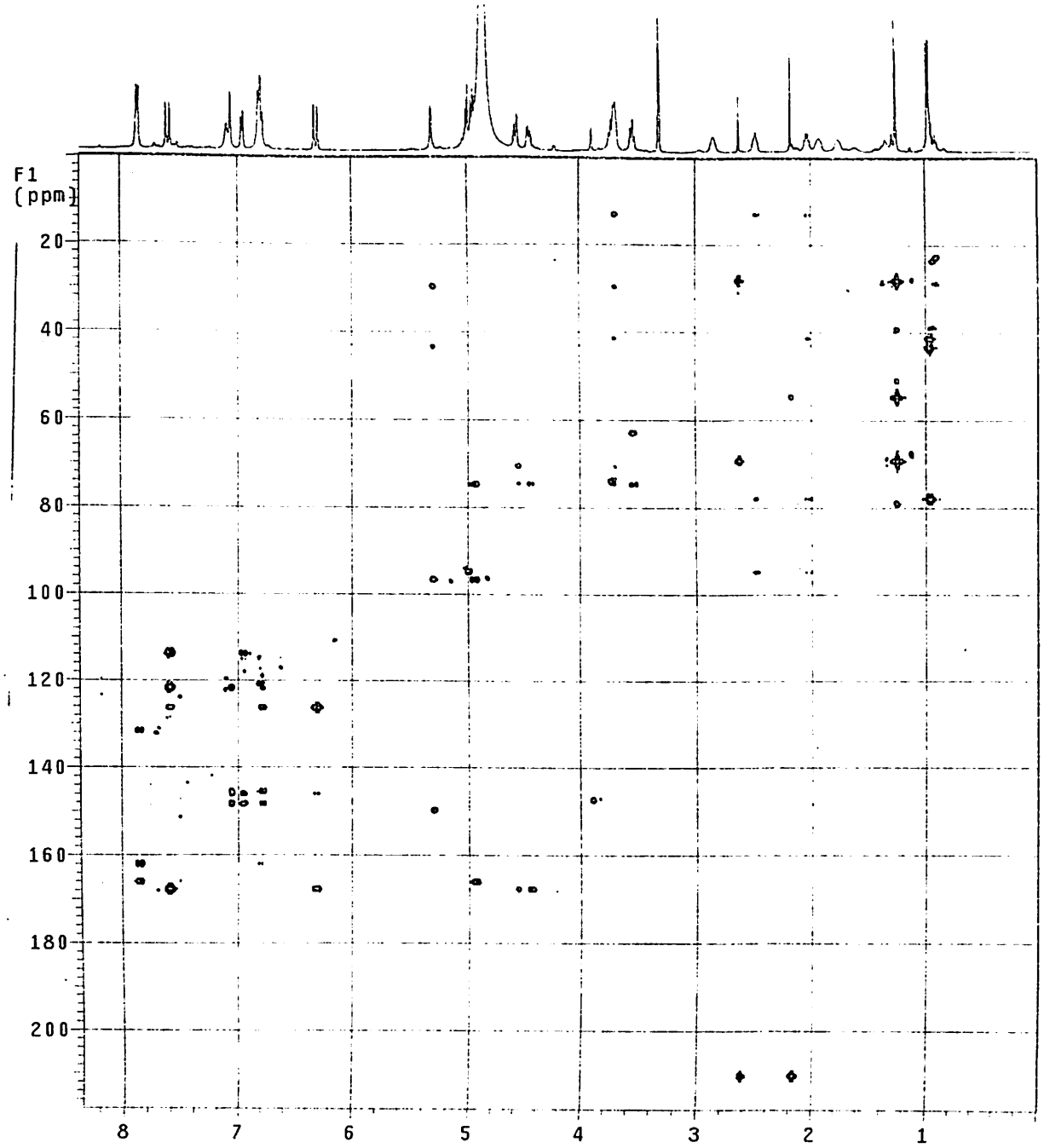
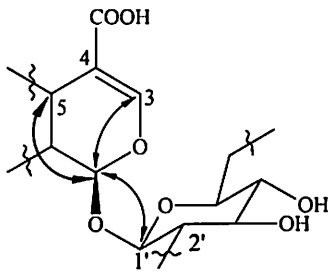
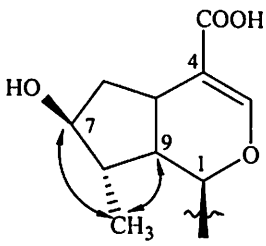
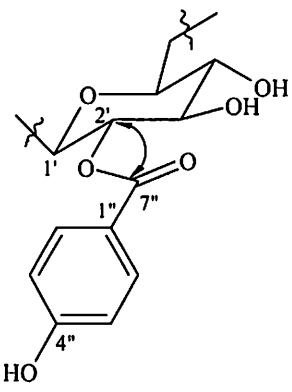
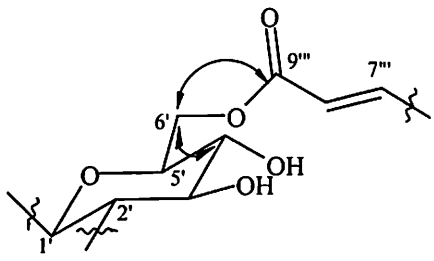
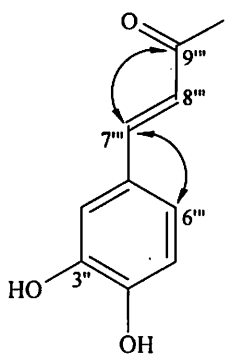



Fig. 3.35: HMBC spectrum (500 MHz, $d_4$-MeOH) of compound **VA-18 (3.07)**

## TABLE 3.25

**HMBC spectral data of VA-18 (2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoyl-8-epiloganic acid, 3.07)**

**(Fig. 3.35, solvent: $d_4$-MeOH)**

| Chemical shift (δ) | Correlated carbons | Structural units derived |
|---|---|---|
| 5.29 (H-1) | C-1′, C-3, C-5 |  |
| 0.96 (H-10) | C-9, C-7 |  |
| 4.94 (H-2′) | C-7″ |  |
| 4.54, 4.42 (H-6′) | C-9‴ |  |
| 7.59 (H-7‴) | C-6‴, C-9‴ |  |

**SECTION IV**
**(AUCUBIN DERIVATIVES)**

## VA-13

It was obtained as a pale-brown powder, m.p. 151-153 °C. Its molecular formula, $C_{22}H_{26}O_{11}$, was deduced from elemental analysis and LC-MS [*m/z* 465, $(M - H)^-$] data.

The spectral characteristics of VA-13 are: UV (MeOH) 258 nm, IR (KBr): $\nu_{max}$ 3400 (hydroxyl), 1703 (CO, ester), 1640 (C=C), 1590 $cm^{-1}$(aromatic). The $^1H$ NMR (Fig. 3.37, Table 3.26) spectral data showed the presence of a hemiacetal proton (1H, δ 4.87 m, H-1), a pair of olefinic double doublets [ δ 6.34 (1H, dd, *J* = 6.6, 1.8 Hz, H-3) and δ 5.05 (1H, dd, *J* = 6.6, 4.2 Hz, H-4), a trisubstituted olefinic proton (δ 5.79, 1H, s, H-7), two oxymethylene protons (δ 4.94-4.91, 2H, m, H-10) and a methine proton attached to an oxygen bearing carbon (δ 4.35, 1H, m, H-6). The absence of H-3 doublet (δ 7.11), characteristic of a trisubstituted olefinic proton of a mussaenosidic acid nucleus[5,6] as evident in VA-12 and VA-14 and the presence of two olefinic double doublets indicated that C-4 is unsubtituted in VA-13. These spectral data indicated the presence of aucubin skeleton[16] in VA-13.

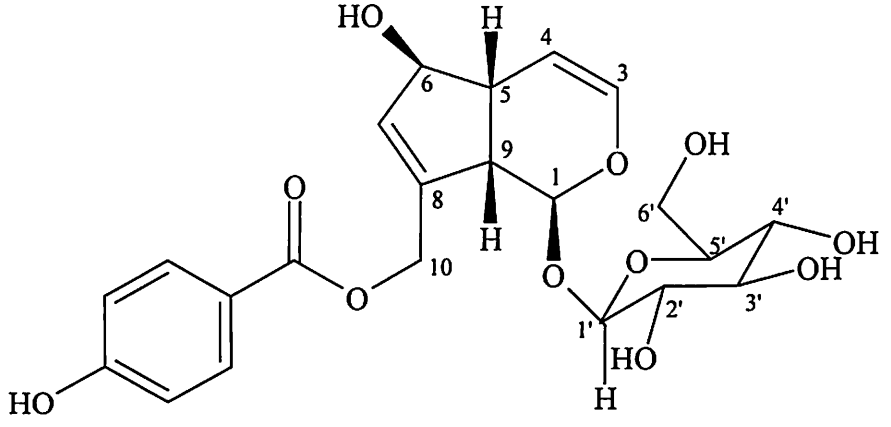
The $^1H$ NMR spectrum showed a series of signals between δ 2.98 and 4.51 assignable to a sugar moiety.[2] The $^1H$ NMR spectrum also contained two AB proton doublets at δ 7.84 (2H, d, *J* = 8.4 Hz) and 6.85 (2H, d, *J* = 8.4 Hz) attributable to a *p*-hydroxybenzoyl moiety.[4]

The $^{13}C$ NMR (Fig. 3.38, Table. 3.27) spectrum contained an ester carbonyl carbon (δ 165.9), three quaternary carbons (δ 162.7, 141.0 and 120.9), two oxymethylene carbons (δ 62.7 and 61.8) and a trisubstituted olefinic methine carbon (δ 132.9). These assignments

were supported by the DEPT (Fig. 3.39) spectral data and the correlations observed in the HMQC (Fig. 3.40, Table 3.28) spectrum.

The absence of a singlet methyl (δ 24.6) in comparison with VA-12 and VA-14 and the presence of a downfield (δ 62.7) oxymethylene carbon indicated the acylation of C-10 hydroxyl in VA-13. The $^{1}H$-$^{1}H$ COSY (Fig. 3.41, Table 3.29), and the HMBC (Fig. 3.42, Table 3.30) spectra confirmed further the aucubin skeleton[16] in VA-13.

A careful comparison of the above spectral data of **VA-13** with those of naturally occurring acylated aucubin derivatives revealed that the physical and spectral data of **VA-13** corroborate well with those recorded for agnuside (10-*O*-*p*-hydroxybenzoylaucubin, **3.08**), isolated earlier from *Vitex agnus-castus*,[17] *Vitex negundo*[7] and *Vitex leucoxylon*.[18]




**3.08**

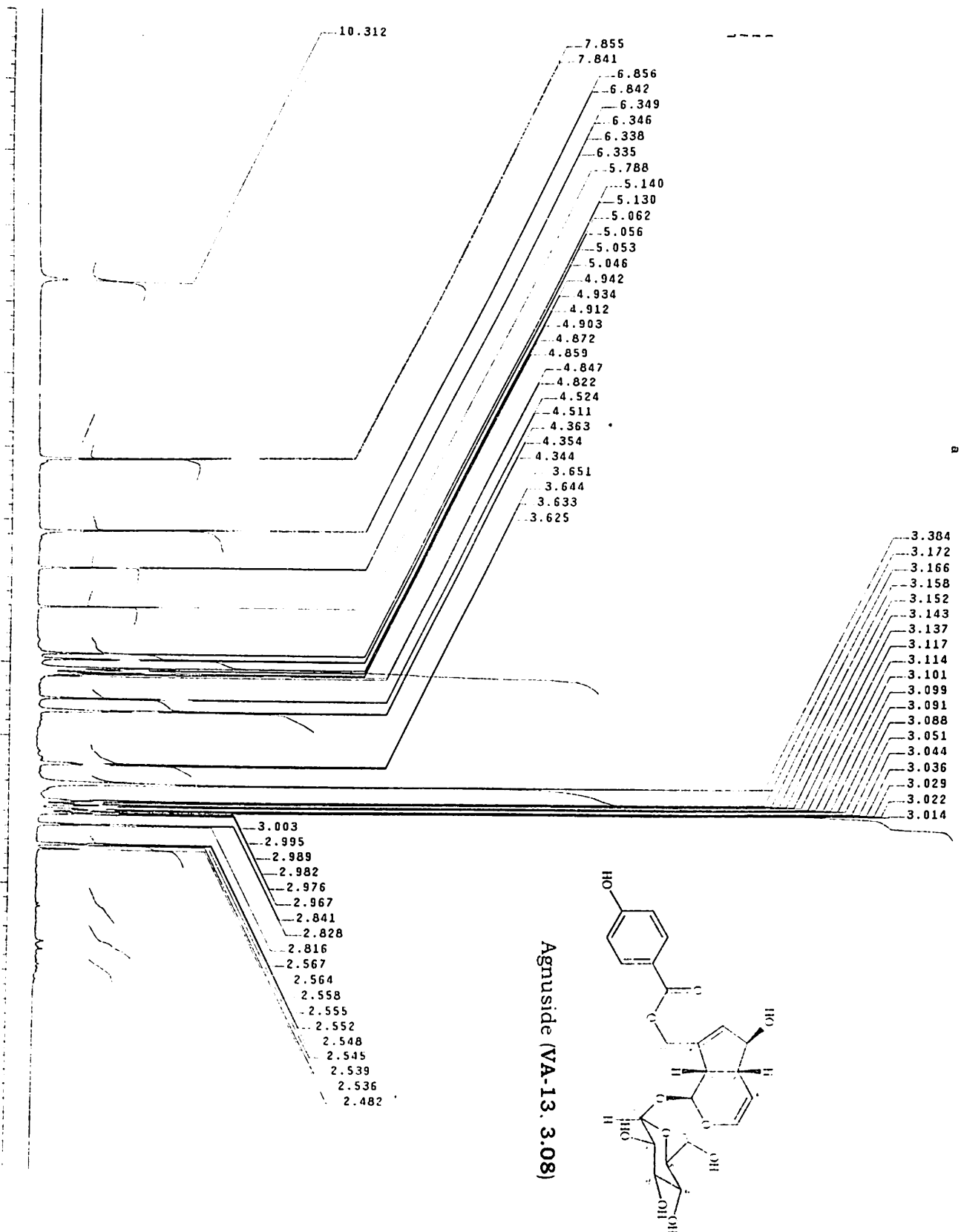



Fig. 3.37: $^1$H NMR spectrum (600 MHz, $d_6$-DMSO) of compound **VA-13 (3.08)**



Agnuside (**VA-13. 3.08**)

## TABLE 3.26

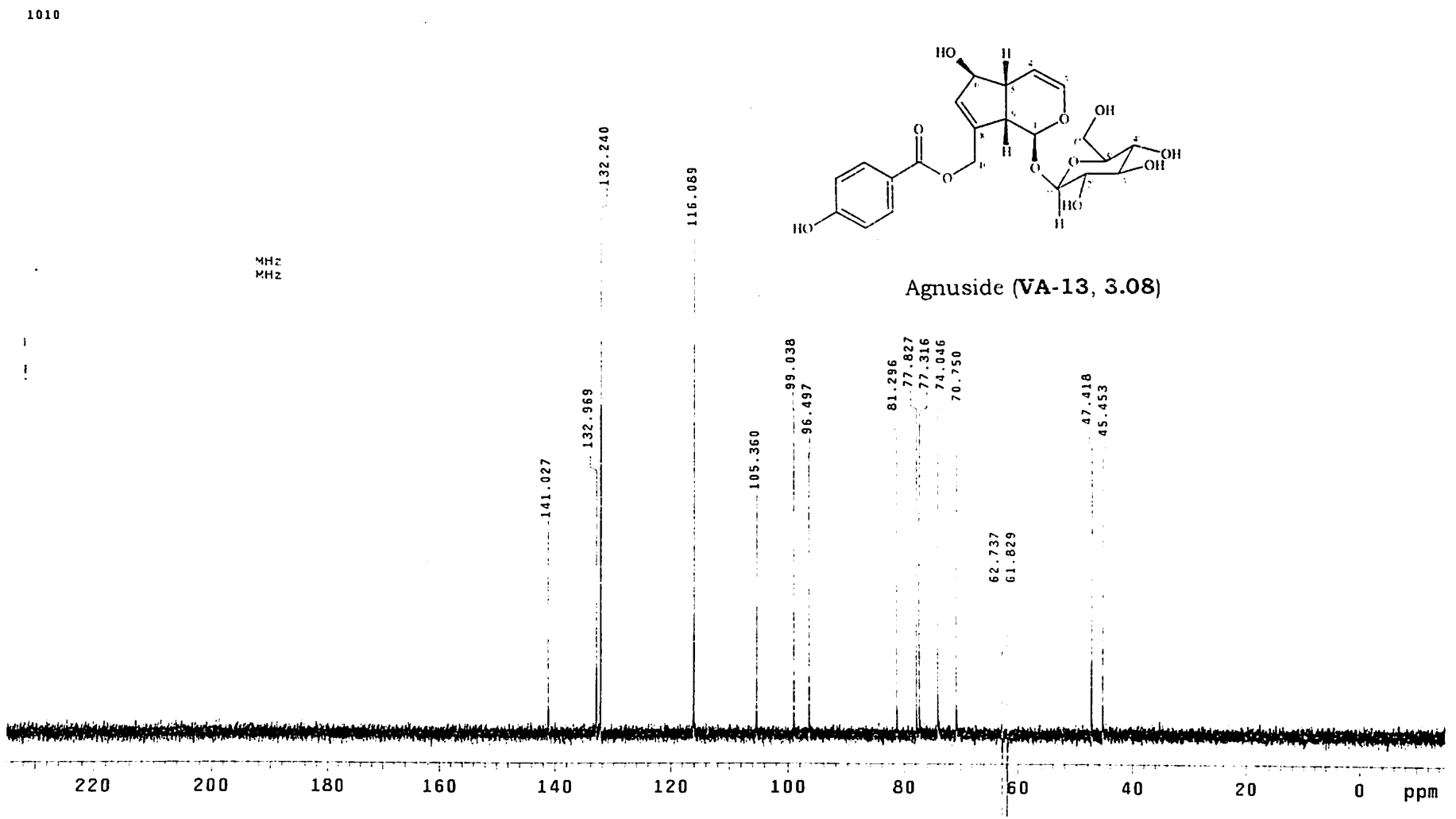
**$^1$H NMR spectral data of VA-13 (agnuside, 3.08)**

**(Fig. 3.37, 600 MHz spectrum, $d_6$-DMSO)**

| **Chemical shift (δ)** | **Proton integration** | **Multiplicity (*J* in Hz)** | **Assignment** |
|---|---|---|---|
| 4.87 | 1H | m | H-1 |
| 6.34 | 1H | dd (6.6,1.8) | H-3 |
| 5.05 | 1H | dd (6.6,4.2) | H-4 |
| 2.55 | 1H | m | H-5 |
| 4.35 | 1H | m | H-6 |
| 5.79 | 1H | s | H-7 |
| 2.83 | 1H | m | H-9 |
| 4.94-4.91 | 2H | m | H-10 |
| 4.51 | 1H | d (7.8) | H-1′ |
| 2.98 | 1H | m | H-2′ |
| 3.11 | 1H | m | H-3′ |
| 3.03 | 1H | m | H-4′ |
| 3.15 | 1H | m | H-5′ |
| 3.64 | 1H | d (4.8) | $H_a$-6′ |
| 3.63 | 1H | d (4.2) | $H_b$-6′ |
| 7.84 | 2H | d (8.4) | H-2″ and H-6″ |
| 6.85 | 2H | d (8.4) | H-3″ and H-5″ |

Agnuside (**VA-13, 3.08**)

Fig. 3.38: $^{13}C$ NMR spectrum (150 MHz, $d_6$-DMSO) of compound **VA-13 (3.08)**




Agnuside (**VA-13, 3.08**)

Fig. 3.39: DEPT spectrum ($d_6$-DMSO) of compound **VA-13 (3.08)**

## TABLE 3.27

**$^{13}$C NMR spectral data of VA-13 (agnuside, 3.08)**

**(Fig. 3.38, 125 MHz spectrum, $d_6$-DMSO)**

| Carbon number | Chemical shift (δ) | Carbon number | Chemical shift (δ) |
|---|---|---|---|
| C-1 | 96.5 | C-2′ | 74.0 |
| C-3 | 140.6 | C-3′ | 77.8 |
| C-4 | 105.4 | C-4′ | 70.7 |
| C-5 | 45.4 | C-5′ | 77.3 |
| C-6 | 81.3 | C-6′ | 61.8 |
| C-7 | 132.9 | C-1″ | 120.9 |
| C-8 | 141.0 | C-2″ and C-6″ | 132.9 |
| C-9 | 47.4 | C-3″ and C-5″ | 116.1 |
| C-10 | 62.7 | C-4″ | 162.7 |
| C-1′ | 99.0 | C-7″ | 165.9 |

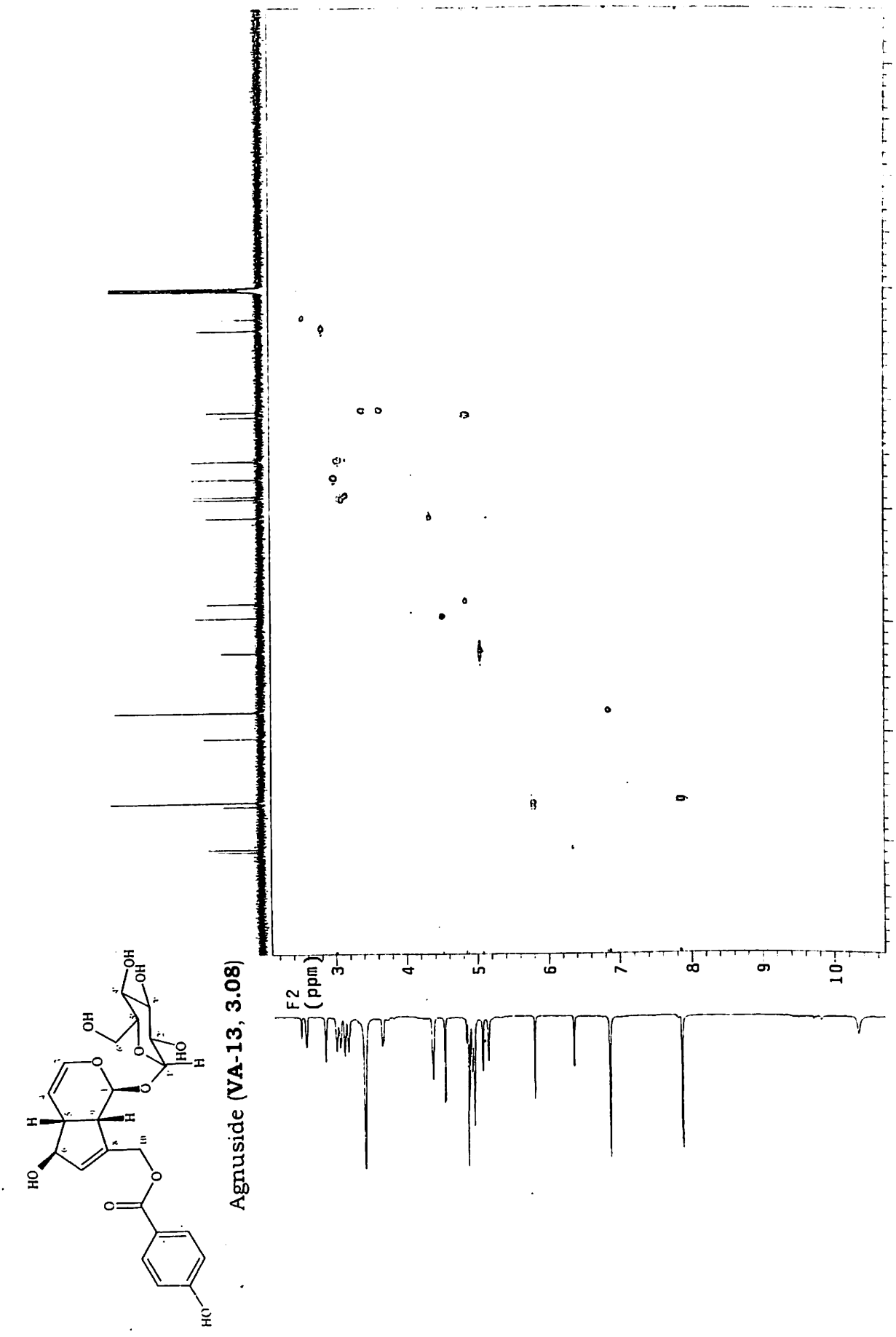


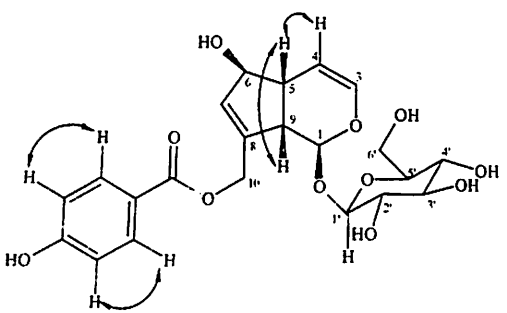

Fig. 3.40: HMQC spectrum (600 MHz, $d_6$-DMSO) of compound **VA-13 (3.08)**

## TABLE 3.28

**HMQC spectral data of VA-13 (agnuside, 3.08)**

**(Fig. 3.40, solvent: $d_6$-DMSO)**

| **Proton chemical shift (δ)** | **Correlated carbon chemical shift (δ)** | **Assignment** |
|---|---|---|
| 4.87 (H-1) | 96.5 | C-1 |
| 6.34 (H-3) | 140.6 | C-3 |
| 5.05 (H-4) | 105.4 | C-4 |
| 2.55 (H-5) | 45.4 | C-5 |
| 4.35 (H-6) | 81.3 | C-6 |
| 5.79 (H-7) | 132.9 | C-7 |
| 2.83 (H-9) | 47.4 | C-9 |
| 4.94-4.91 (H-10) | 62.7 | C-10 |
| 4.51 (H-1′) | 99.0 | C-1′ |
| 2.98 (H-2′) | 74.0 | C-2′ |
| 3.11 (H-3′) | 77.8 | C-3′ |
| 3.03 (H-4′) | 70.7 | C-4′ |
| 3.15 (H-5′) | 77.3 | C-5′ |
| 3.63,3.64 (H-6′) | 61.8 | C-6′ |
| 7.84 (H-2″ and H-6″) | 132.9 | C-2″ and C-6″ |
| 6.85 (H-3″ and H-5″) | 116.1 | C-3″ and C-5″ |



Agnuside (**VA-13**, **3.08**)

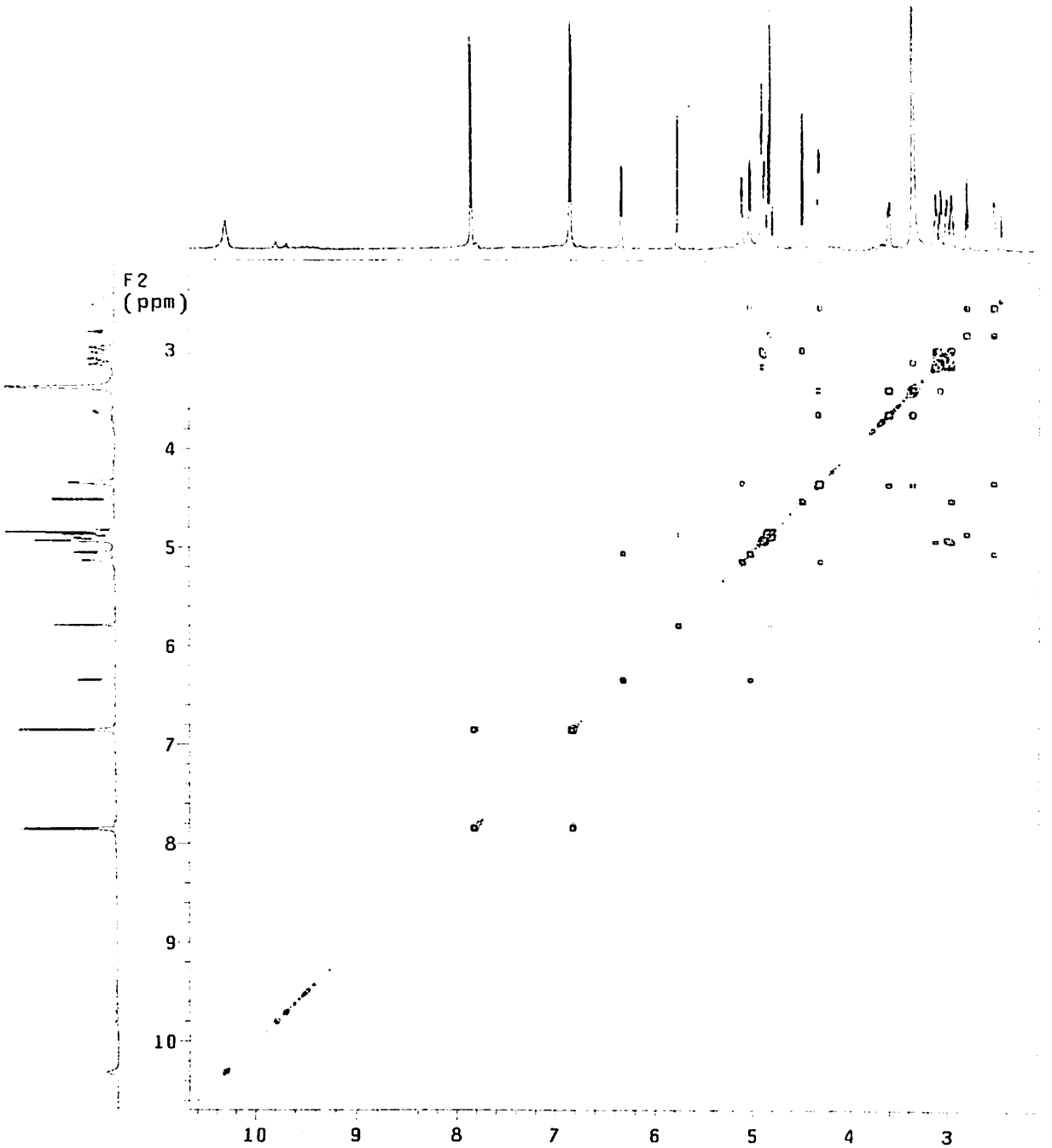



Fig. 3.41: $^{1}H$-$^{1}H$ COSY spectrum (600 MHz, $d_6$-DMSO) of compound **VA-13** (**3.08**)

## TABLE 3.29

**$^1$H-$^1$H COSY spectral data of VA-13 (agnuside, 3.08)**

**(Fig. 3.41, solvent: $d_6$-DMSO)**

| **Chemical shifts of coupled protons** | **Type of coupling** | **Assignment** |
|---|---|---|
| 2.55 and 2.83 | vicinal | H-5 and H-9 |
| 4.51and 2.98 | vicinal | H-1′and H-2′ |
| 5.05 and 2.55 | vicinal | H-4 and H-5 |
| 7.84 and 6.85 | ortho | H-2″ and H-3″ |

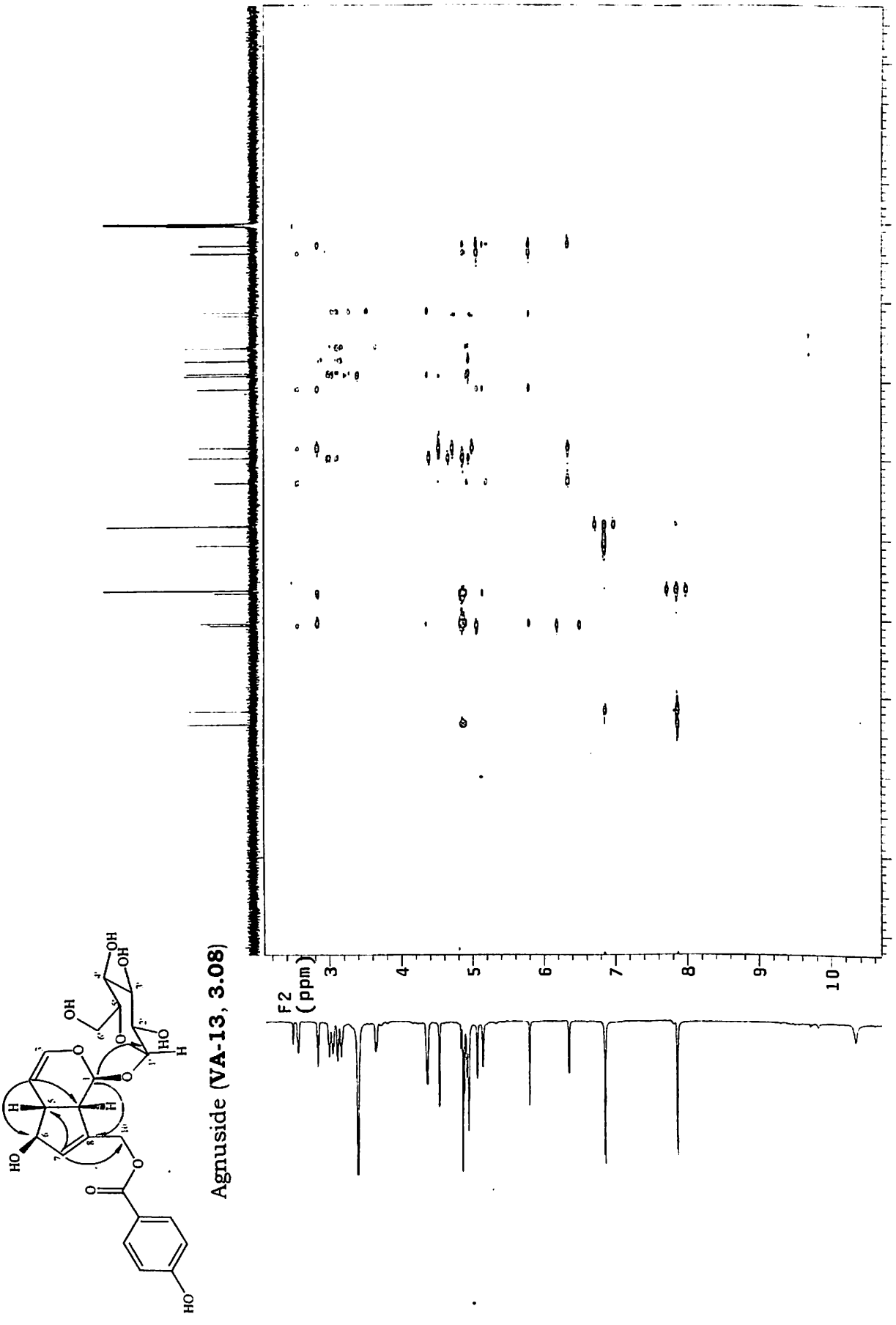
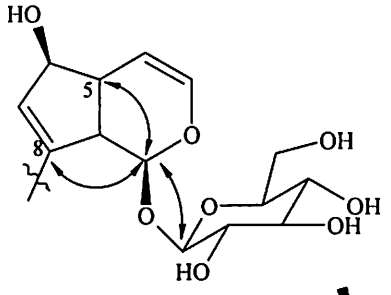
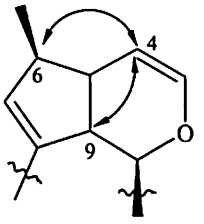
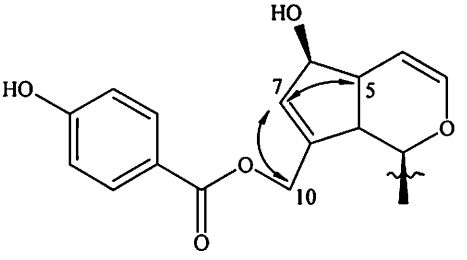



Fig. 3.42: HMBC spectrum (600 MHz, $d_6$-DMSO) of compound **VA-13 (3.08)**

## TABLE 3.30

**HMBC spectral data of VA-13 (agnuside, 3.08)**

**(Fig. 3.42, solvent: $d_6$-DMSO)**

| Chemical shift (δ) | Correlated carbon | Structural units derived |
|---|---|---|
| 4.87 (H-1) | C-1′, C-5, C-8 |  |
| 5.05 (H-4) | C-6, C-9 |  |
| 5.79 (H-7) | C-5, C-9, C-10 |  |

## EXPERIMENTAL

**Negundoside (VA-16, 3.01, 2.0 g)**

It was obtained as white crystalline solid from methanol, m.p. 161-163 °C.

| | |
|---|---|
| $[\alpha]_D^{25}$ | : −125° (*c* 0.5, MeOH) |
| Found | : C, 55.62%; H, 5.67% |
| $C_{23}H_{28}O_{12}$ requires | : C, 55.64%; H, 5.64% |
| UV (MeOH) | : $\lambda_{max}$ 255 nm |
| IR (KBr) | : $\nu_{max}$ 3331, 1717, 1681, 1642, 1602, 1508, $cm^1$ |
| $^1H$ NMR | : Fig. 3.01, Table 3.01 |
| $^{13}C$ NMR | : Fig. 3.02, Table 3.02 |
| DEPT | : Fig. 3.03 |

**6′-*O*-*trans*-feruloylnegundoside (VA-12, 3.02, 0.92 g)**

It was obtained as pale-yellow amorphous powder, m.p. 155-156 °C

| | |
|---|---|
| $[\alpha]_D^{25}$ | : −74.6° (*c* 0.5, MeOH) |
| Found | : C, 58.90%; H, 5.26% |
| $C_{33}H_{36}O_{15}$ requires | : C, 58.92%; H, 5.35% |
| UV (MeOH) | : $\lambda_{max}$ 247 and 327 nm |
| IR (KBr) | : $\nu_{max}$ 3415, 1698, 1633, 1601, 1515, 1452, 1271, 1168, 980 and 850 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 3.04, Table 3.03 |
| $^{13}C$ NMR | : Fig. 3.05, Table 3.04 |
| DEPT | : Fig. 3.06 |
| HMQC | : Fig. 3.07, Table 3.05 |
| HMBC | : Fig. 3.08, Table 3.06 |

**6′-*O*-*trans*-caffeoylnegundoside (VA-14, 3.03, 2.0 g)**

It was obtained as pale-yellow amorphous powder, m.p. 168-169 °C

| | |
|---|---|
| $[\alpha]_D^{25}$ | : −109.3° (*c* 0.5, MeOH) |
| Found | : C, 58.31%; H, 5.11% |
| $C_{32}H_{34}O_{15}$ requires | : C, 58.35%; H, 5.16% |
| UV (MeOH) | : $\lambda_{max}$ 249 and 331 nm |
| IR (KBr) | : $\nu_{max}$ 3398, 1695, 1633, 1604, 1517 and 1448 1272, 1168, 1117, 980 and 852 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 3.10, Table 3.07 |
| $^{13}C$ NMR | : Fig. 3.11, Table 3.08 |
| DEPT | : Fig. 3.12 |
| HMQC | : Fig. 3.13, Table 3.09 |
| HMBC | : Fig. 3.14, Table 3.10 |

**2′-*O*-*p*-hydroxybenzoylgardoside (VA-21, 3.04, 13 mg)**

It was obtained as colorless amorphous powder, m.p. 215-216 °C

| | |
|---|---|
| $[\alpha]_D^{25}$ | : −41.3° (*c* 0.5, MeOH) |
| Found | : C, 55.82%; H, 5.22% |
| $C_{23}H_{26}O_{12}$ requires | : C, 55.87%; H, 5.26% |
| UV (MeOH) | : $\lambda_{max}$ 255 nm |
| IR (KBr) | : $\nu_{max}$ 3368, 1703, 1636, 1603, 1512, 1271, 1170, 1079, 902 and 852 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 3.16, Table 3.11 |
| DEPT | : Fig. 3.17 |
| $^1H$-$^1H$ COSY | : Fig. 3.18, Table 3.12 |
| $^{13}C$ NMR | : Fig. 3.19, Table 3.13 |

**6′-*O*-*trans*-caffeoylnegundoside (VA-14, 3.03, 2.0 g)**

It was obtained as pale-yellow amorphous powder, m.p. 168-169 °C

| | |
|---|---|
| $[\alpha]_D^{25}$ | : −109.3° (*c* 0.5, MeOH) |
| Found | : C, 58.31%; H, 5.11% |
| $C_{32}H_{34}O_{15}$ requires | : C, 58.35%; H, 5.16% |
| UV (MeOH) | : $\lambda_{max}$ 249 and 331 nm |
| IR (KBr) | : $\nu_{max}$ 3398, 1695, 1633, 1604, 1517 and 1448 1272, 1168, 1117, 980 and 852 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 3.10, Table 3.07 |
| $^{13}C$ NMR | : Fig. 3.11, Table 3.08 |
| DEPT | : Fig. 3.12 |
| HMQC | : Fig. 3.13, Table 3.09 |
| HMBC | : Fig. 3.14, Table 3.10 |

**2′-*O*-*p*-hydroxybenzoylgardoside (VA-21, 3.04, 13 mg)**

It was obtained as colorless amorphous powder, m.p. 215-216 °C

| | |
|---|---|
| $[\alpha]_D^{25}$ | : −41.3° (*c* 0.5, MeOH) |
| Found | : C, 55.82%; H, 5.22% |
| $C_{23}H_{26}O_{12}$ requires | : C, 55.87%; H, 5.26% |
| UV (MeOH) | : $\lambda_{max}$ 255 nm |
| IR (KBr) | : $\nu_{max}$ 3368, 1703, 1636, 1603, 1512, 1271, 1170, 1079, 902 and 852 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 3.16, Table 3.11 |
| DEPT | : Fig. 3.17 |
| $^1H$-$^1H$ COSY | : Fig. 3.18, Table 3.12 |
| $^{13}C$ NMR | : Fig. 3.19, Table 3.13 |

**2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoylgardoside (VA-17, 3.05, 45 mg)**

It was obtained as pale-yellow amorphous powder, m.p. 191-192 °C

| | |
|---|---|
| $[\alpha]_D^{25}$ | : −32.6° (*c* 0.5, MeOH) |
| Found | : C, 58.50%; H, 4.85% |
| $C_{32}H_{32}O_{15}$ requires | : C, 58.53%; H, 4.87% |
| UV (MeOH) | : $\lambda_{max}$ 250 and 330 nm |
| IR (KBr) | : $\nu_{max}$ 3406, 1698, 1633, 1604, 1516 and 1449 1272, 1168, 1113, 985, 852 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 3.20, Table 3.14 |
| $^{13}C$ NMR | : Fig. 3.21, Table 3.15 |
| DEPT | : Fig. 3.22 |
| HMQC | : Fig. 3.23, Table 3.16 |
| HMBC | : Fig. 3.24, Table 3.17 |

**2′-*O*-*p*-hydroxybenzoyl-8-epiloganic acid (VA-22, 3.06, 20 mg)**

It was obtained as colorless amorphous powder, m.p. 219-220 °C

| | |
|---|---|
| $[\alpha]_D^{25}$ | : −119.9° (*c* 0.75, MeOH) |
| Found | : C, 55.61%; H, 5.62% |
| $C_{23}H_{28}O_{12}$ requires | : C, 55.64%; H, 5.64% |
| UV (MeOH) | : $\lambda_{max}$ 255 nm |
| IR (KBr) | : $\nu_{max}$ 3405, 1703, 1648, 1609, 1516, 1275, 1171, 1074, 904 and 856 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 3.26, Table 3.18 |
| $^1H$-$^1H$ COSY | : Fig. 3.27, Table 3.19 |
| $^{13}C$ NMR | : Fig. 3.28, Table 3.20 |
| DEPT | : Fig. 3.29 |

**2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoyl-8-epiloganic acid (VA-18, 3.07, 95 mg)**

It was obtained as pale-yellow amorphous powder, m.p. 158-160 °C

| | |
|---|---|
| $[\alpha]_D^{25}$ | : −88.6° (*c* 0.5, MeOH) |
| Found | : C, 58.30%; H, 5.13% |
| $C_{32}H_{34}O_{15}$ requires | : C, 58.35%; H, 5.16% |
| UV (MeOH) | : $\lambda_{max}$ 249 and 330 nm |
| IR (KBr) | : $\nu_{max}$ 3400, 1698, 1604, 1517, 1449, 1272, 1168, 1113, 985 and 852 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 3.30, Table 3.21 |
| $^1H$-$^1H$ COSY | : Fig. 3.31, Table 3.22 |
| $^{13}C$ NMR | : Fig. 3.32, Table 3.23 |
| DEPT | : Fig. 3.33 |
| HMQC | : Fig. 3.34, Table 3.24 |
| HMBC | : Fig. 3.35, Table 3.25 |

**Agnuside (VA-13, 3.08, 118 mg)**

It was obtained as pale-brown powder, m.p. 151-153 °C.

| | |
|---|---|
| Found | : C, 59.22%; H, 5.86% |
| $C_{22}H_{26}O_{11}$ requires | : C, 59.19%; H, 5.82% |
| UV (MeOH) | : $\lambda_{max}$ 258 nm |
| IR (KBr) | : $\nu_{max}$ 3400, 1703, 1640 and 1590 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 3.37, Table 3.26 |
| $^{13}C$ NMR | : Fig. 3.38, Table 3.27 |
| DEPT | : Fig. 3.39 |
| HMQC | : Fig. 3.40, Table 3.28 |
| $^1H$-$^1H$ COSY | : Fig. 3.41, Table 3.29 |
| HMBC | : Fig. 3.42, Table 3.30 |

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# STRUCTURE ELUCIDATION OF ALTISSINONE, A NEW LIGNAN FROM *V. ALTISSIMA*

The details of isolation of altissinone (**VA-05**), a lignan have been described in **chapter-2**. Structure elucidation of this lignan, belonging to the tetrahydrofuranoid group has been presented in the following pages.

## VA-05

It was obtained as pale green flakes from hexane-acetone, m.p. 151-152 °C, $[\alpha]_D^{25}$ –40.3° (*c* 0.5, $CHCl_3$). The molecular formula, $C_{21}H_{20}O_8$, was deduced from elemental analysis and LC-MS [*m/z* 423 $(M+Na)^+$] data.

The UV ($CHCl_3$) spectrum of VA-05 exhibited absorption maxima at 226 nm. Its IR (KBr) spectrum showed bands at $\nu_{max}$ 3483 (hydroxyl), 1654 (carbonyl), 1592 and 1485 (aromatic), and 1041 and 942 $cm^{-1}$ (methylenedioxy group). The $^1H$ NMR (Fig. 4.01, Table 4.01) spectrum showed the presence of a methine proton at δ 4.57 (1H, d, *J* = 9.0 Hz), characteristic of H-2 of 2-substitutedtetrahydrofuran moiety,[1] two methine protons (δ 2.85, 1H, m, H-3 and δ 4.03, 1H, m, H-4), and two methylene groups at δ 3.63 (1H, dd, *J* = 11.0, 6.5 Hz, $H_a$-3a) and δ 3.73 (1H, dd, *J* = 11.0, 5.0 Hz, $H_b$-3a) and [δ 4.17 (1H, dd, *J* = 9.0, 6.0 Hz, $H_a$-5 and δ 4.22 (1H, t, *J* = 9.0 Hz, $H_b$-5). These spectral data supported the presence of a tetrahydrofuranoid skeleton[2] in VA-05. The $^1H$ NMR spectrum also showed the presence of a piperonyl unit[3] constituted by a 1,2,4-trisubstitutedphenyl moiety [δ 6.77 (1H, d, *J* = 8.0 Hz), 6.85 (1H, dd, *J* = 8.0, 1.5 Hz) and 6.97 (1H, d, *J* = 1.5 Hz)] and a methylenedioxy group at δ 5.95 (2H, s).

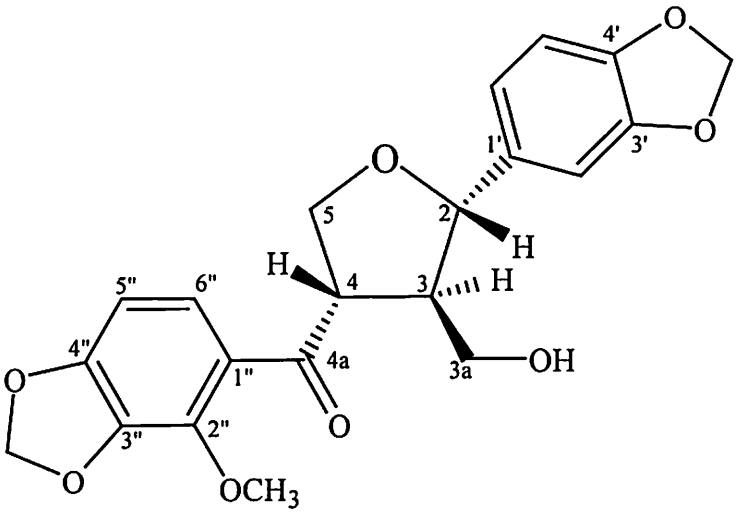
In addition, the $^1H$ NMR spectrum contained a methoxyl group (δ 4.11, 3H, s), a methylenedioxy group (2H, δ 6.03, s) and two aromatic AB protons at δ 6.59 (1H, d, *J* = 8.5 Hz, H-5″) and 7.27 (1H, d, *J* = 8.5 Hz, H-6″), consistent with the presence of a 2-methoxy-3,4-methylenedioxyphenyl unit.[4]

The $^{13}C$ NMR (Fig. 4.02, Table 4.02) spectrum showed the presence of a ketone carbonyl ($\delta_C$ 200.3), two oxymethylene carbons ($\delta_C$ 70.8, C-5 and $\delta_C$ 62.2, C-3a), an oxymethine carbon ($\delta_C$ 83.9), a methoxyl group ($\delta_C$ 60.1) and two methylenedioxy carbons ($\delta_C$ 101.0 and

101.8). These assignments were supported by the DEPT (Fig. 4.03) spectrum and correlations observed in the HMQC (Fig. 4.04, Table 4.03) spectrum. From the above data, the gross structure of VA-05 was deduced as a 2,3,4-trisubstitutedtetrahydrofuranoid lignan having a piperonyl, hydroxymethyl and 2-methoxy-3,4-methylenedioxybenzoyl units.

The position of these units on tetrahydrofuranoid skeleton was determined by the correlations observed in the HMBC spectrum. In the HMBC (Fig. 4.05, Table 4.04) spectrum, H-6″, H-4, and H-5 have shown correlation with the ketone (δ 200.3), this supported the presence of ketone at C-4a. Further, the H-3a, H-5 and H-6′ protons showed correlations with C-2 (δ 83.9). Thus, the structure of the lignan could be derived as 2-(3′,4′-methylenedioxyphenyl)-3-hydroxymethyl-4-(2″-methoxy-3″,4″-methylenedioxybenzoyl) tetrahydrofuran (**4.01**). The configuration of **4.01** at C-2, C-3 and C-4 asymmetric centers was assumed to be identical with that of (−)-sesaminone,[5,6] based on the similar chemical shifts and same sign of optical rotation observed. The *trans*-orientation of C-2 aryl and C-3 hydroxymethyl groups was supported further by the observed chemical shift (δ 4.57, d) and coupling constant ($J$ = 9.0 Hz) of H-2 proton.[7]

Based on the foregoing, the structure of VA-05, a new tetrahydrofuranoid lignan, named altissinone, was established as (–)-2-(3′,4′-methylenedioxyphenyl)-3-hydroxymethyl-4-(2″-methoxy-3″,4″-methylenedioxybenzoyl) tetrahydrofuran (**4.01**). Compound **4.01** could have formed biogenetically through an oxidative cleavage of previously known 2-methoxysesamin.[4]




**4.01**

In view of the isolation of a new 2,3,4-trisubstituted tetrahydrofuranoid lignan, altissinone (**4.01**), the author considered appropriate to refer to other naturally occurring 2,3,4-trisubstituted tetrahydrofuranoid lignans and the details are appended at the end of this chapter (Table 4.05).

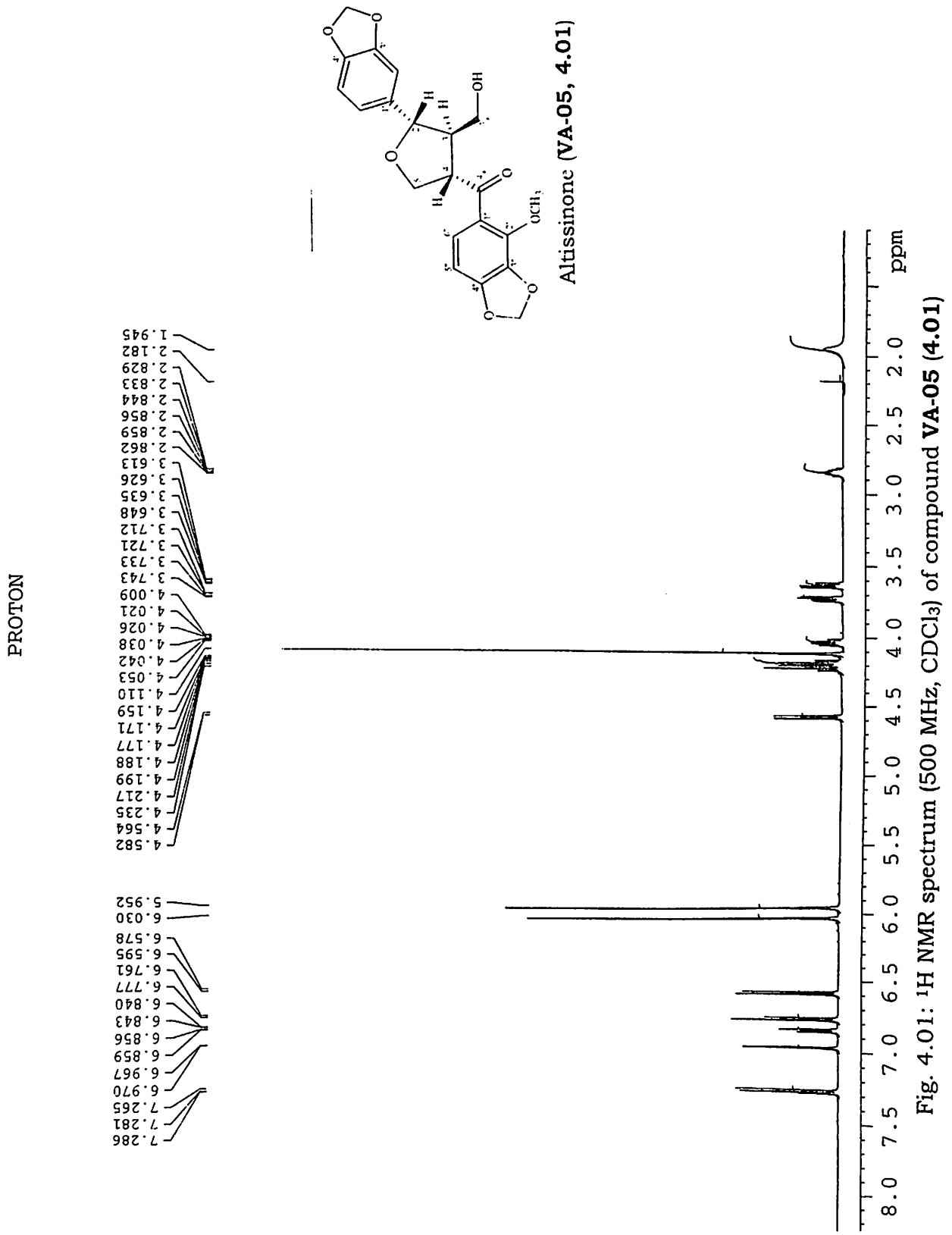



Fig. 4.01: $^1$H NMR spectrum (500 MHz, $CDCl_3$) of compound **VA-05 (4.01)**

## TABLE 4.01

**$^1$H NMR spectral data of VA-05 (Altissinone, 4.01)**

**(Fig. 4.01, 500 MHz spectrum, $CDCl_3$)**

| **Chemical shift (δ)** | **Proton integration** | **Multiplicity (*J* in Hz)** | **Assignment** |
|---|---|---|---|
| 4.57 | 1H | d (9.0) | H-2 |
| 2.85 | 1H | m | H-3 |
| 3.63 | 1H | dd (11.0, 6.5) | $H_a$-3a |
| 3.73 | 1H | dd (11.0, 5.0) | $H_b$-3a |
| 4.03 | 1H | m | H-4 |
| 4.17 | 1H | dd (9.0, 6.0) | $H_a$-5 |
| 4.22 | 1H | t (9.0) | $H_b$-5 |
| 6.97 | 1H | d (1.5) | H-2′ |
| 6.77 | 1H | d (8.0) | H-5′ |
| 6.85 | 1H | dd (8.0, 1.5) | H-6′ |
| 6.59 | 1H | d (8.5) | H-5″ |
| 7.27 | 1H | d (8.5) | H-6″ |
| 4.11 | 3H | s | $-OCH_3$ |
| 5.95 | 2H | s | $-OCH_2O-$ |
| 6.03 | 2H | s | $-OCH_2O-$ |

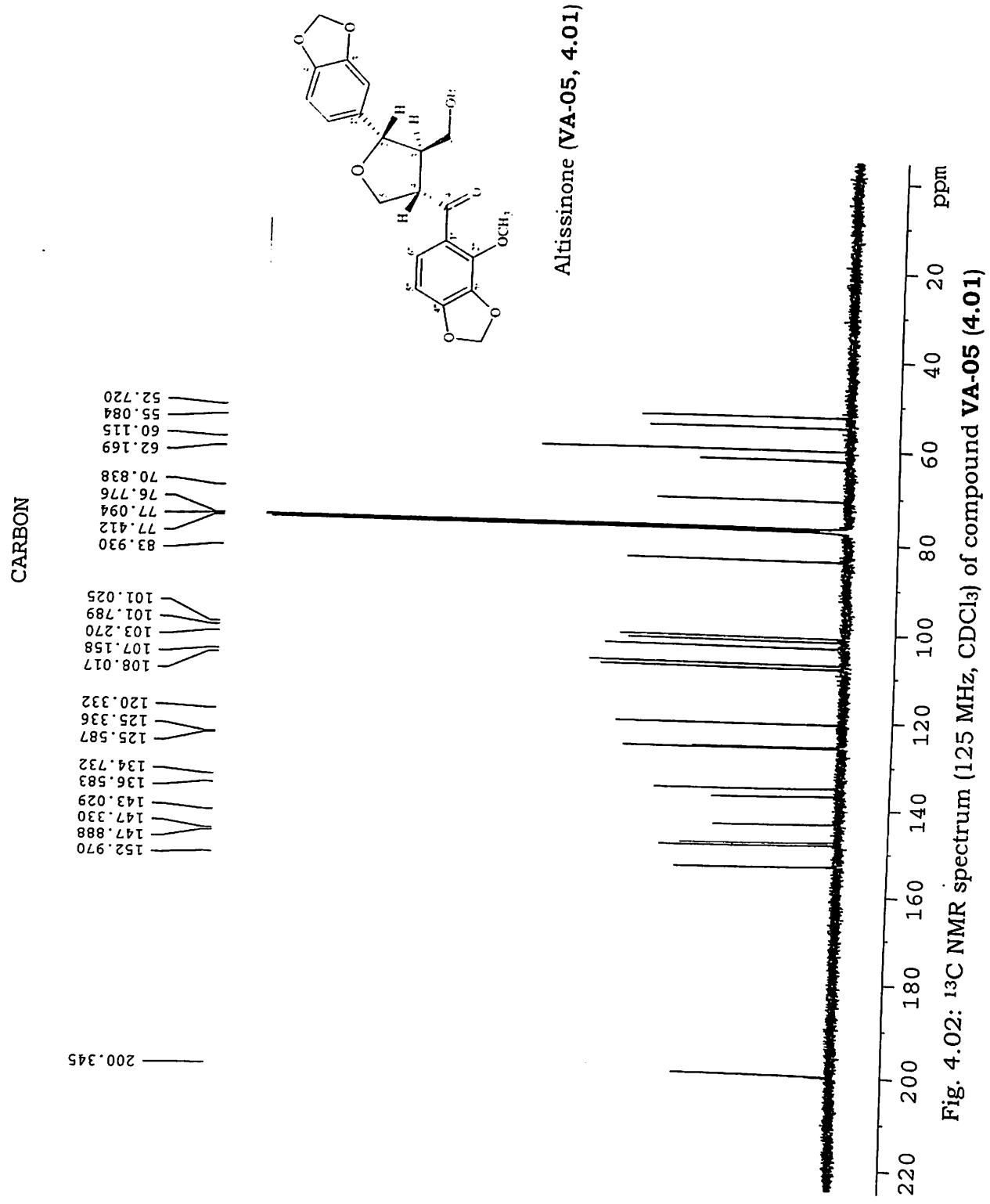
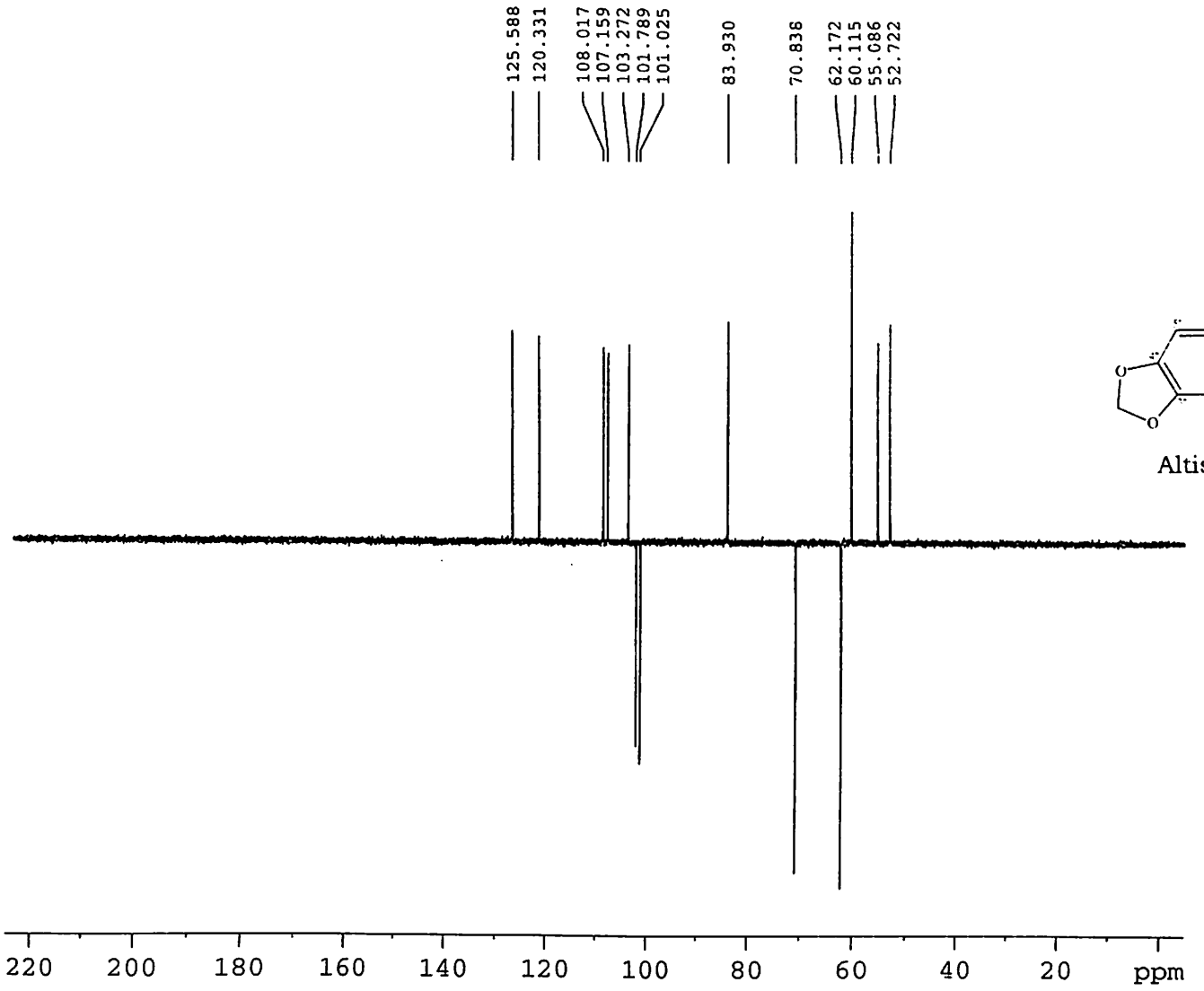
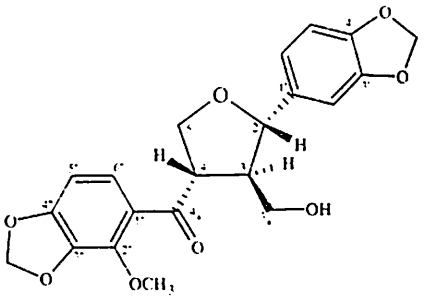



Fig. 4.02: $^{13}C$ NMR spectrum (125 MHz, $CDCl_3$) of compound **VA-05 (4.01)**




Altissinone (**VA-05, 4.01**)

Fig. 4.03: DEPT spectrum ($CDCl_3$) of compound **VA-05 (4.01)**

## TABLE 4.02

**$^{13}C$ NMR spectral data of VA-05 (Altissinone, 4.01)**

**(Fig. 4.02, 125 MHz spectrum, $CDCl_3$)**

| Chemical shift (δ) | DEPT | Assignment |
|---|---|---|
| 83.9 | CH | C-2 |
| 52.7 | CH | C-3 |
| 62.2 | $CH_2$ | C-3a |
| 55.1 | CH | C-4 |
| 200.3 | C | C-4a |
| 70.8 | $CH_2$ | C-5 |
| 134.7 | C | C-1′ |
| 107.2 | CH | C-2′ |
| 147.3 | C | C-3′ |
| 147.8 | C | C-4′ |
| 108.0 | CH | C-5′ |
| 120.3 | CH | C-6′ |
| 125.3 | C | C-1″ |
| 143.0 | C | C-2″ |
| 136.6 | C | C-3″ |
| 152.9 | C | C-4″ |
| 103.3 | CH | C-5″ |
| 125.6 | CH | C-6″ |
| 60.1 | $CH_3$ | $-OCH_3$ |
| 101.0 | $CH_2$ | $-OCH_2O-$ |
| 101.8 | $CH_2$ | $-OCH_2O-$ |

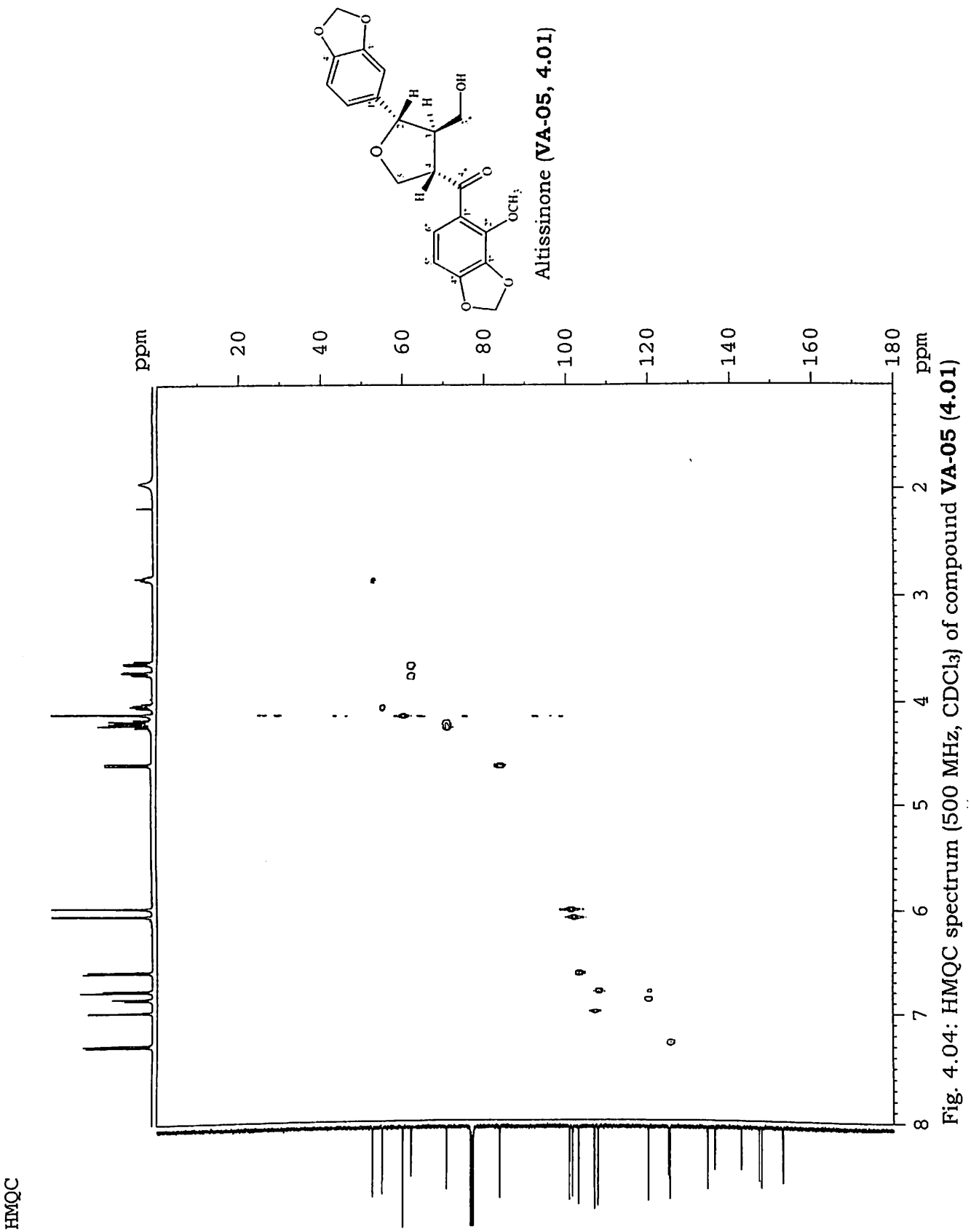



Fig. 4.04: HMQC spectrum (500 MHz, $CDCl_3$) of compound **VA-05 (4.01)**

## TABLE 4.03

**HMQC spectral data of VA-05 (Altissinone, 4.01)**

**(Fig. 4.04, solvent: $CDCl_3$)**

| **Proton chemical shift (δ)** | **Correlated carbon chemical shift (δ)** | **Assignment** |
|---|---|---|
| 4.57 (H-2) | 83.9 | C-2 |
| 2.85 (H-3) | 52.7 | C-3 |
| 3.63 and 3.73 ($H_a$-3a and $H_b$-3a) | 62.2 | C-3a |
| 4.03 (H-4) | 55.1 | C-4 |
| 4.17 and 4.22 ($H_a$-5 and $H_b$-5) | 70.8 | C-5 |
| 6.97 (H-2′) | 107.2 | C-2′ |
| 6.77 (H-5′) | 108.0 | C-5′ |
| 6.85 (H-6′) | 120.3 | C-6′ |
| 6.59 (H-5″) | 103.3 | C-5″ |
| 7.27 (H-6″) | 125.6 | C-6″ |
| 4.11 ($OCH_3$) | 60.1 | -$OCH_3$ |
| 5.95 (-$OCH_2O$-) | 101.0 | -$OCH_2O$- |
| 6.03 (-$OCH_2O$-) | 101.8 | -$OCH_2O$- |

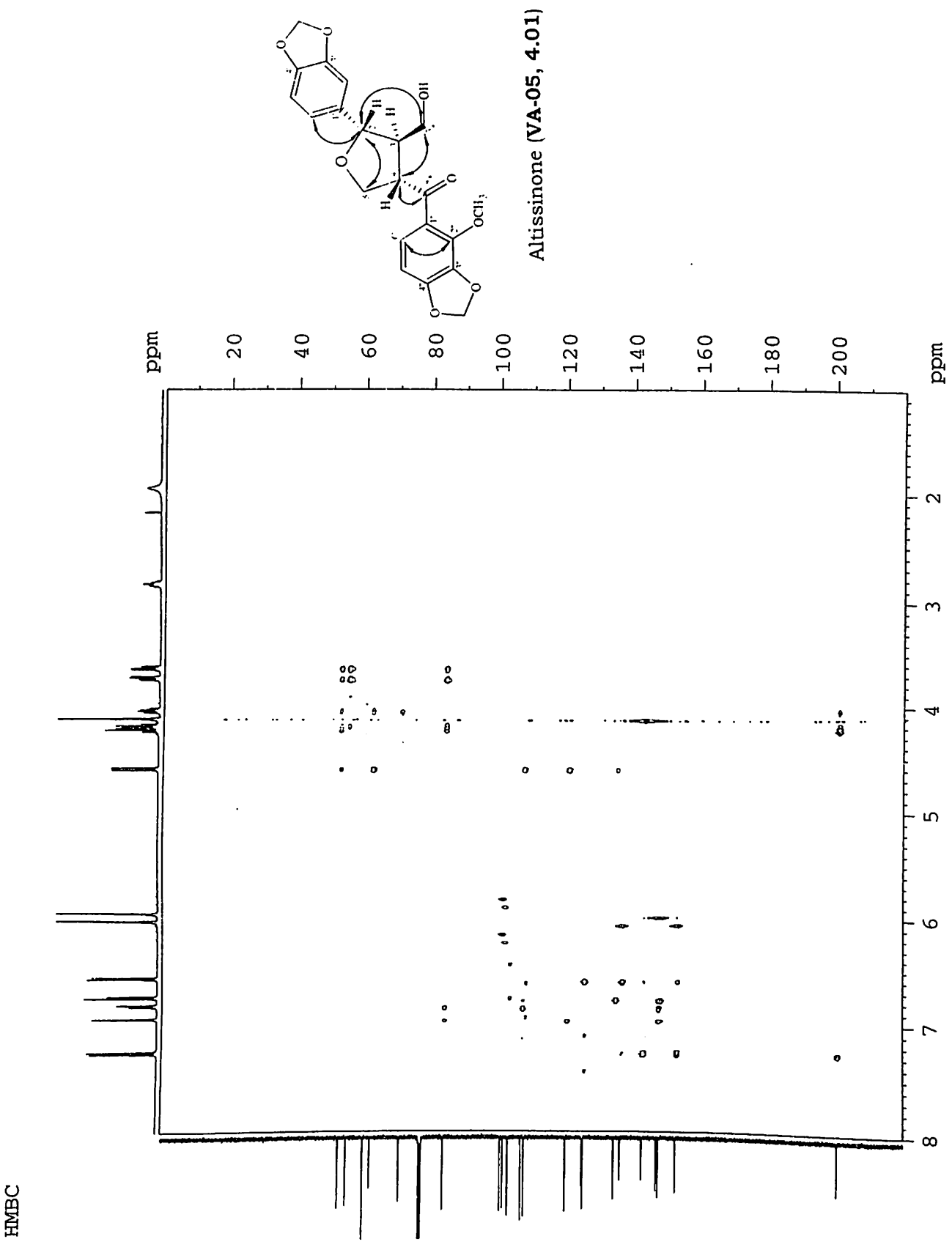
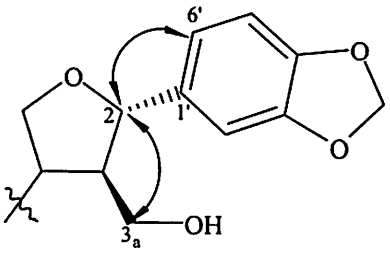
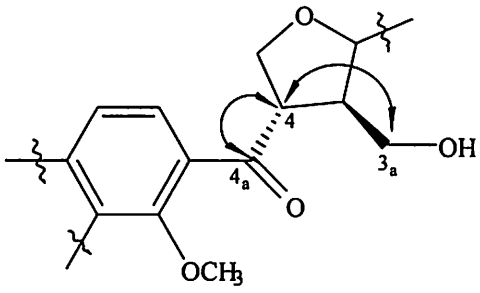
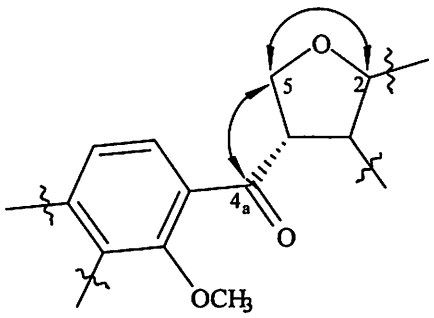
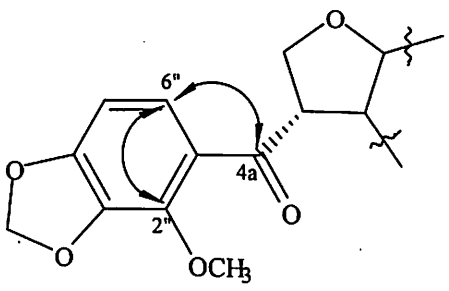



Fig. 4.05: HMBC spectrum (500 MHz, $CDCl_3$) of compound **VA-05 (4.01)**

## TABLE 4.04

**HMBC spectral data of VA-05 (Altissinone, 4.01)**

**(Fig. 4.05, solvent: $CDCl_3$)**

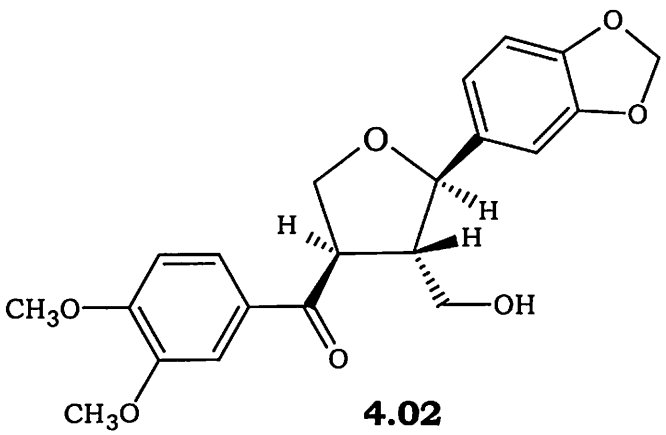
| Chemical shift (δ) | Correlated carbons | Structural units derived |
|---|---|---|
| 4.57 (H-2) | C-3a, C-6′ |  |
| 4.03 (H-4) | C-3a, C-4a |  |
| 4.22 (H-5) | C-2. C-4a |  |
| 7.27 (H-6″) | C-4a, C-2″ |  |

# TABLE 4.05

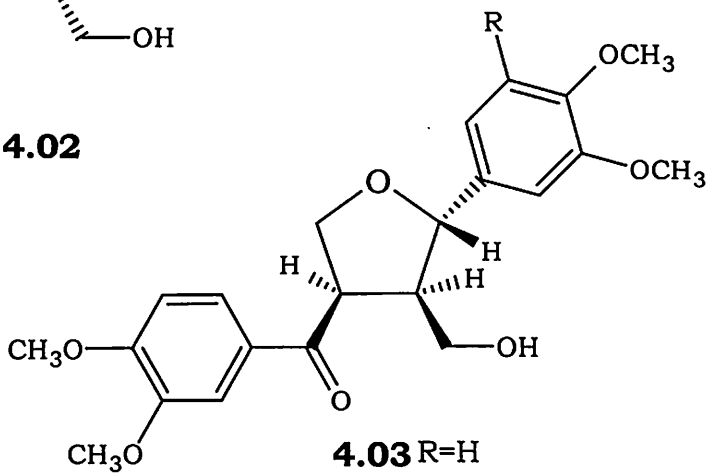
## Naturally occurring 2,3,4-trisubstituted tetrahydrofuranoid lignans

| S.No. | Name | Source | Reference |
|---|---|---|---|
| 1 | Magnolone (**4.02**) | *Magnolia coco* | 3 |
| 2 | Magnone-A (**4.03**) | *Magnolia fargesii* | 8 |
| 3 | Magnone-B (**4.04**) | *Magnolia fargesii* | 8 |
| 4 | Hernone (**4.05**) | *Hernandia nymphaefolia* | 6 |
| 5 | Nymphone (**4.06**) | *Hernandia nymphaefolia* | 6 |
| 6 | Sylvone (**4.07**) | *Piper sylvaticum* | 2 |
| 7 | Sesaminone (**4.08**) | *Streptomyces* sp. IT-44 | 4 |
| 8 | Episesaminone (**4.09**) | *Sesamum indicum* | 9 |
| 9 | Dihydrosesamin (**4.10**) | *Daphne tangutica* | 10 |
| 10 | Dihydrosesartemin (**4.11**) | *Virola elongata* | 11 |
| 11 | Dihydroyangambin (**4.12**) | *Virola elongata* | 11 |
| 12 | Lariciresinol (**4.13**) | *Justicia diffusa* | 12 |
| 13 | Lariciresinol-4-methyl ether (**4.14**) | *Araucaria angustifolia* | 13 |
| 14 | Acuminatin (**4.15**) | *Helichrysum acuminatum* | 14 |

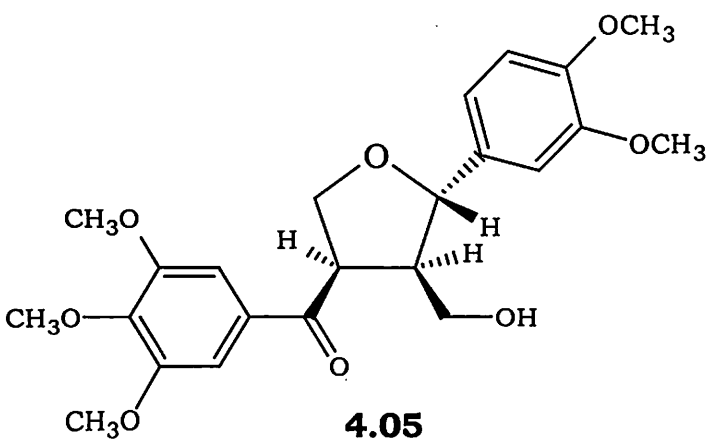
# Naturally occurring 2,3,4-trisubstituted tetrahydrofuranoid lignans



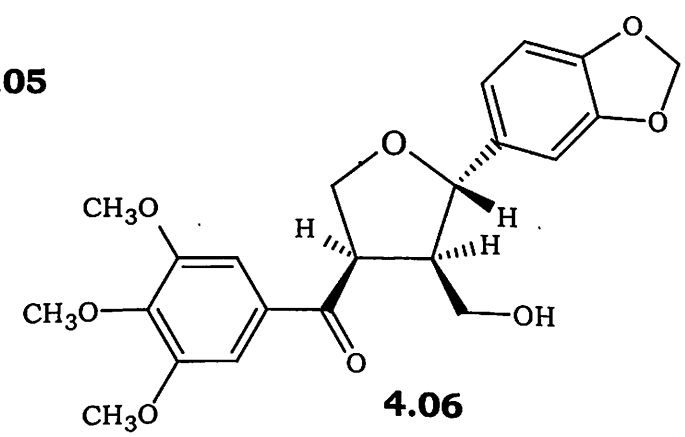
**4.02**



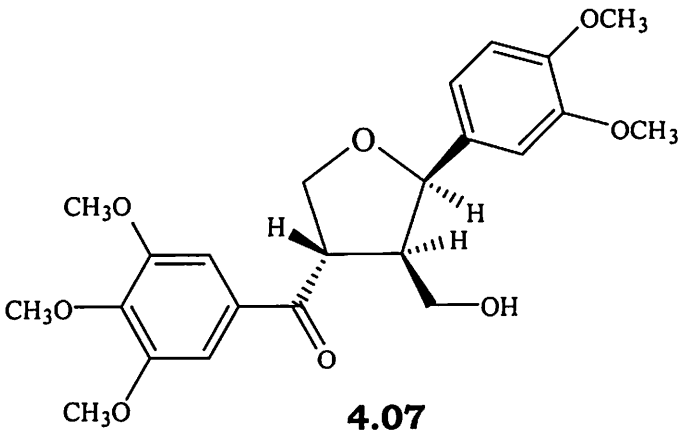
**4.03** R=H
**4.04** R=$OCH_3$



**4.05**



**4.06**



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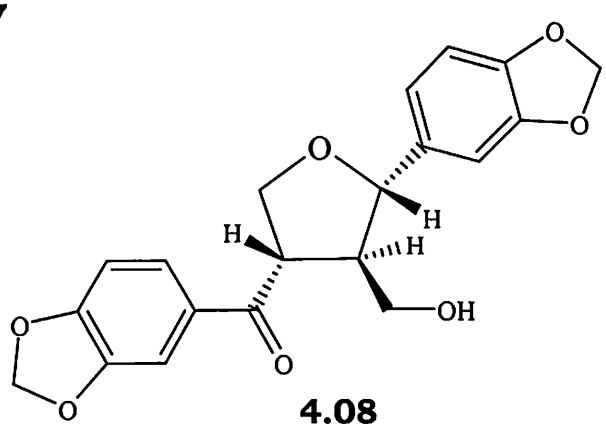
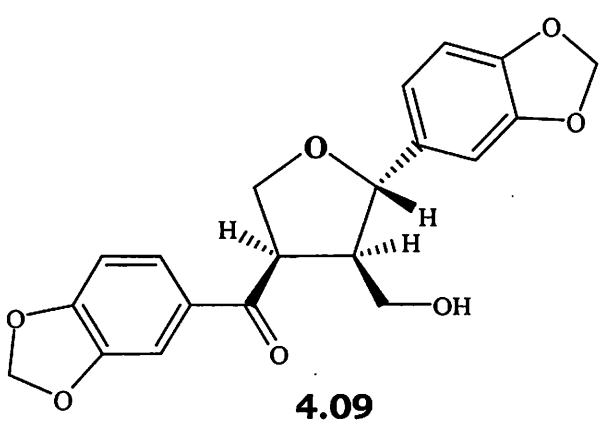


**4.08**



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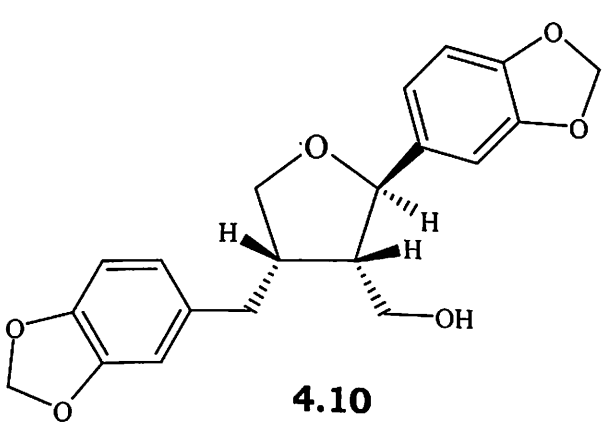

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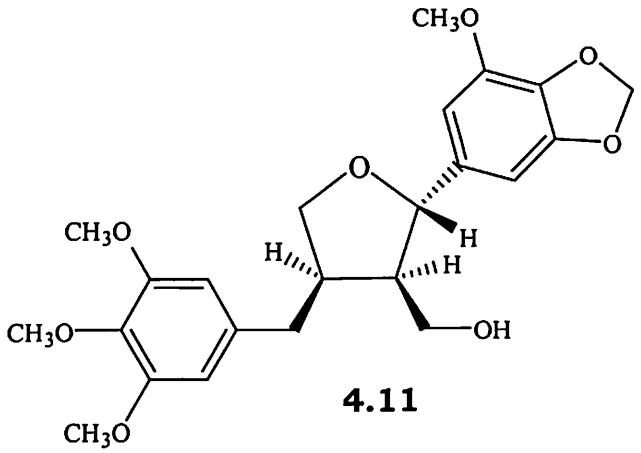

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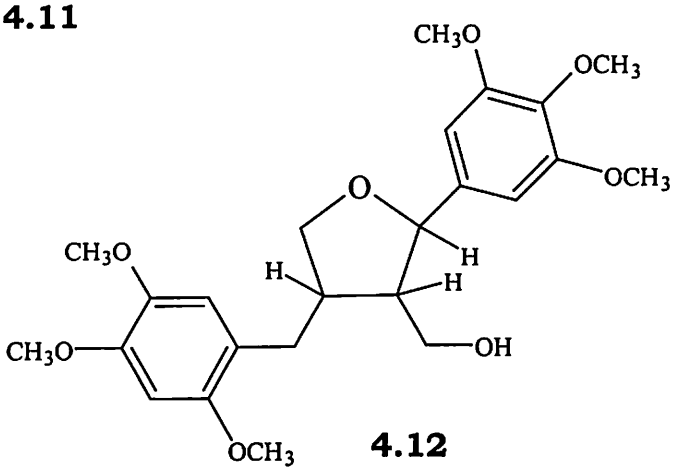
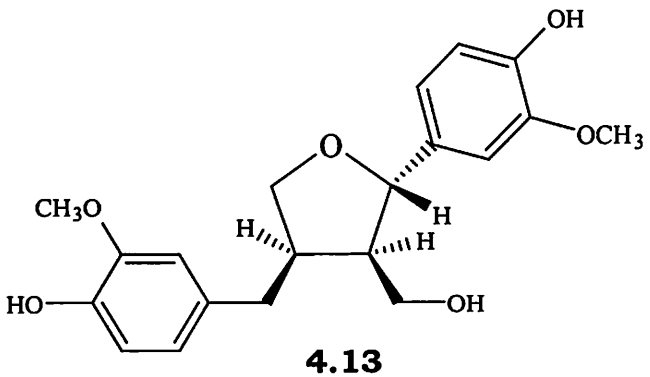
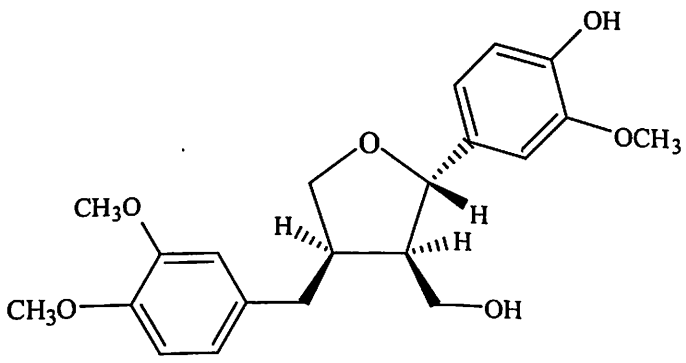


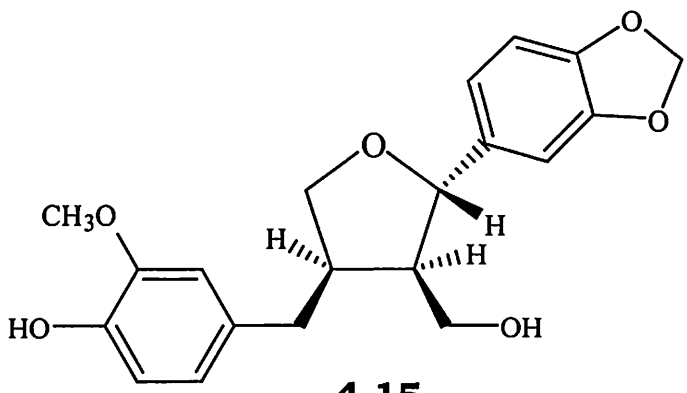
**4.12**



**4.13**



**4.14**




**4.15**

## EXPERIMENTAL

### Altissinone

It was obtained as pale-green flakes from hexane-acetone, m.p. 151-152 °C.

| | |
|---|---|
| $[\alpha]_D^{25}$ | : –40.3° (*c* 0.5, $CHCl_3$) |
| Found | : C, 62.99%; H, 5.36% |
| $C_{21}H_{20}O_8$ requires | : C, 63.00%; H, 5.00% |
| UV ($CHCl_3$) | : $\lambda_{max}$ 226 nm |
| IR (KBr) | : $\nu_{max}$ 3483, 1654, 1592, 1485, 1246 and 1041 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 4.01, Table 4.01 |
| $^{13}C$ NMR | : Fig. 4.02, Table 4.02 |
| DEPT | : Fig. 4.03 |
| HMQC | : Fig. 4.04, Table 4.03 |
| HMBC | : Fig. 4.05, Table 4.04 |

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# *Chapter-5*

## STRUCTURE ELUCIDATION OF TRITERPENOIDS ISOLATED FROM *V. ALTISSIMA*

The details of isolation of nine triterpenoids (**VA-01**-**VA-03 and VA-06**-**VA-11**) have been described in **Chapter-2**. Structure elucidation of these triterpenoids belonging to ursane (**VA-01**, **VA-07**, **VA-02**, **VA-06**, **VA-11**, **VA-09** and **VA-10**) and oleanane (**VA-03** and **VA-08**) series is presented in **section-I** and **section-II** respectively, in the following pages.

## SECTION-I
## (Ursane derivatives)

### VA-01

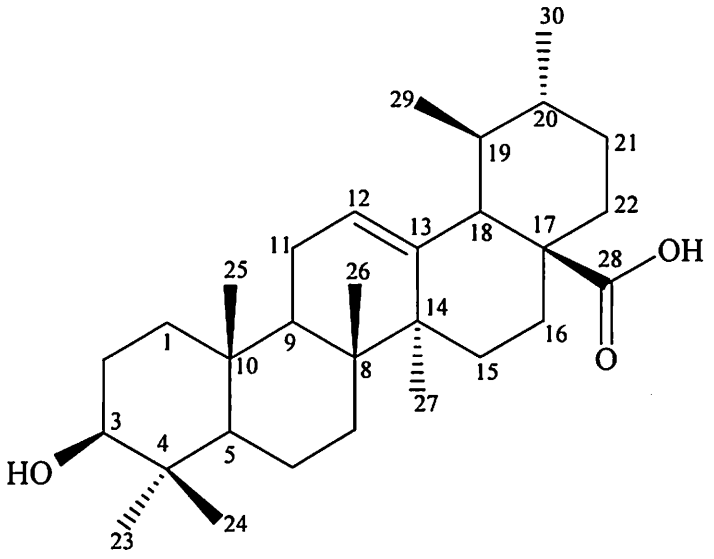
It was obtained as a white amorphous powder from methanol, m.p. 285-287 °C. Its molecular formula, $C_{30}H_{48}O_3$, was deduced based on elemental analysis and LC-MS [*m/z* 455 $(M - H)^-$] data. It showed positive LB test for triterpenoids.

The IR (KBr) spectrum of VA-01 showed bands at $\nu_{max}$ 3430 (hydroxyl) and 1691 $cm^{-1}$ (COOH). The $^1H$ NMR spectral (Fig. 5.01, Table 5.01) data showed the presence of a hydroxymethine proton (δ 3.44, 1H, dd, *J* = 9.0, 6.5 Hz, H-3), an olefinic methine proton (δ 5.48, 1H, br s, H-12) and a methine proton (δ 2.63, 1H, d, *J* = 11.2 Hz) attributable to H-18 of ursane drivatives.[1] In addition, the $^1H$ NMR spectrum showed the presence of five tertiary methyl groups (δ 1.24, 1.22, 1.04, 1.01 and 0.88, each 3H, s) and two secondary methyl groups (δ 0.99, 3H, d, *J* = 7.0 Hz, and δ 0.94, 3H, d, *J* = 6.5 Hz) characteristic of ursane skeleton.[1,2]

Literature survey on ursane derivatives revealed that the physical, spectral and optical rotation data of VA-01 were in good agreement with those recorded for ursolic acid (3β-hydroxyurs-12-en-28-oic acid, **5.01**), isolated earlier from *Salzmania nitida*.[3]

The identity of VA-01 as ursolic acid was ascertained further by direct comparison with authentic sample (co-HPTLC and mmp) kindly supplied by Laila Impex, R & D centre, Vijayawada.

Based on the foregoing, the structure of VA-01 was established as ursolic acid (**5.01**).




**5.01**

Like most triterpenoids, ursolic acid is ubiquitous in plant kingdom and is known to exhibit a broad range of biological activities.[4] Ursolic acid is a potent anti-inflammatory agent[5] and inhibits the lipoxygenase and cyclooxygenase enzymes.[6,7] Ursolic acid and its derivatives have been reported to possess anti-HIV activity.[8] It is also known to exhibit antitumor activity.[9,10]

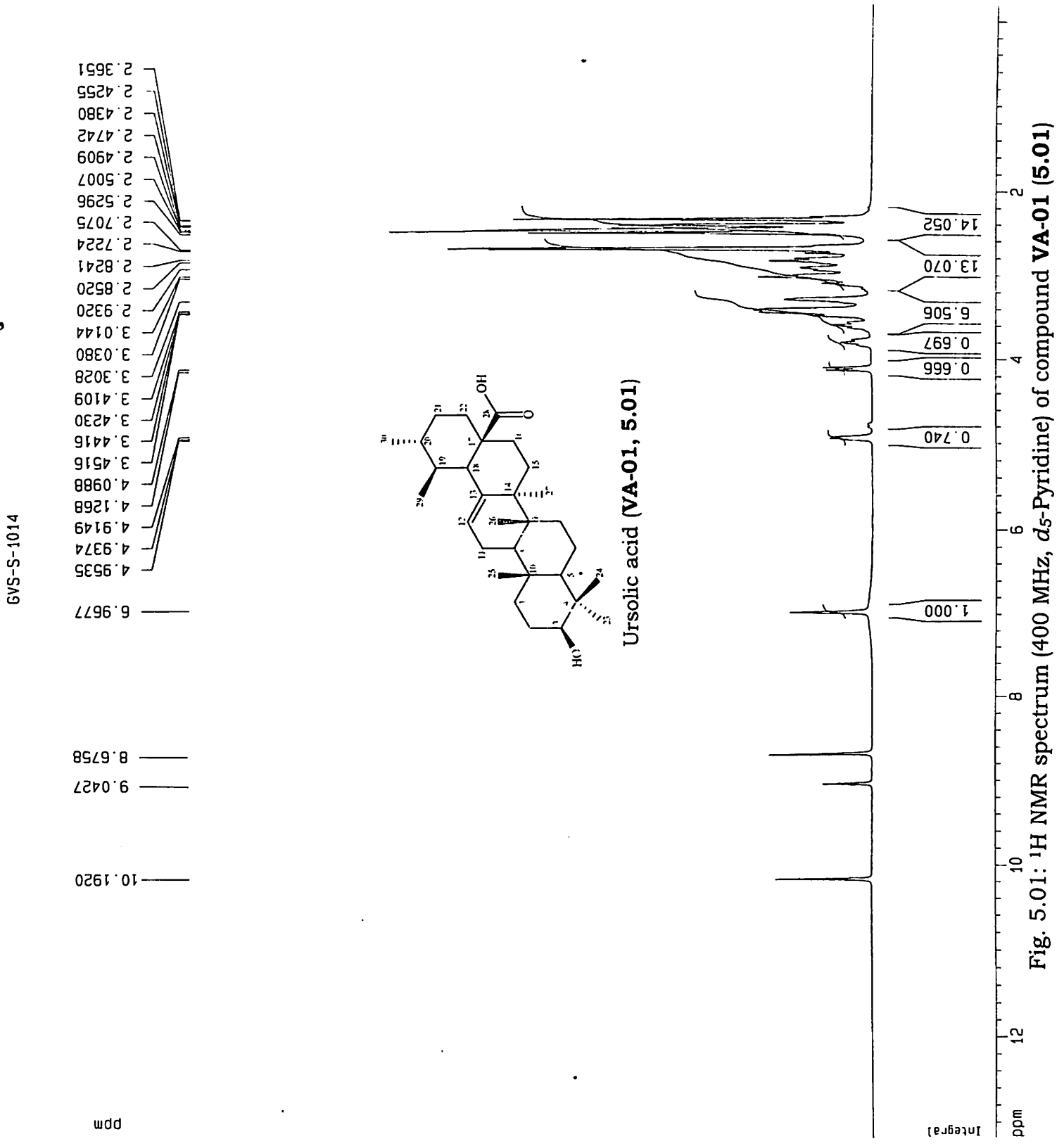



Fig. 5.01: $^1$H NMR spectrum (400 MHz, $d_5$-Pyridine) of compound **VA-01 (5.01)**

## TABLE 5.01

**$^1$H NMR spectral data of VA-01 (Ursolic acid, 5.01)**

**(Fig. 5.01, 400 MHz spectrum, $d_5$-Pyridine)**

| **Chemical shift (δ)** | **Proton integration** | **Multiplicity (*J* in Hz)** | **Assignment** |
|---|---|---|---|
| 3.44 | 1H | dd (9.0, 6.5) | H-3 |
| 5.48 | 1H | br s | H-12 |
| 2.63 | 1H | d (11.2) | H-18 |
| 1.24 | 3H | s | H-23 |
| 1.01 | 3H | s | H-24 |
| 0.88 | 3H | s | H-25 |
| 1.04 | 3H | s | H-26 |
| 1.22 | 3H | s | H-27 |
| 0.99 | 3H | d (7.0) | H-29 |
| 0.94 | 3H | d (6.5) | H-30 |

## VA-07

It was obtained as a white amorphous powder from methanol, m.p. 243-245 °C. The molecular formula, $C_{30}H_{48}O_4$, was deduced from elemental analysis and LC-MS [*m/z* 471 $(M - H)^-$] data. It gave positive LB test for triterpenoids.

The IR (KBr) spectrum of VA-07 showed bands at $\nu_{max}$ 3424 (hydroxyl) and 1693 $cm^{-1}$ (COOH). The $^1H$ NMR spectral (Fig. 5.02, Table 5.02) data showed the presence of two hydroxymethine protons [δ 4.09 (1H, ddd, *J* = 11.0, 9.0, 4.3 Hz) and δ 3.40 (1H, d, *J* = 9.4 Hz), a trisubstituted olefinic proton (δ 5.41, br s, H-12) and a methine proton at δ 2.61, (d, *J* = 11.3 Hz), characteristic of H-18 of ursane skeleton.[1] The $^1H$ NMR spectrum also showed, the presence of two secondary methyl signals located at δ 0.99 (3H, d, *J* = 6.2 Hz) and δ 0.95 (3H, d, *J* = 6.2 Hz), as expected.[1,2]

The $^{13}C$ NMR spectral (Fig. 5.03, Table 5.03) data showed the presence of a carboxylic acid (δ 180.1), two oxygenated methine carbons (δ 83.9 and 68.7) and two olefinic carbons (δ 125.6 and 139.4) characteristic of C-12 and C-13 of ursane derivatives.[11,12]

A comparison of the above data with those of ursolic acid (**5.01**) revealed that VA-07 contains an additional hydroxyl group. The chemical shifts and coupling constants of two hydroxyl methine protons revealed the vicinal substitution of hydroxyl groups. In view of the ubiquitous presence of an oxygen function at C-3 in triterpenoids, the second hydroxyl was placed on C-2. The nature and magnitude of coupling constants ($J_{ax\text{-}ax}$ = 9.4 Hz) suggested that C-2 and C-3 protons possess diaxial coupling thereby indicating that the stereochemistry of C-2 and C-3 hydroxyls is 2α-OH and 3β-OH respectively.[13]

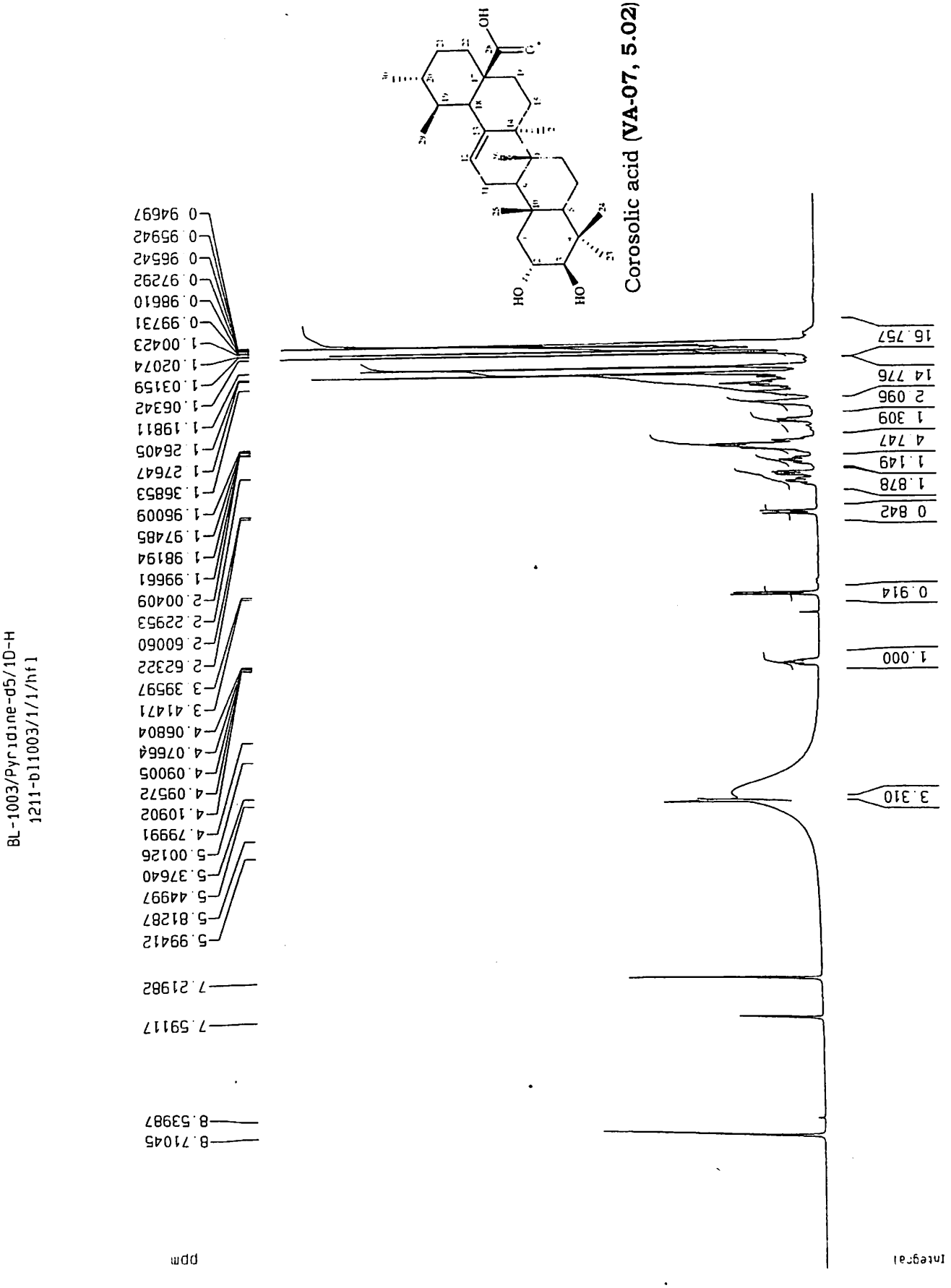



Fig. 5.02: $^1$H NMR spectrum (500 MHz, $d_5$-Pyridine) of compound **VA-07 (5.02)**

## TABLE 5.02

**$^1$H NMR spectral data of VA-07 (Corosolic acid, 5.02)**

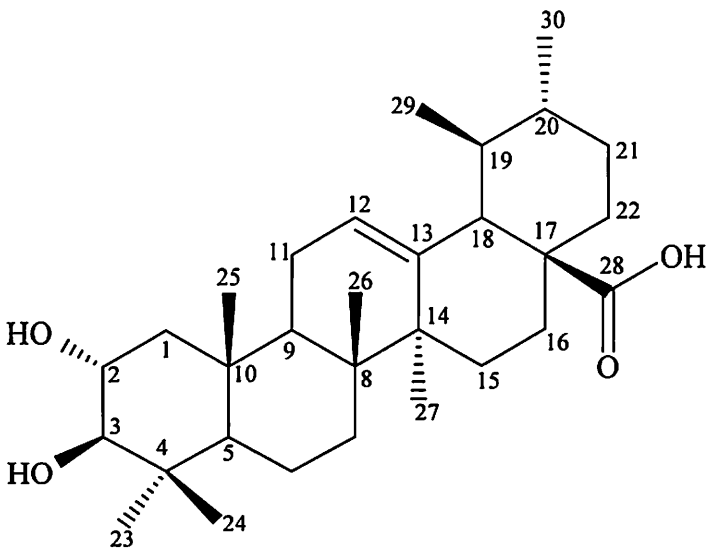
**(Fig. 5.02, 500 MHz spectrum, $d_5$-Pyridine)**

| **Chemical shift (δ)** | **Proton integration** | **Multiplicity (*J* in Hz)** | **Assignment** |
|---|---|---|---|
| 4.09 | 1H | ddd (11.0, 9.0, 4.3) | H-2 |
| 3.40 | 1H | d (9.4) | H-3 |
| 5.41 | 1H | br s | H-12 |
| 2.61 | 1H | d (11.3) | H-18 |
| 1.28 | 3H | s | H-23 |
| 1.03 | 3H | s | H-24 |
| 0.97 | 3H | s | H-25 |
| 1.06 | 3H | s | H-26 |
| 1.19 | 3H | s | H-27 |
| 0.95 | 3H | d (6.2) | H-29 |
| 0.99 | 3H | d (6.2) | H-30 |

The above physical and spectral data of VA-07 were found to corroborate well with those recorded for corosolic acid (2α,3β-dihydroxyurs-12-en-28-oic acid, **5.02**), isolated earlier from *Lagerstroemia speciosa.*[14]

The identity of VA-07 as corosolic acid was ascertained further by direct comparison with authentic sample (co-HPTLC and mmp) kindly supplied by Laila Impex, R & D centre, Vijayawada.

Based on the above the structure of VA-07 was deduced as corosolic acid (**5.02**).




**5.02**

Corosolic acid has been reported to exhibit hypoglycemic activity and was shown to be a glucose transport activator.[14] The leaves of *Lagerstroemia speciosa* (known as Banaba in Philippines) are used as an antidiabetic drug and the decoction of the leaves has been clinically tested and found to reduce blood sugar levels.[15]

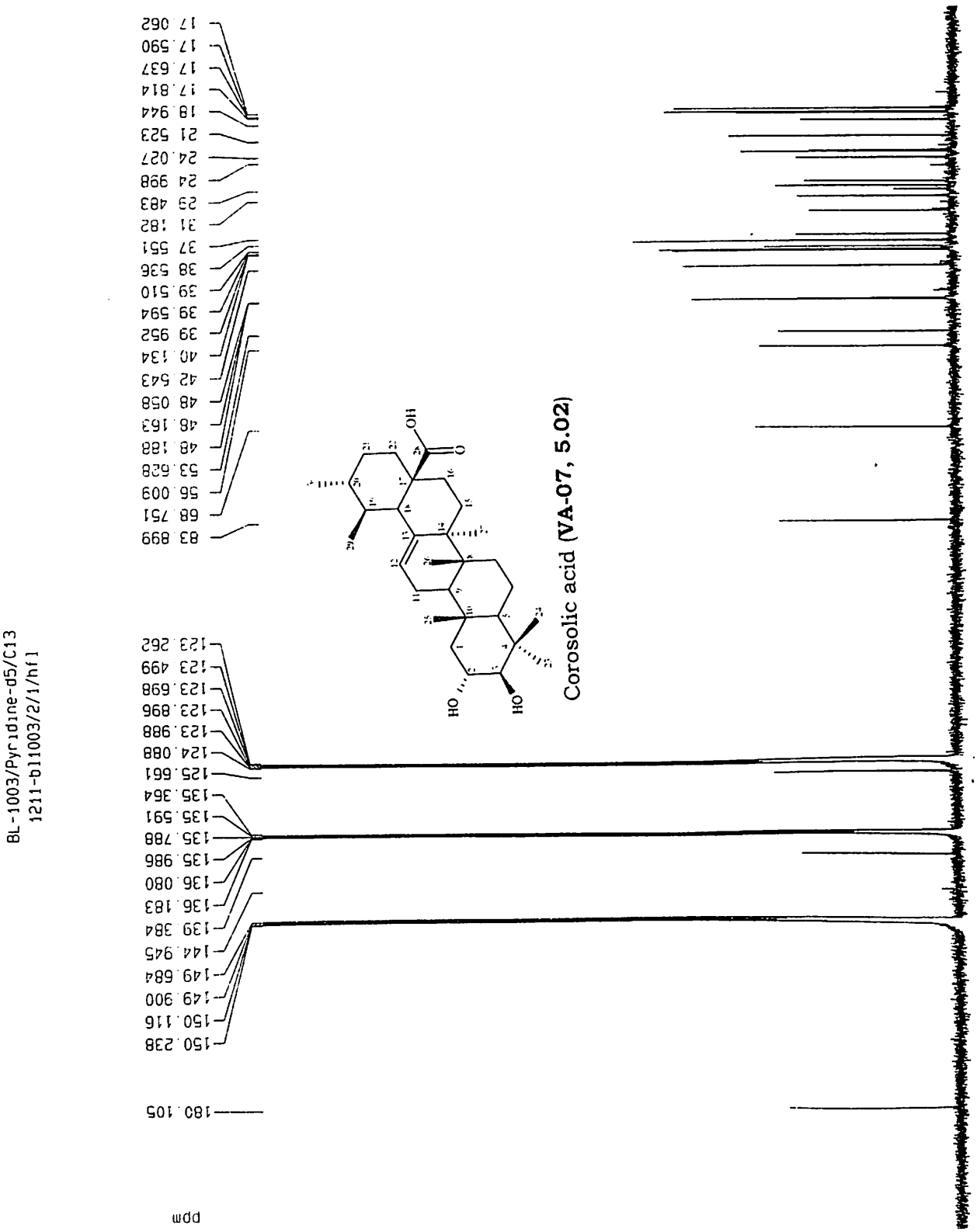



Fig. 5.03: $^{13}C$ NMR spectrum (125 MHz, $d_5$-Pyridine) of compound **VA-07 (5.02)**

## TABLE 5.03

**$^{13}C$ NMR spectral data of VA-07 (Corosolic acid, 5.02)**

**(Fig. 5.03, 125 MHz spectrum, $d_5$-Pyridine)**

| Chemical shift (δ) | DEPT | Assignment | Chemical shift (δ) | DEPT | Assignment |
|---|---|---|---|---|---|
| 48.0 | $CH_2$ | C-1 | 24.9 | $CH_2$ | C-16 |
| 68.7 | CH | C-2 | 48.2 | C | C-17 |
| 83.9 | CH | C-3 | 53.6 | CH | C-18 |
| 39.0 | C | C-4 | 39.6 | CH | C-19 |
| 56.0 | CH | C-5 | 39.9 | CH | C-20 |
| 18.9 | $CH_2$ | C-6 | 31.2 | $CH_2$ | C-21 |
| 33.6 | $CH_2$ | C-7 | 37.5 | $CH_2$ | C-22 |
| 40.1 | C | C-8 | 29.5 | $CH_3$ | C-23 |
| 48.2 | CH | C-9 | 17.6 | $CH_3$ | C-24 |
| 38.5 | C | C-10 | 17.0 | $CH_3$ | C-25 |
| 23.8 | $CH_2$ | C-11 | 17.6 | $CH_3$ | C-26 |
| 125.6 | CH | C-12 | 24.0 | $CH_3$ | C-27 |
| 139.4 | C | C-13 | 180.1 | C | C-28 |
| 42.6 | C | C-14 | 17.8 | $CH_3$ | C-29 |
| 28.7 | $CH_2$ | C-15 | 21.5 | $CH_3$ | C-30 |

## VA-02

It was obtained as a white amorphous powder from methanol, m.p. 195-197 °C. The molecular formula, $C_{30}H_{48}O_4$, was deduced from elemental analysis and LC-MS [*m/z* 471 $(M - H)^-$] data.

The IR (KBr) spectrum of VA-02 showed bands at $\nu_{max}$ 3433 (hydroxyl), and 1693 $cm^{-1}$ (COOH). The $^1H$ NMR spectral (Fig. 5.04, Table 5.04) data showed the presence of two hydroxymethine protons [δ 4.28 (d, *J* = 9.1 Hz, H-2) and δ 3.75 (br s, H-3)], a trisubstituted olefinic proton (δ 5.44, br s, H-12) and a methine doublet at δ 2.59 (d, *J* = 10.7 Hz) characteristic of H-18 of ursane skeleton.[1] The $^1H$ NMR spectrum showed the presence of five tertiary methyl groups and two poorly resolved secondary methyl groups.

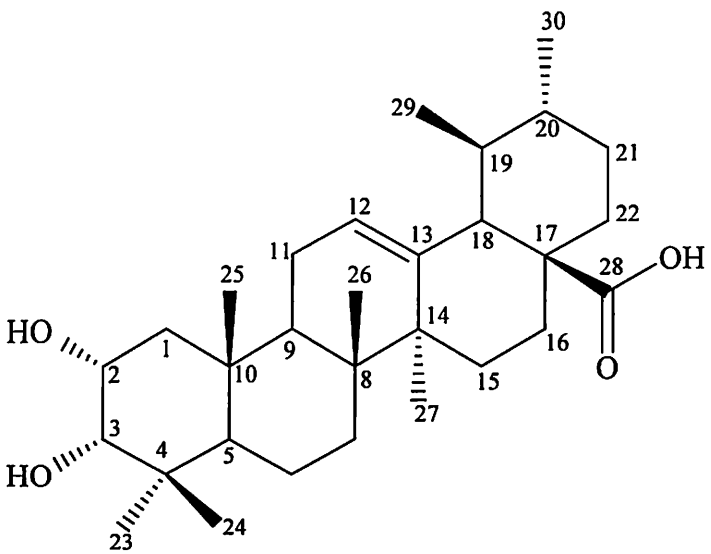
The above data of VA-02 in comparison with corosolic acid (**5.02**) revealed that VA-02 was also a 2,3-dihydroxy derivative of urs-12-en-28-oic acid. The relative stereochemistry at C-2 and C-3 centres could not be derived due to poorly resolved hydroxymethine proton signals. Therefore, the stereochemistry of the vicinal hydroxyls was deduced from the NMR spectral (Fig. 5.05 and 5.06, Table 5.05 and 5.06) data of the diacetyl derivative (VA-02a) wherein H-2 and H-3 were well resolved and the stereochemistry could be assigned as follows. The $^1H$ NMR spectral (Fig. 5.05, Table 5.05) data of VA-02a showed the presence of two downfield acetoxymethine protons [δ 5.23 (1H, ddd, *J* = 11.4, 4.0, 3.0 Hz, H-2) and δ 4.98 (1H, d, *J* = 2.4 Hz, H-3)] and two secondary methyl groups located at δ 0.95 (3H, d, *J* = 6.0 Hz) and δ 0.86 (3H, d, *J* = 6.0 Hz).

The coupling constant $^3J_{H\text{-}2,H\text{-}3}$ 2.4 Hz in VA-02a is consistent with the *cis* orientation of H-2 and H-3 protons[13] and consequently, the acetoxyls could be assigned as 2α-OAc and 3α-OAc.

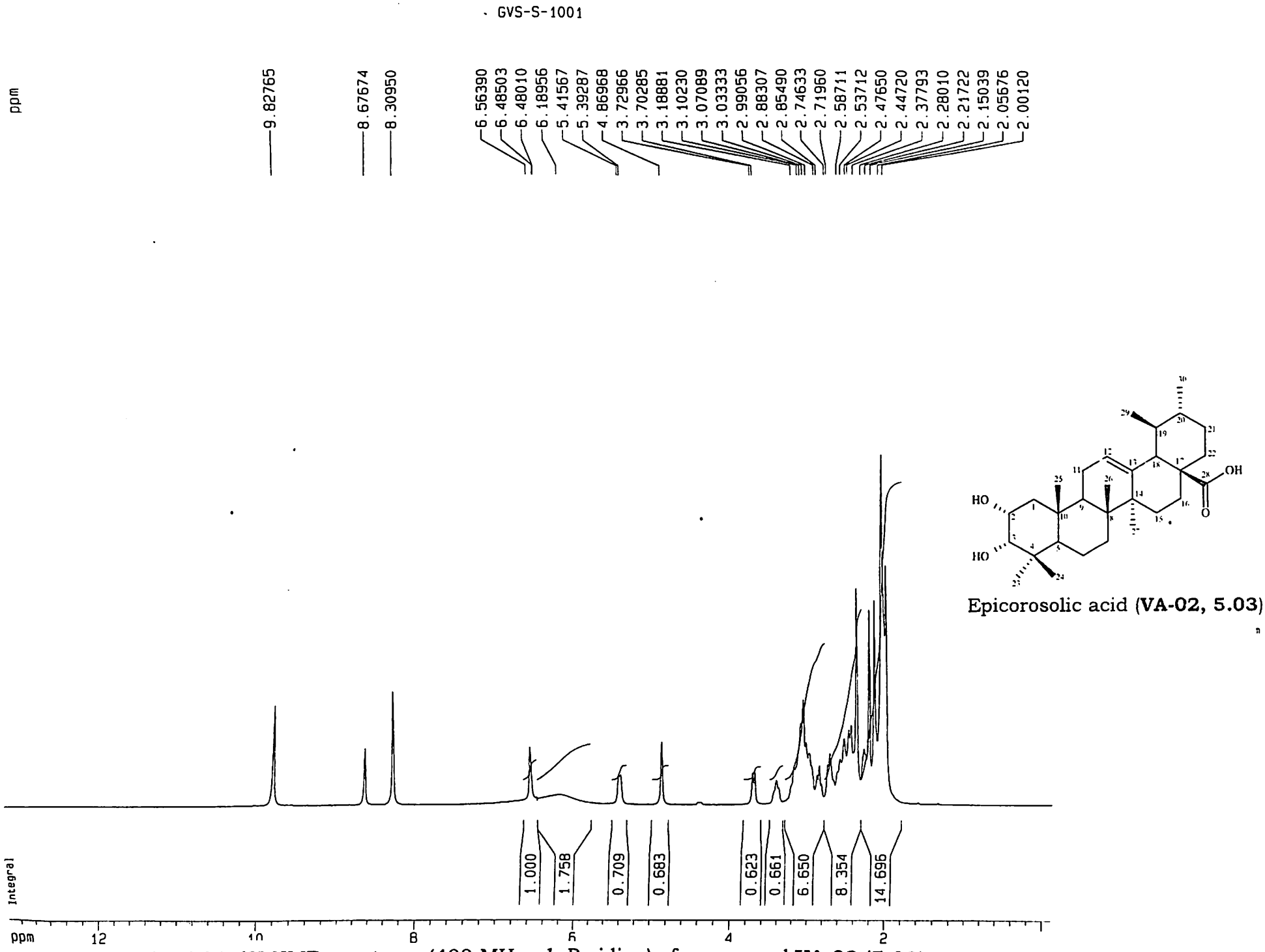
Thus, the relative disposition of H-2 and H-3 in VA-02 was assigned as β-orientation and the corresponding hydroxyl groups have been deduced as 2α-OH and 3α-OH (**5.03**).

Literature survey on ursane derivatives revealed that the physical and spectral data of VA-02 were in good agreement with those recorded for epicorosolic acid (2α,3α-dihydroxyurs-12-en-28-oic acid, **5.03**) isolated earlier from *Nepeta eriostachia,*[16] *Prunus serotina*[17] and *Prunus lusitanica.*[17]

Based on the foregoing, the structure of VA-02 was established as epicorosolic acid (**5.03**).




**5.03**







Epicorosolic acid (**VA-02, 5.03**)

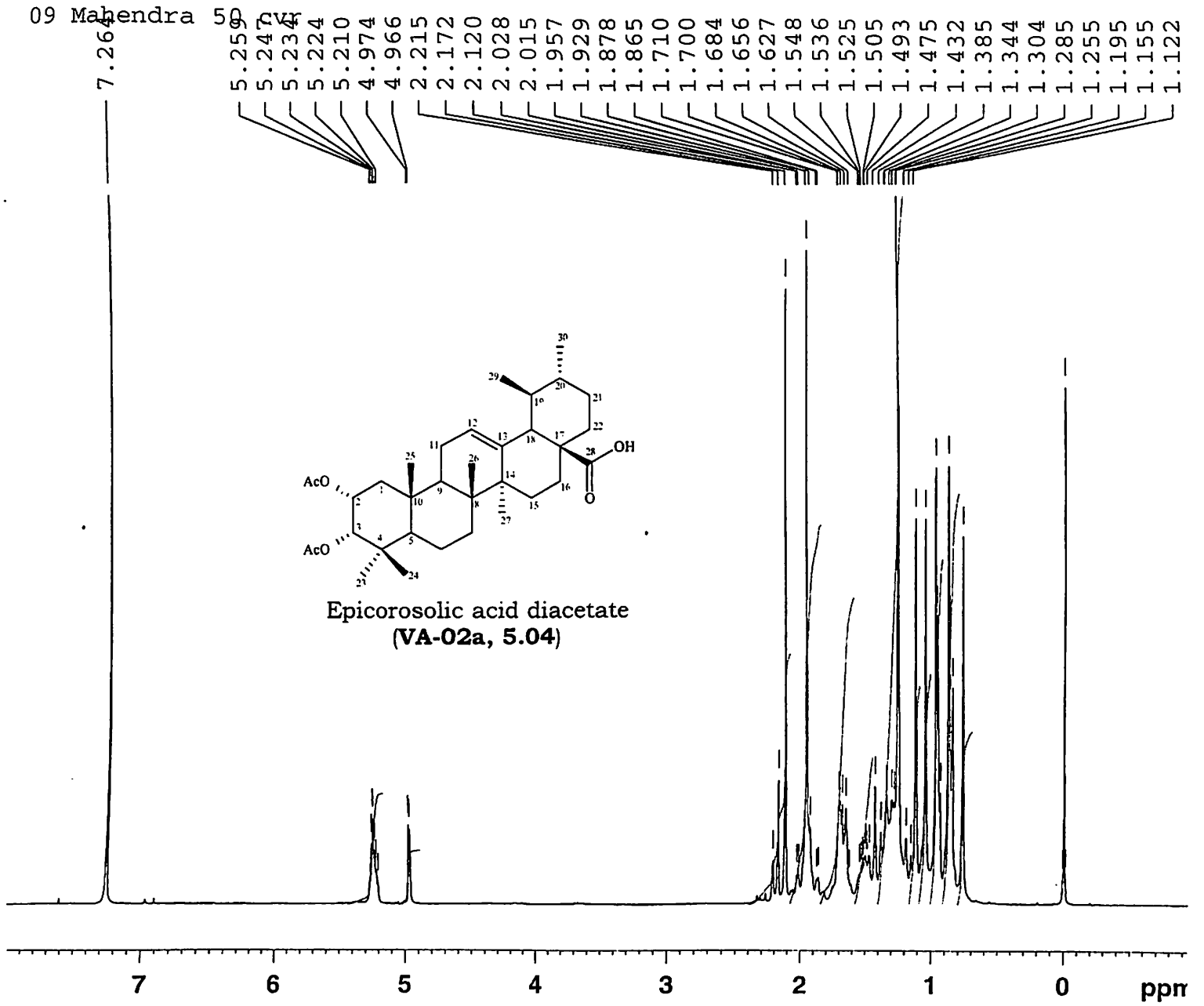
Fig. 5.04: $^1$H NMR spectrum (400 MHz, $d_5$-Pyridine) of compound **VA-02 (5.03)**

## TABLE 5.04

**$^1$H NMR spectral data of VA-02 (epicorosolic acid, 5.03)**

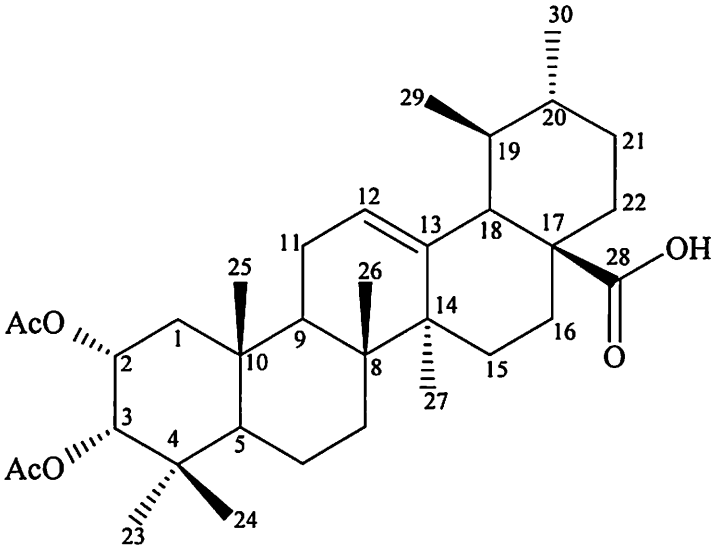
**(Fig. 5.04, 400 MHz spectrum, $d_5$-Pyridine)**

| **Chemical shift (δ)** | **Proton integration** | **Multiplicity (*J* in Hz)** | **Assignment** |
|---|---|---|---|
| 4.28 | 1H | d (9.1) | H-2 |
| 3.75 | 1H | br s | H-3 |
| 5.44 | 1H | br s | H-12 |
| 2.59 | 1H | d (10.7) | H-18 |
| 1.26 | 3H | s | H-23 |
| 1.02 | 6H | br s | H- 24 and H-30 |
| 0.94 | 6H | br s | H-25 and H-29 |
| 1.09 | 3H | s | H-26 |
| 1.16 | 3H | s | H-27 |




Epicorosolic acid diacetate
**(VA-02a, 5.04)**

Fig. 5.05: $^1$H NMR spectrum (300 MHz, $CDCl_3$) of compound **VA-02a (5.04)**

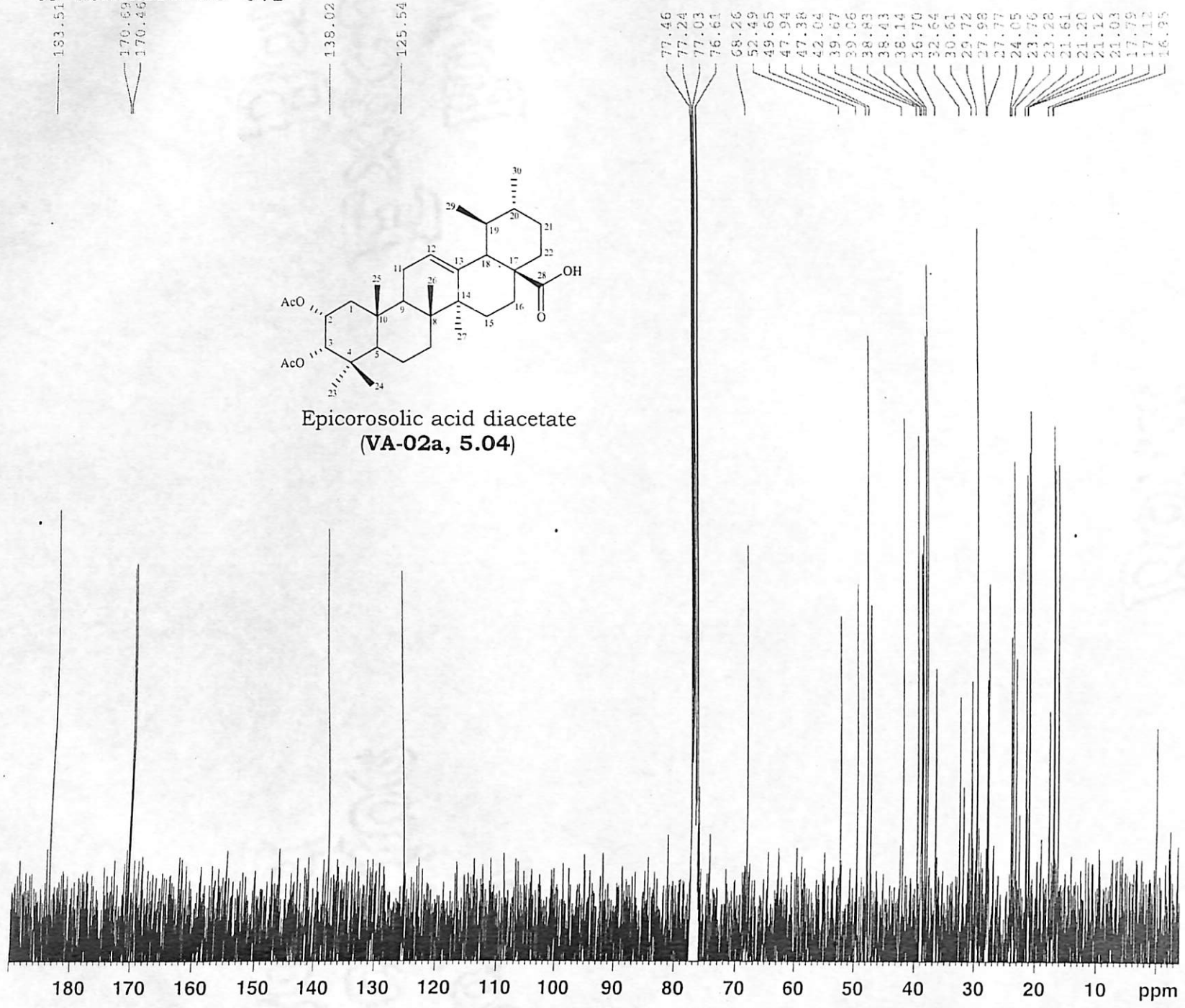



**5.04**

## TABLE 5.05

**$^1$H NMR spectral data of VA-02a (epicorosolic acid diacetate, 5.04) (Fig. 5.05, 300 MHz spectrum, $CDCl_3$)**

| Chemical shift (δ) | Proton integration | Multiplicity (*J* in Hz) | Assignment |
|---|---|---|---|
| 5.23 | 1H | ddd (11.4, 4.0, 3.0) | H-2 |
| 4.98 | 1H | d (2.4) | H-3 |
| 5.27 | 1H | br s | H-12 |
| 2.23 | 1H | d (11.2) | H-18 |
| 0.89 | 3H | s | H-23 |
| 0.98 | 3H | s | H-24 |
| 1.06 | 3H | s | H-25 |
| 0.79 | 3H | s | H-26 |
| 1.14 | 3H | s | H-27 |
| 0.86 | 3H | d (6.0) | H-29 |
| 0.95 | 3H | d (6.0) | H-30 |
| 2.13 | 3H | s | $-OCOCH_3$ |
| 1.97 | 3H | s | $-OCOCH_3$ |




Epicorosolic acid diacetate
(**VA-02a, 5.04**)

Fig. 5.06: $^{13}C$ NMR spectrum (75 MHz, $CDCl_3$) of compound **VA-02a** (**5.04**)

## TABLE 5.06

**$^{13}C$ NMR spectral data of VA-02a (epicorosolic acid diacetate, 5.04)**

**(Fig. 5.06, 75 MHz spectrum, $CDCl_3$)**

| Chemical shift (δ) | DEPT | Assignment | Chemical shift (δ) | DEPT | Assignment |
|---|---|---|---|---|---|
| 39.0 | $CH_2$ | C-1 | 52.4 | CH | C-18 |
| 68.2 | CH | C-2 | 39.0 | CH | C-19 |
| 77.0 | CH | C-3 | 38.8 | CH | C-20 |
| 38.4 | C | C-4 | 30.6 | $CH_2$ | C-21 |
| 49.6 | CH | C-5 | 36.6 | $CH_2$ | C-22 |
| 17.7 | $CH_2$ | C-6 | 27.7 | $CH_3$ | C-23 |
| 32.6 | $CH_2$ | C-7 | 21.6 | $CH_3$ | C-24 |
| 39.6 | C | C-8 | 16.3 | $CH_3$ | C-25 |
| 47.3 | CH | C-9 | 16.9 | $CH_3$ | C-26 |
| 38.1 | C | C-10 | 23.7 | $CH_3$ | C-27 |
| 23.3 | $CH_2$ | C-11 | 183.5 | C | C-28 |
| 125.5 | CH | C-12 | 17.1 | $CH_3$ | C-29 |
| 138.0 | C | C-13 | 21.2 | $CH_3$ | C-30 |
| 42.0 | C | C-14 | 170.0 | C | -O$\underline{C}$O$CH_3$ |
| 27.9 | $CH_2$ | C-15 | 170.2 | C | -O$\underline{C}$O$CH_3$ |
| 24.0 | $CH_2$ | C-16 | 21.1 | $CH_3$ | -OCO$\underline{C}H_3$ |
| 47.9 | C | C-17 | 21.0 | $CH_3$ | -OCO$\underline{C}H_3$ |

## VA-06

It was obtained as a white amorphous powder from methanol, m.p. 268-270 °C. Its molecular formula, $C_{30}H_{48}O_5$, was deduced from elemental analysis and LC-MS [*m/z* 487 $(M - H)^-$] data. It gave positive LB test for triterpenoids.

The IR (KBr) spectrum of VA-06 showed bands at $\nu_{max}$ 3340 (hydroxyl) and 1689 $cm^{-1}$ (COOH). The $^1H$ NMR spectral (Fig. 5.07, Table 5.07) data showed the presence of a carboxylic acid (δ 11.85, s), two hydroxymethine protons [δ 3.79 (1H, m) and δ 3.22 (1H, br s), a trisubstituted methine proton (δ 5.16, br s, H-12) and a methine proton (δ 2.35, s, H-18), in addition to secondary methyl group (δ 0.82, 3H, d, *J* = 6.3 Hz) and a down field tertiary methyl group (δ 1.27) indicative of a modified ursane skeleton.[1]

The $^{13}C$ NMR spectral data (Fig. 5.08, Table 5.08) showed the presence of a carboxylic acid (δ 179.8, C-28), three hydroxymethine carbons (δ 78.7, 72.4 and 65.5). In addition, two olefinic carbons (δ 127.6, C-12 and 139.5, C-13) characteristic of ursane derivatives[11,12] were also present. These assignments were supported by the DEPT (Fig. 5.09) spectral data.

A comparison of the above data with those of epicorosolic acid (**5.03**) revealed that VA-06 is a derivative of epicorosolic acid having an additional hydroxyl group. A fact supported further by the presence of three hydoxymethine carbons in VA-06.

VA-06 on acetylation with acetic anhydride-pyridine yielded only a diacetyl derivative (VA-06a), indicating the presence of either a hindered or tertiary hydroxyl group. The presence of only one secondary methyl group (δ 0.82, 3H, d, *J* = 6.3 Hz) and a tertiary methyl group located at δ 1.27 (characteristic of oxygenated methyl group) led to the

placement of hydroxyl at C-19 in VA-06[18]. The nature of H-18, which appeared as a singlet at δ 2.35, rather than the usual doublet, also supported the position of the hydroxyl.

The stereochemistry at C-2 and C-3 could not be established unambiguously due to poorly resolved hydroxymethine proton signals (H-2 and H-3). Therefore, the diacetyl derivative (VA-06a) was prepared and its $^1$H NMR spectral (Fig. 5.10, Table 5.09) data showed the presence of two well resolved acetoxymethine protons [δ 5.24 (1H, ddd, $J$ = 9.3,4.9,2.7 Hz) and δ 4.97 (1H, d, $J$ = 2.5 Hz) attributable to H-2 and H-3 as expected. The above spectral data in comparison with those of diacetylepicorosolic acid (**5.04**) confirmed the cis orientation of H-2 and H-3 in VA-06a also. Thus, the stereochemistry of H-2 and H-3 was assigned as β-orientation[19] and the corresponding hydroxyl in VA-06 as 2α-OH and 3α-OH.

Literature survey on trihydroxy ursane derivatives revealed that the physical and spectral data of VA-06 corroborate well with those recorded for euscaphic acid (2α,3α,19α-trihydroxyurs-12-en-28-oic acid, **5.05**) isolated earlier from *Rosa davurica*[20] and *Myrianthus arboreus*.[21]

Based on the foregoing, the structure of VA-06 was deduced as euscaphic acid (**5.05**)

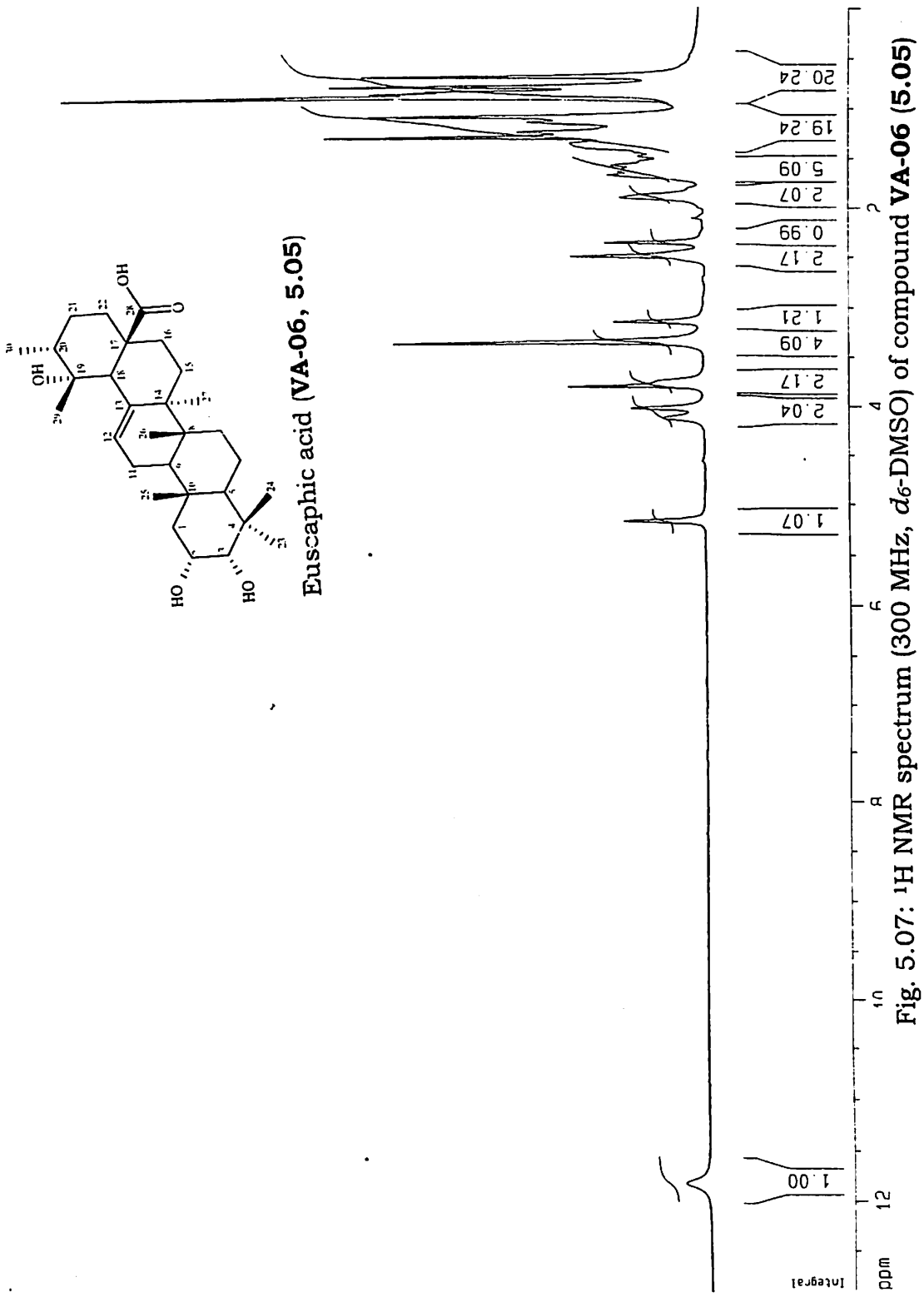
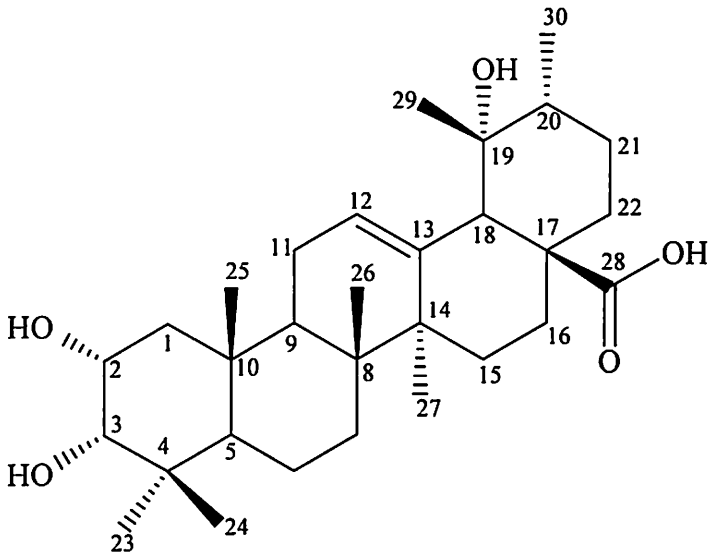



Fig. 5.07: $^1$H NMR spectrum (300 MHz, $d_6$-DMSO) of compound **VA-06 (5.05)**




**5.05**

## TABLE 5.07

**$^1$H NMR spectral data of VA-06 (Euscaphic acid, 5.05)**

**(Fig. 5.07, 300 MHz spectrum, $d_6$-DMSO)**

| **Chemical shift (δ)** | **Proton integration** | **Multiplicity (*J* in Hz)** | **Assignment** |
|---|---|---|---|
| 11.85 | 1H | s | COOH |
| 3.79 | 1H | m | H-2 |
| 3.22 | 1H | br s | H-3 |
| 5.16 | 1H | br s | H-12 |
| 2.35 | 1H | s | H-18 |
| 0.76 | 3H | s | H-23 |
| 0.87 | 6H | s | H-24 and H-25 |
| 0.67 | 3H | s | H-26 |
| 1.07 | 3H | s | H-27 |
| 1.27 | 3H | s | H-29 |
| 0.82 | 3H | d (6.3) | H-30 |

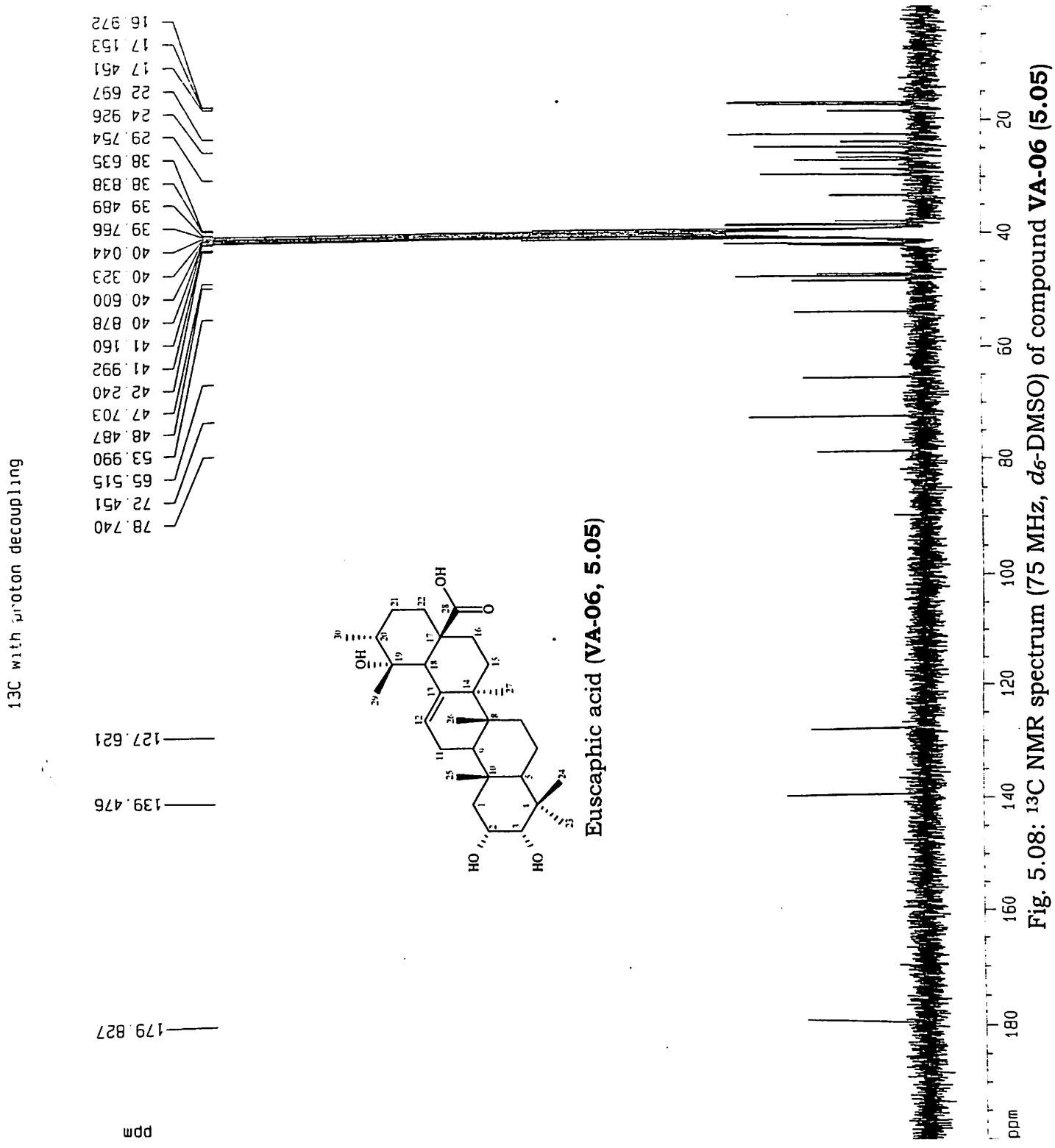



Fig. 5.08: $^{13}C$ NMR spectrum (75 MHz, $d_6$-DMSO) of compound **VA-06 (5.05)**

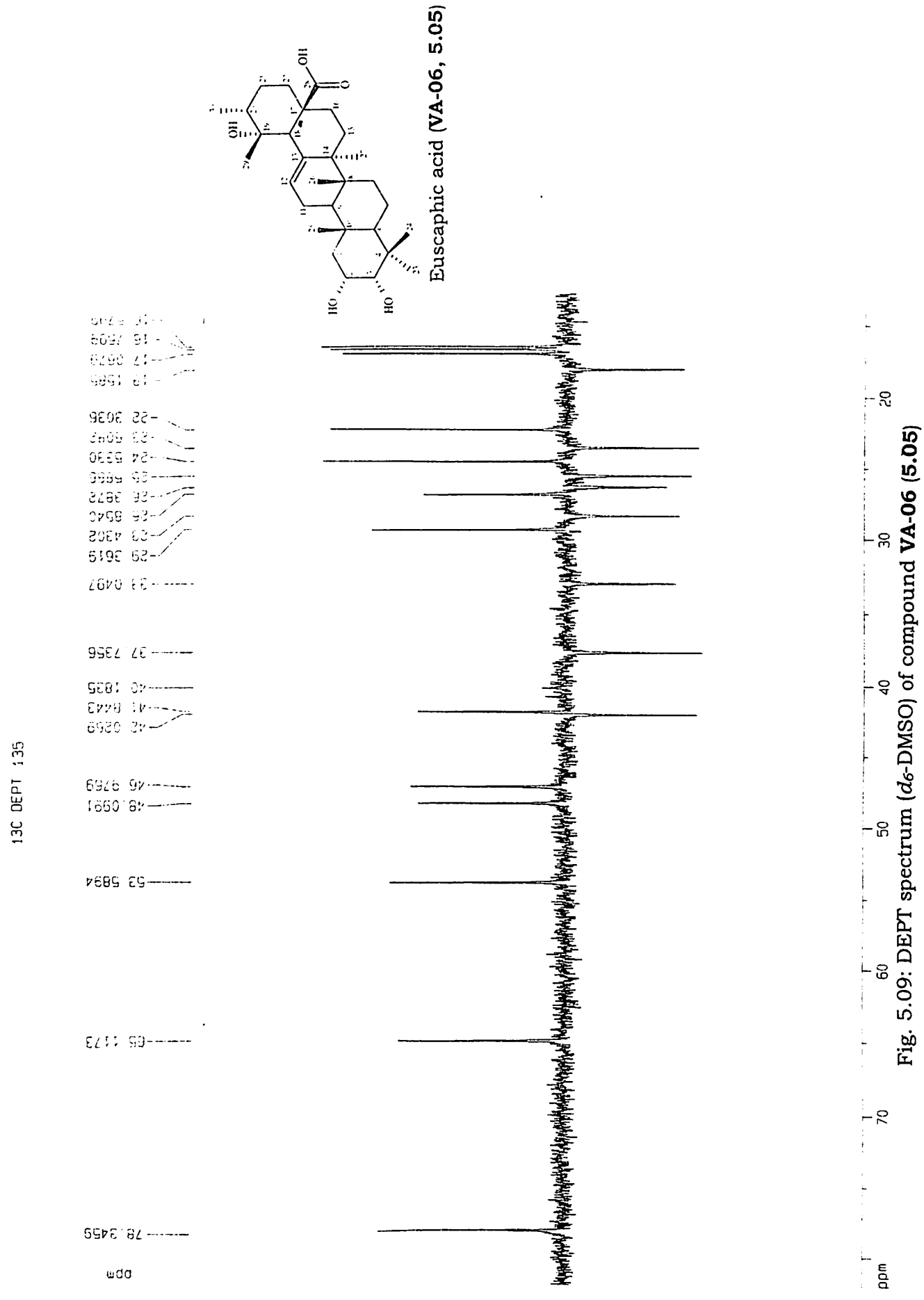


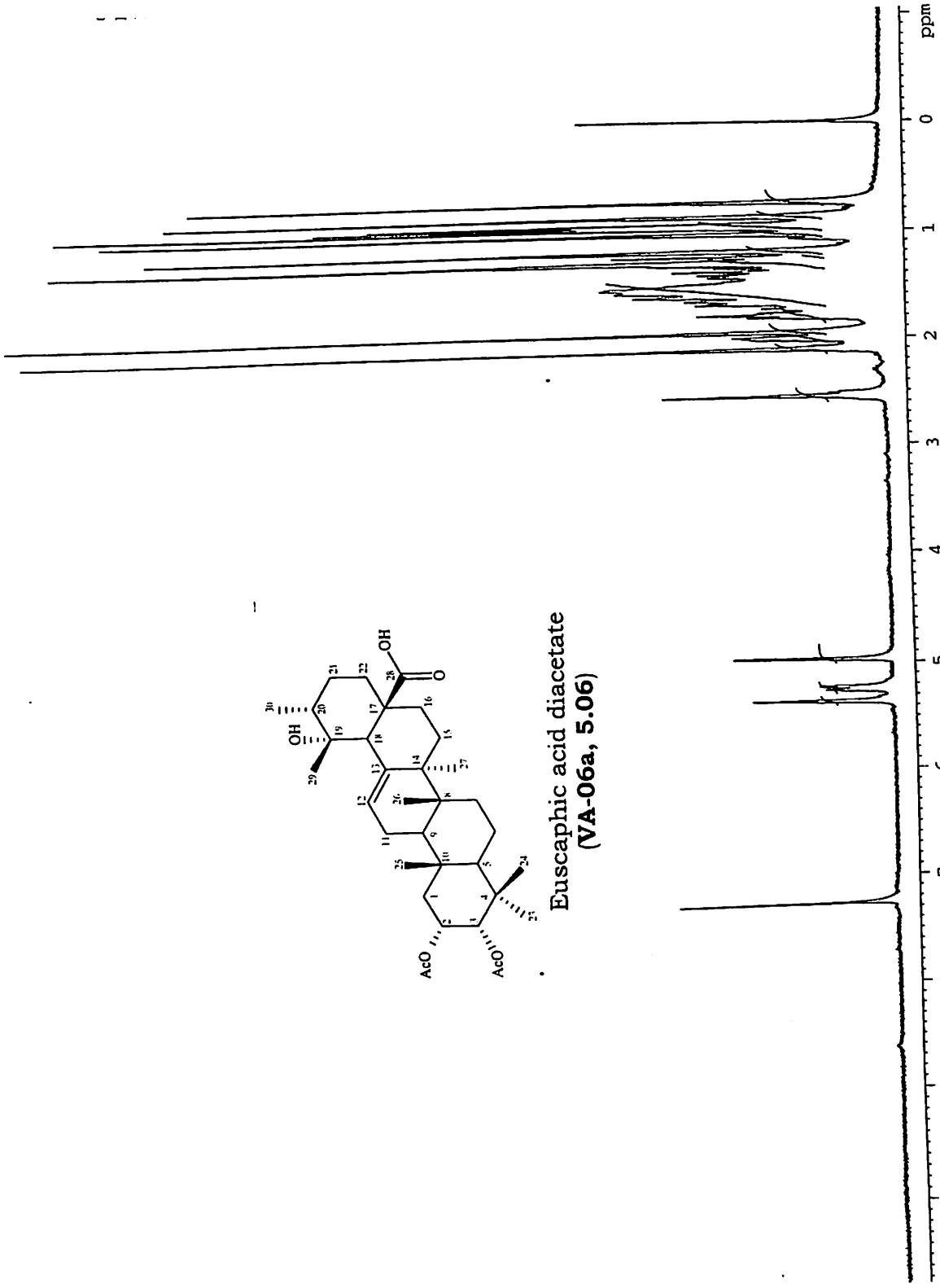

Fig. 5.09: DEPT spectrum ($d_6$-DMSO) of compound **VA-06 (5.05)**

## TABLE 5.08

**$^{13}C$ NMR spectral data of VA-06 (Euscaphic acid, 5.05)**

**(Fig. 5.08, 75 MHz spectrum, $d_6$-DMSO)**

| Chemical shift (δ) | DEPT | Assignment | Chemical shift (δ) | DEPT | Assignment |
|---|---|---|---|---|---|
| 42.4 | $CH_2$ | C-1 | 25.9 | $CH_2$ | C-16 |
| 65.5 | CH | C-2 | 47.7 | C | C-17 |
| 78.7 | CH | C-3 | 53.9 | CH | C-18 |
| 38.8 | C | C-4 | 72.4 | C | C-19 |
| 48.5 | CH | C-5 | 42.2 | CH | C-20 |
| 18.5 | $CH_2$ | C-6 | 26.8 | $CH_2$ | C-21 |
| 33.4 | $CH_2$ | C-7 | 38.1 | $CH_2$ | C-22 |
| 39.5 | C | C-8 | 29.7 | $CH_3$ | C-23 |
| 47.4 | CH | C-9 | 22.7 | $CH_3$ | C-24 |
| 38.6 | C | C-10 | 17.1 | $CH_3$ | C-25 |
| 24.0 | $CH_2$ | C-11 | 17.5 | $CH_3$ | C-26 |
| 127.6 | CH | C-12 | 24.9 | $CH_3$ | C-27 |
| 139.5 | C | C-13 | 179.8 | C | C-28 |
| 41.9 | C | C-14 | 27.2 | $CH_3$ | C-29 |
| 28.6 | $CH_2$ | C-15 | 16.9 | $CH_3$ | C-30 |

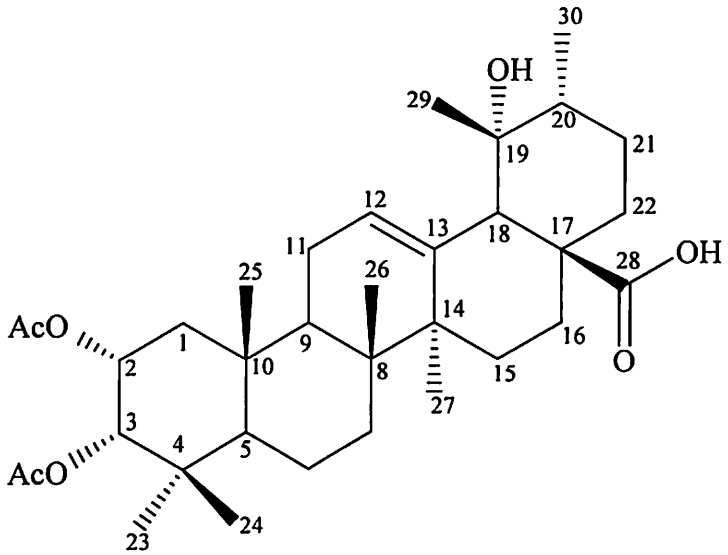






Euscaphic acid diacetate
**(VA-06a, 5.06)**

Fig. 5.10: $^1$H NMR spectrum (400 MHz, $CDCl_3$) of compound **VA-06a (5.06)**




**5.06**

## TABLE 5.09

**$^1$H NMR spectral data of VA-06a (Euscaphic acid diacetate, 5.06) (Fig. 5.10, 400 MHz spectrum, $CDCl_3$)**

| Chemical shift (δ) | Proton integration | Multiplicity (*J* in Hz) | Assignment |
|---|---|---|---|
| 5.24 | 1H | ddd (9.3,4.9,2.7) | H-2 |
| 4.97 | 1H | d (2.5) | H-3 |
| 5.36 | 1H | br s | H-12 |
| 2.55 | 1H | s | H-18 |
| 0.88 | 3H | s | H-23 |
| 0.98 | 3H | s | H-24 |
| 1.04 | 3H | s | H-25 |
| 0.74 | 3H | s | H-26 |
| 1.21 | 3H | s | H-27 |
| 1.31 | 3H | s | H-29 |
| 0.95 | 3H | d (6.6) | H-30 |
| 1.96 | 3H | s | $-OCOCH_3$ |
| 2.11 | 3H | s | $-OCOCH_3$ |

## VA-11

It was obtained as a white amorphous powder from methanol. The IR (KBr) spectrum showed bands at 3434 (hydroxyl) and 1737 (ester). $^1$H NMR spectrum showed the presence of signals indicative of a triterpenoid skeleton and it is devoid of any acetyl groups. But the spectrum indicated the presence of impurities, which could not be removed completely. As such the impure triterpenoid was acetylated with acetic anhydride-pyridine and the details of acetylation and purification of the acetyl derivative were noted in chapter-2. Thus, the structure of VA-11 has been deduced based on the spectral data of its acetyl derivative (VA-11a).

The acetyl derivative (VA-11a) was obtained as a white amorphous powder from methanol, m.p. 182-184 °C. The molecular formula, $C_{48}H_{70}O_{16}$, was deduced from elemental analysis and LC-MS [*m/z* 901 $(M - H)^-$ ]data.

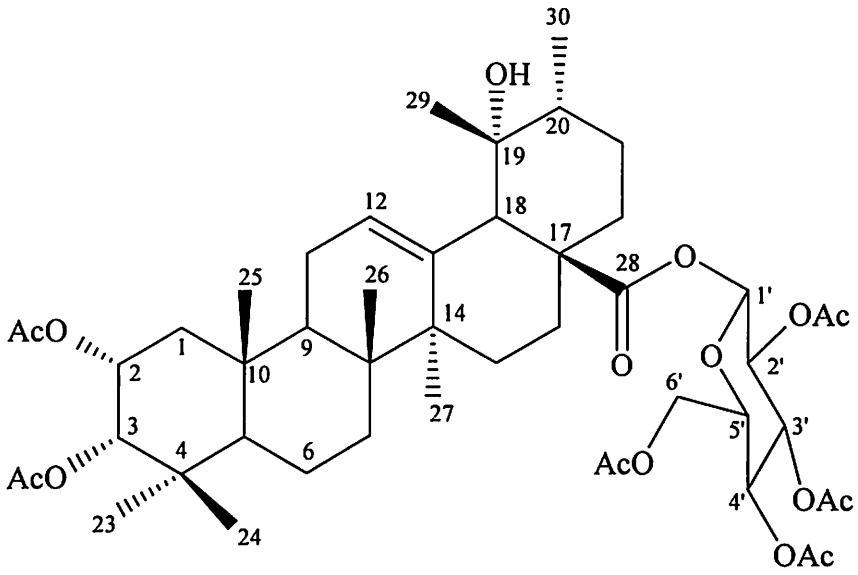
The IR (KBr) spectrum of VA-11a showed a broad band at $\nu_{max}$ 1748 cm$^{-1}$ (ester carbonyls). The $^1$H NMR spectral (Fig. 5.11, Table 5.10) data showed the presence of two acetoxymethine protons [δ 5.24 (1H, m, H-2) and δ 4.97 (1H, d, *J* = 2.2 Hz, H-3)], a trisubstituted olefinic proton (δ 5.39, br s, H-12), a secondary methyl group (δ 0.94, 3H, d, *J* = 6.5 Hz, H-30) and a methine proton at δ 2.54 (1H, s, H-18) characteristic of ursane derivatives.[1]

The $^1$H NMR spectrum also indicated the presence of a sugar unit as revealed by the anomeric proton signal at δ 5.53 (1H, d, *J* = 7.9 Hz, H-1′) and acetoxymethine and methylene protons in the region δ 3.79 - 5.21. The coupling constant (*J* = 7.9 Hz) of the anomeric proton (H-1′) was consistent with the β-configuration of the sugar unit.

The $^{13}C$ NMR spectral (Fig. 5.12, Table 5.11) data showed the presence of an ester carbonyl (δ 175.7), three oxygenated carbons (δ 77.1, 73.1 and 68.2) and an anomeric carbon (δ 91.8). In addition the $^{13}C$ NMR spectrum showed the presence of six acetyl carbons (δ 170.6, 170.5, 170.4, 170.1, 169.4 and 168.9) and signal at δ 72.8, 72.5, 69.9, 68.0 and 61.5 attributable to sugar unit. These assignments were supported by the DEPT (Fig. 5.13) spectral data.

The above spectral data in comparison with VA-06a revealed that VA-11a also contains an euscaphic acid (2α,3α,19α-trihydroxyurs-12-en) unit[20,21] but, having an additional sugar unit. The absence of free carboxyl group and the presence of an ester carbonyl (δ 175.7) and the upfield shift of anomeric carbon[22] (δ 91.8) in $^{13}C$ NMR spectrum supported the presence of a C-28 ester glucoside in VA-11a.

Based on the above the structure of VA-11a was deduced as euscaphic acid ester glucoside hexaacetate (2α,3α-diacetoxy-19α-hydroxyurs-12-en-28-oic acid 28-*O*-β-D-tetraacetoxyglucopyranoside, **5.07**) and thus the original compound VA-11 could be derived as euscaphic acid glucoside (2α,3α,19α-trihydroxyurs-12-en-28-oic acid 28-*O*-β-D-glucopyranoside, **5.08**), isolated earlier from *Rosa sterilis*.[23]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26
14
AcO
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9
2
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O
2'
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6'
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4
6
AcO
4'
OAc
23
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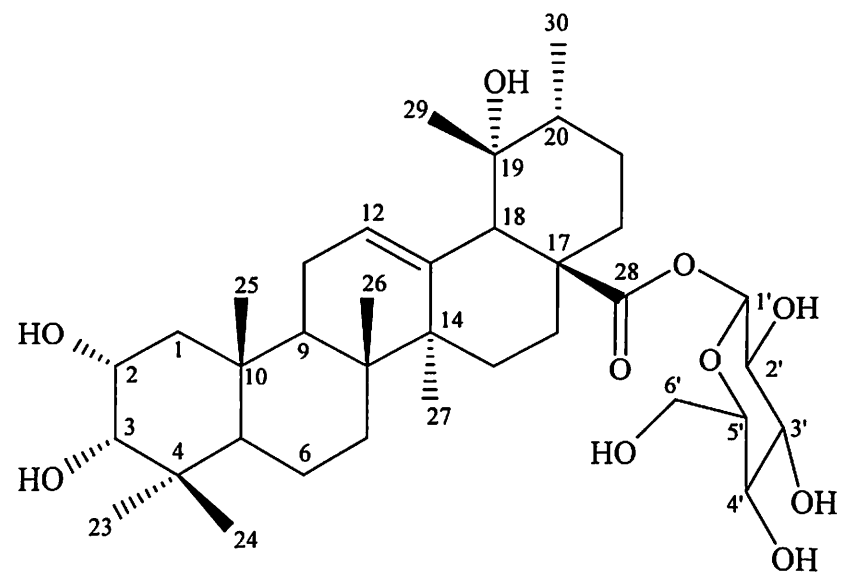
OAc

**5.07**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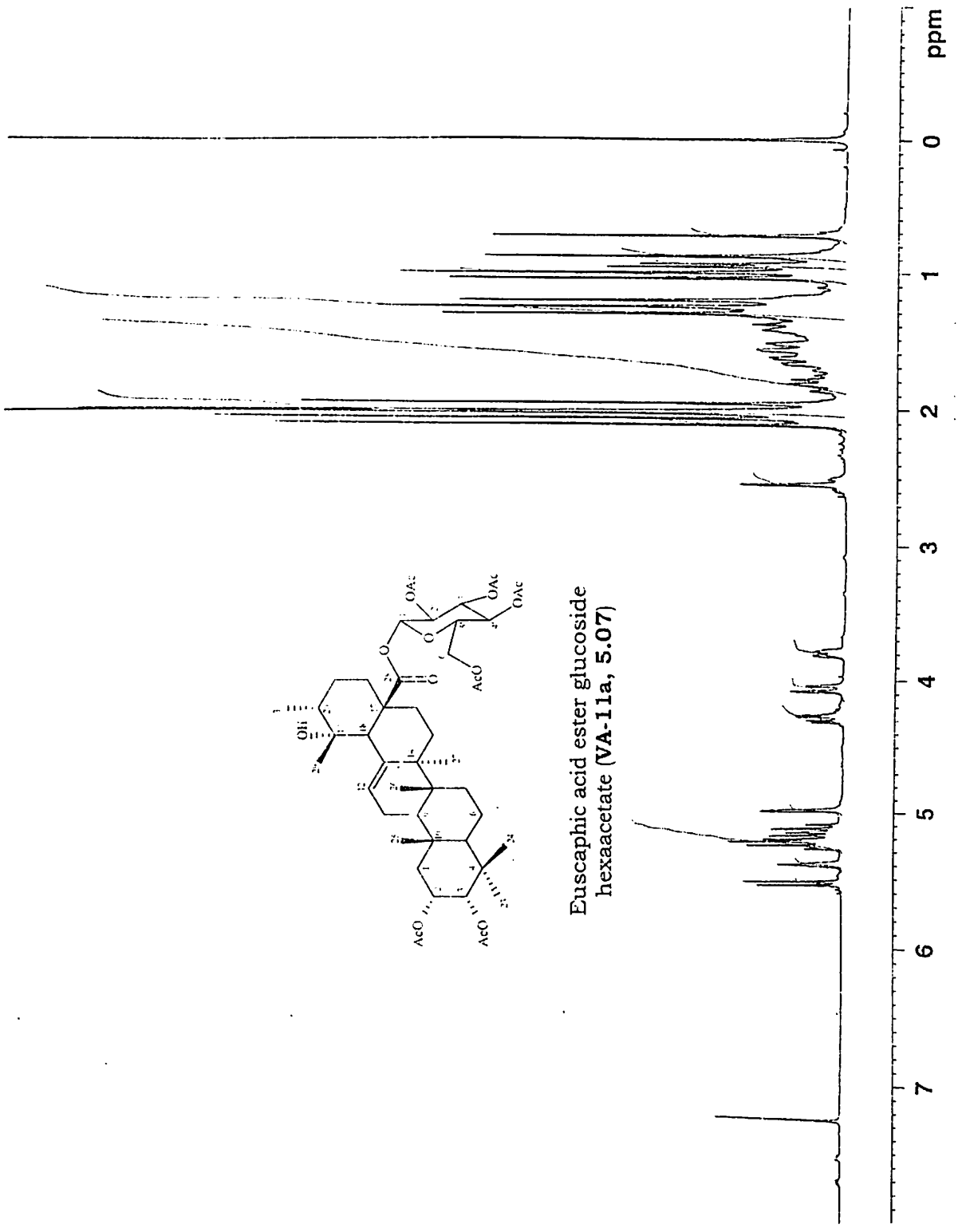
OH

**5.08**




Euscaphic acid ester glucoside hexaacetate (**VA-11a, 5.07**)

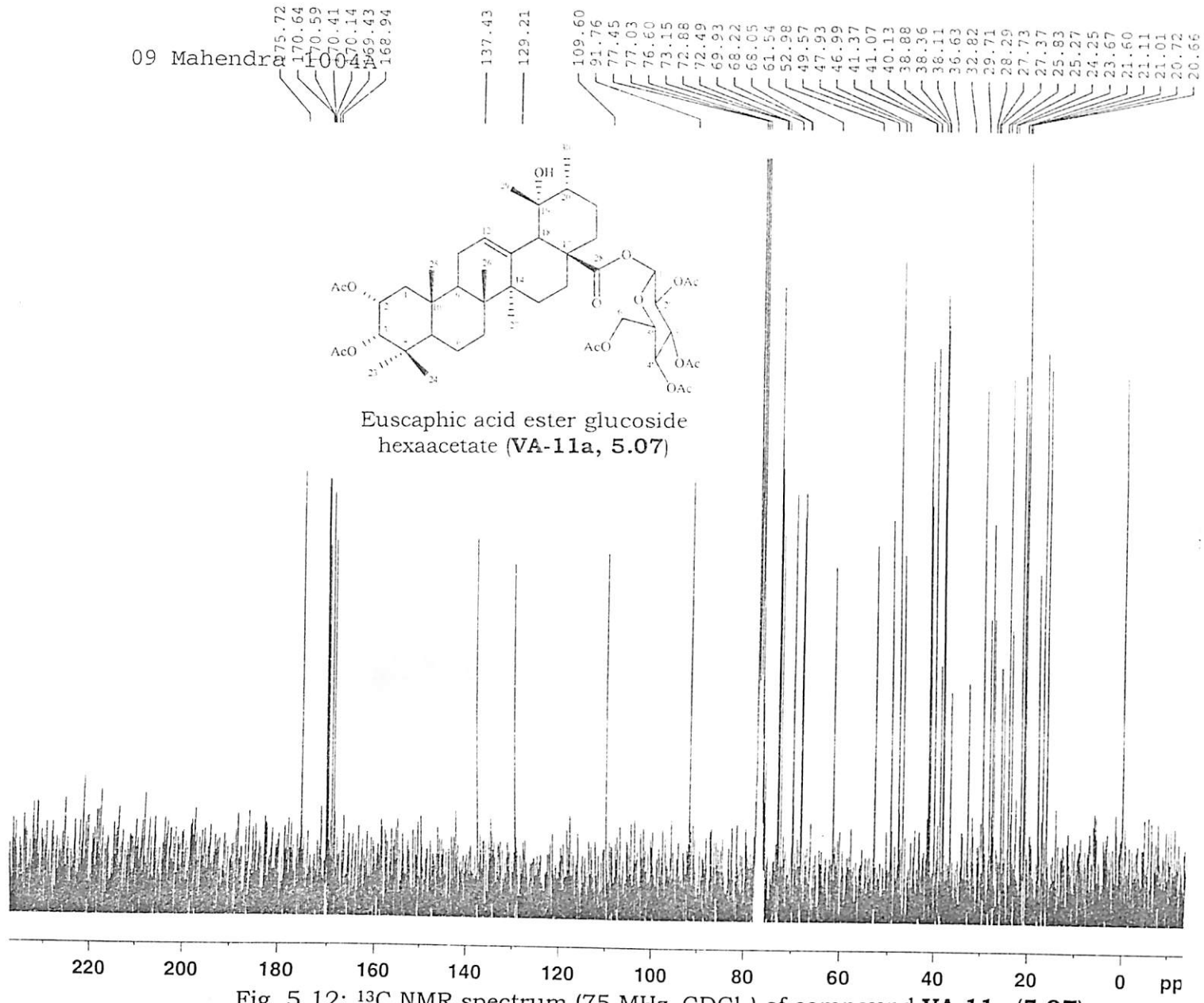
Fig. 5.11: $^{1}H$ NMR spectrum (300 MHz, $CDCl_3$) of compound **VA-11a (5.07)**

## TABLE 5.10

**$^1$H NMR spectral data of VA-11a (Euscaphic acid ester glucoside hexaacetate, 5.07)**

**(Fig. 5.11, 300 MHz spectrum, $CDCl_3$)**

| **Chemical shift (δ)** | **Proton integration** | **Multiplicity (*J* in Hz)** | **Assignment** |
|---|---|---|---|
| 5.24 | 1H | m | H-2 |
| 4.97 | 1H | d (2.2) | H-3 |
| 5.39 | 1H | br s | H-12 |
| 2.54 | 1H | s | H-18 |
| 0.87 | 3H | s | H-23 |
| 0.99 | 3H | s | H-24 |
| 1.04 | 3H | s | H-25 |
| 0.72 | 3H | s | H-26 |
| 1.20 | 3H | s | H-27 |
| 1.31 | 3H | s | H-29 |
| 0.94 | 3H | d (6.5) | H-30 |
| 5.53 | 1H | d (7.9) | H-1′ |
| 5.21-5.08 | 3H | m | H-2′-H-4′ |
| 3.79 | 1H | m | H-5′ |
| 4.27 | 1H | dd (12.4, 4.4) | $H_a$-6′ |
| 4.04 | 1H | dd (12.4, 2.1) | $H_b$-6′ |
| 2.11 | 3H | s | |
| 2.07 | 3H | s | |
| 2.02 | 6H | s | OAc × 6 |
| 2.02 | 3H | s | |
| 1.96 | 3H | s | |




Euscaphic acid ester glucoside hexaacetate (**VA-11a, 5.07**)

Fig. 5.12: $^{13}C$ NMR spectrum (75 MHz, $CDCl_3$) of compound **VA-11a (5.07)**

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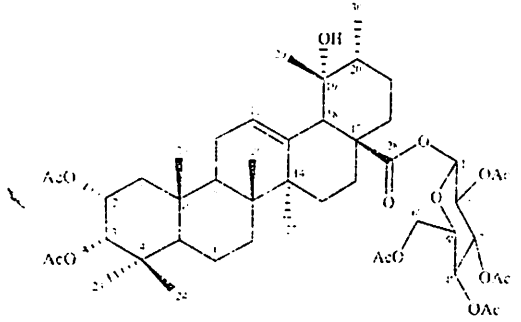


Euscaphic acid ester glucoside hexaacetate (**VA-11a, 5.07**)




Fig. 5.13: DEPT spectrum ($CDCl_3$) of compound **VA-11a (5.07)**

## TABLE 5.11

**$^{13}C$ NMR spectral data of VA-11a (Euscaphic acid ester glucoside hexaacetate, 5.07)**

**(Fig. 5.12, 75 MHz spectrum, $CDCl_3$)**

| Chemical shift (δ) | DEPT | Assignment | Chemical shift (δ) | DEPT | Assignment |
|---|---|---|---|---|---|
| 38.8 | $CH_2$ | C-1 | 16.2 | $CH_3$ | C-25 |
| 68.2 | CH | C-2 | 16.9 | $CH_3$ | C-26 |
| 77.1 | CH | C-3 | 24.2 | $CH_3$ | C-27 |
| 38.4 | C | C-4 | 175.7 | C | C-28 |
| 49.6 | CH | C-5 | 27.4 | $CH_3$ | C-29 |
| 17.9 | $CH_2$ | C-6 | 16.1 | $CH_3$ | C-30 |
| 32.8 | $CH_2$ | C-7 | 91.8 | CH | C-1′ |
| 40.1 | C | C-8 | 69.9 | CH | C-2′ |
| 46.9 | CH | C-9 | 72.8 | CH | C-3′ |
| 38.1 | C | C-10 | 68.0 | CH | C-4′ |
| 23.7 | $CH_2$ | C-11 | 72.5 | CH | C-5′ |
| 129.2 | CH | C-12 | 61.5 | $CH_2$ | C-6′ |
| 137.4 | C | C-13 | 170.6 | C | -$OCOCH_3$ |
| 41.4 | C | C-14 | 170.5 | C | -$OCOCH_3$ |
| 28.3 | $CH_2$ | C-15 | 170.4 | C | -$OCOCH_3$ |
| 25.3 | $CH_2$ | C-16 | 170.1 | C | -$OCOCH_3$ |
| 47.9 | C | C-17 | 169.4 | C | -$OCOCH_3$ |
| 52.9 | CH | C-18 | 168.9 | C | -$OCOCH_3$ |
| 73.1 | C | C-19 | 21.6 | $CH_3$ | -$OCOCH_3$ |
| 41.1 | CH | C-20 | 21.1 | $CH_3$ | -$OCOCH_3$ |
| 25.8 | $CH_2$ | C-21 | 21.0 | $CH_3$ | -$OCOCH_3$ |
| 36.6 | $CH_2$ | C-22 | 20.7 | $CH_3$ | -$OCOCH_3$ |
| 27.7 | $CH_3$ | C-23 | 20.6 | $CH_3$ | -$OCOCH_3$ |
| 21.6 | $CH_3$ | C-24 | 20.6 | $CH_3$ | -$OCOCH_3$ |

## VA-09

It was obtained as a white amorphous powder, m.p. 278-281 °C The molecular formula, $C_{30}H_{48}O_5$, was deduced from LC-MS [*m/z* 487 $(M - H)^-$] data and $^{13}C$ NMR spectral data. It gave positive LB test for triterpenoids.

The IR (KBr) spectrum of VA-09 showed bands at $\nu_{max}$ 3445 (hydroxyl) and 1694 $cm^{-1}$ (COOH). The $^1H$ NMR spectral data (Fig. 5.14, Table 5.12) showed the presence of two hydroxymethine protons (δ 4.57, br s and δ 4.43, m), an olefinic proton (δ 5.45, 1H, br s, H-12) and a methine proton (δ 2.60, 1H, d, *J* = 11.2 Hz) characteristic of H-18 of ursane derivatives.[1] In addition the $^1H$ NMR spectral data showed the presence of an AB set of signals at δ 4.09 and δ 3.80 (*J* = 10.8 Hz) attributable to hydroxymethyl group.[24]

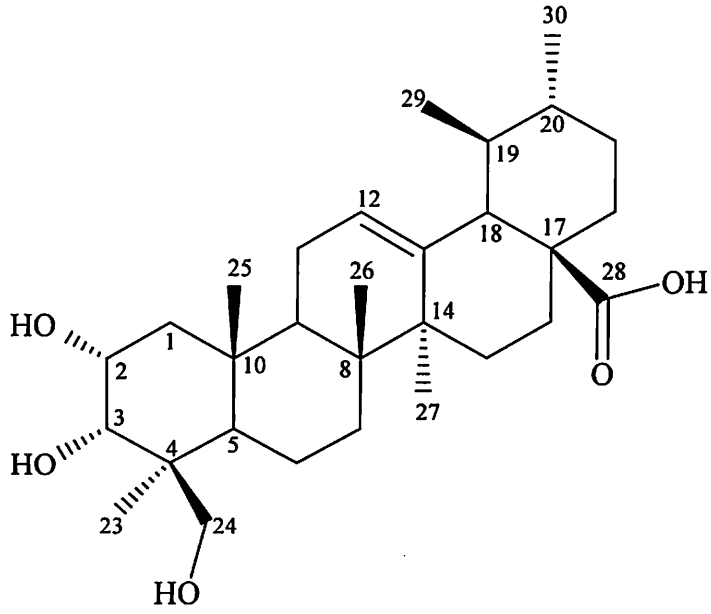
The $^{13}C$ NMR spectral data (Fig. 5.15, Table 5.13) showed the presence of a carboxylic acid (δ 179.9), two hydroxymethine carbons (δ 74.3, C-3 and δ 66.3, C-2), an hydroxymethylene carbon (δ 65.3) and two olefinic carbons (δ 125.6 and δ 139.3) attributable to C-12 and C-13 of ursane derivatives.[11]

The above spectral data in comparison with those of epicorosolic acid (**5.03**) and corosolic acid (**5.02**) indicated that VA-09 also contains a 2α,3α-dihydroxyurs-12-en-28-oic acid skeleton with an additional hydroxyl group. The presence of only four tertiary methyl groups instead of the usual five tertiary methyl groups suggest that the additional hydroxyl group could be present on one of the tertiary methyl groups in VA-09. The downfield shift of C-4 ($\delta_C$ 45.2) and upfield shift of C-5 ($\delta_C$ 49.5) indicated the presence of hydroxyl group on C-23 or C-24.[24] The absence of an usual upfield methyl signal ($\delta_C$ 17-18) characteristic of C-24 and the upfield shift to the extent of 3.0 ppm observed in the chemical shift ($\delta_C$ 26.1) of C-23 methyl group revealed

that the location of hydroxyl is indeed on C-24 in VA-09. A fact supported further by the appearance of hydroxymethylene doublets at δ 4.09 and δ 3.08 indicative of axial orientation of hydroxymethyl group on C-4. An equatorial hydroxymethylene proton are expected to resonate at upfield than δ 3.08.[25]

Literature survey on trihydroxy ursane derivatives revealed that the above spectral data corroborate well with those recorded for 2α,3α,24-trihydroxyurs-12-en-28-oic acid (**5.09**) isolated earlier from *Rhododendron japonicum,*[26] *Prunella vulgaris,*[27] and *Prunus serrulata.*[28]

Based on the foregoing, the structure of VA-09 was deduced as 2α,3α,24-trihydroxyurs-12-en-28-oic acid (**5. 09**)




**5.09**

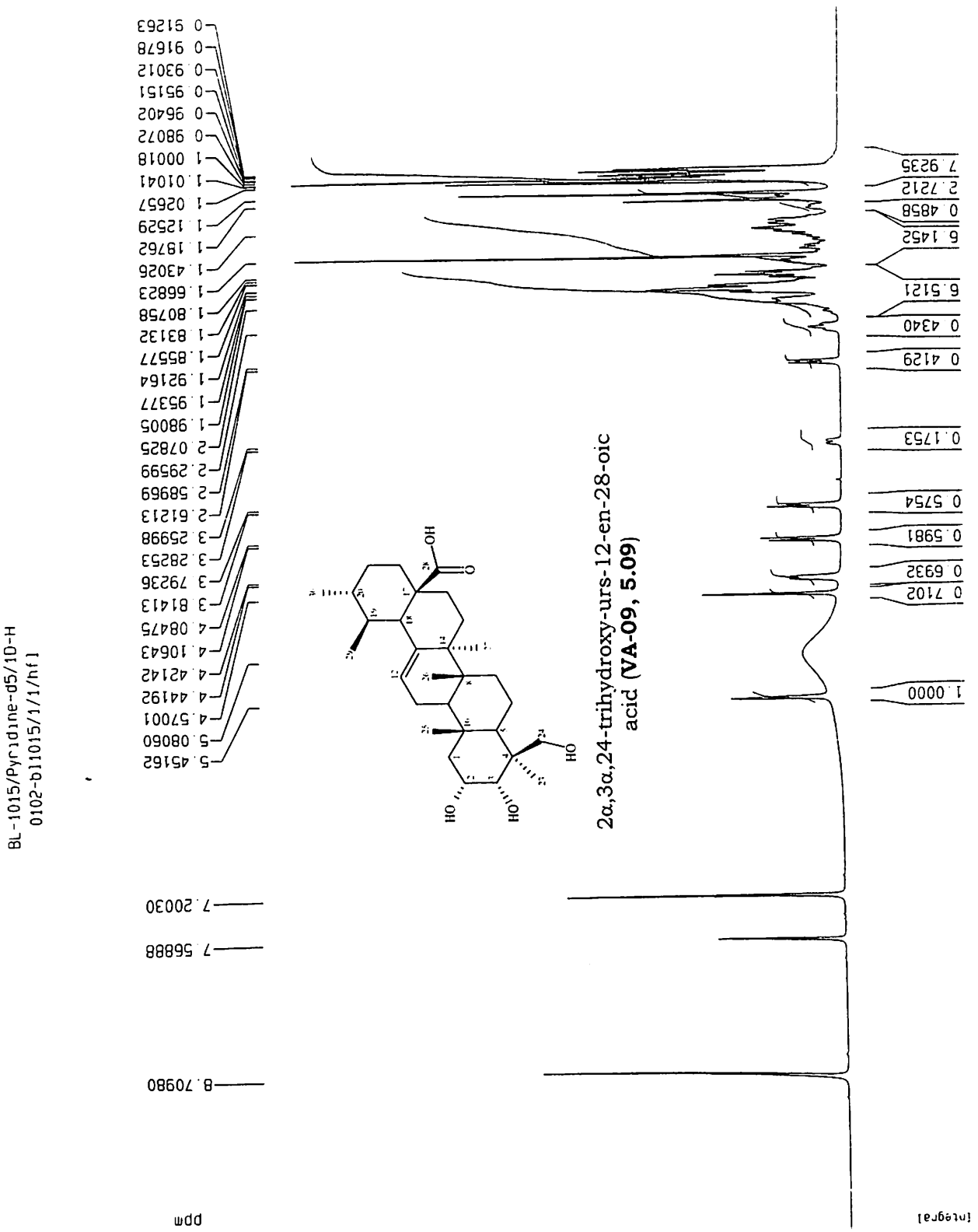



Fig. 5.14: $^1$H NMR spectrum (500 MHz, $d_5$-Pyridine) of compound **VA-09 (5.09)**

## TABLE 5.12

**$^1$H NMR spectral data of VA-09 (2α,3α,24-trihydroxyurs-12-en-28-oic acid, 5.09)**
**(Fig. 5.14, 500 MHz spectrum, $d_5$-Pyridine)**

| **Chemical shift (δ)** | **Proton integration** | **Multiplicity (*J* in Hz)** | **Assignment** |
|---|---|---|---|
| 4.43 | 1H | m | H-2 |
| 4.57 | 1H | br s | H-3 |
| 5.45 | 1H | br s | H-12 |
| 2.60 | 1H | d (11.2) | H-18 |
| 4.09 | 1H | d (10.8) | $H_a$-24 |
| 3.80 | 1H | d (10.8) | $H_b$-24 |
| 1.67 | 3H | s | H-23 |
| 1.12 | 3H | s | H-25 |
| 1.00 | 3H | s | H-26 |
| 1.18 | 3H | s | H-27 |
| 0.92 | 3H | d (6.2) | H-29 |
| 0.96 | 3H | d (6.6) | H-30 |

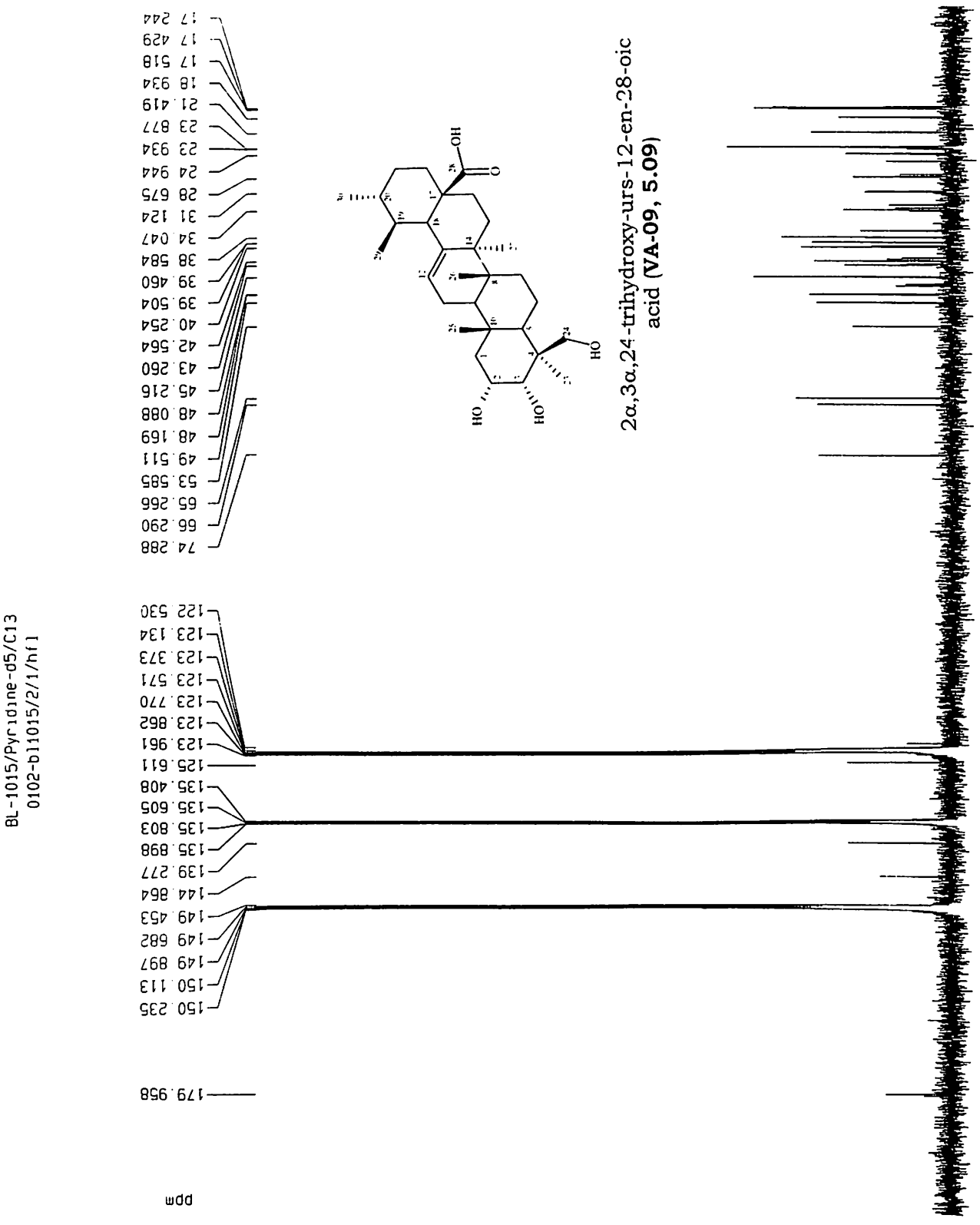



Fig. 5.15: $^{13}C$ NMR spectrum (125 MHz, $d_5$-Pyridine) of compound **VA-09 (5.09)**

## TABLE 5.13

**$^{13}C$ NMR spectral data of VA-09 (2α,3α,24-trihydroxyurs-12-en-28-oic acid, 5.09)**
**(Fig. 5.15, 125 MHz spectrum, $d_5$-Pyridine)**

| Chemical shift (δ) | DEPT | Assignment | Chemical shift (δ) | DEPT | Assignment |
|---|---|---|---|---|---|
| 43.3 | $CH_2$ | C-1 | 24.9 | $CH_2$ | C-16 |
| 66.3 | CH | C-2 | 48.1 | C | C-17 |
| 74.3 | CH | C-3 | 53.6 | CH | C-18 |
| 45.2 | C | C-4 | 39.4 | CH | C-19 |
| 49.5 | CH | C-5 | 39.5 | CH | C-20 |
| 18.9 | $CH_2$ | C-6 | 31.1 | $CH_2$ | C-21 |
| 34.0 | $CH_2$ | C-7 | 37.5 | $CH_2$ | C-22 |
| 40.2 | C | C-8 | 26.1 | $CH_3$ | C-23 |
| 48.2 | CH | C-9 | 65.3 | $CH_2$ | C-24 |
| 38.6 | C | C-10 | 17.2 | $CH_3$ | C-25 |
| 23.9 | $CH_2$ | C-11 | 17.4 | $CH_3$ | C-26 |
| 125.6 | CH | C-12 | 23.9 | $CH_3$ | C-27 |
| 139.3 | C | C-13 | 179.9 | C | C-28 |
| 42.6 | C | C-14 | 17.1 | $CH_3$ | C-29 |
| 28.7 | $CH_2$ | C-15 | 21.4 | $CH_3$ | C-30 |

## VA-10

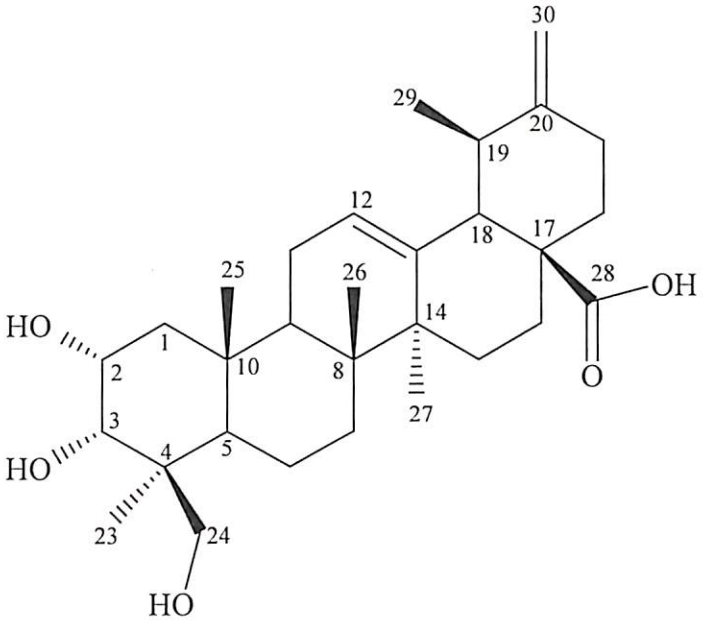
It was obtained as a white amorphous powder, m.p. 258-260 °C. The molecular formula, $C_{30}H_{46}O_5$, was deduced from elemental analysis and LC-MS [*m/z* 485 $(M - H)^-$] data.

The IR (KBr) spectrum of VA-10 showed bands at $\nu_{max}$ 3429 (hydroxyl) and 1684 $cm^{-1}$ (COOH). The $^1H$ NMR spectral data (Fig. 5.16, Table 5.14) showed the presence of two hydroxymethine protons [δ 4.62 (1H, br s, H-3) and δ 4.48 (1H, m, H-2), an olefinic proton (δ 5.36, 1H, br s, H-12), a methine proton (δ 2.77, 1H, d, *J* = 10.0 Hz, H-18) of an ursane derivative[1] and AB signals at δ 4.14 and δ 3.83 (*J* = 10.4 Hz, H-24). In addition the $^1H$ NMR spectral data showed the presence of two olefinic proton signals at δ 4.77, br s and δ 4.75, br s, attributable to an exocyclicmethylene group.[29] The presence of an exocyclicmethylene group is supported by the $^{13}C$ NMR signals at δ 156.0 and δ 105.6.

The $^{13}C$ NMR spectral data (Fig. 5.17, Table 5.15) showed the presence of two hydroxymethine carbons (δ 67.6, C-2 and 75.5, C-3), an hydroxymethylene carbon (δ 66.5, C-24) and two olefinic carbons (δ 126.2, C-12 and δ 140.9, C-13). These assignments were supported by the DEPT (Fig. 5.18) spectral data and correlations observed in the HMQC (Fig. 5.19) spectrum.

A careful scrutiny of the above spectral data in comparison with those of **5.09** indicated that in VA-10, a secondary methyl group present in **5.09** is replaced by an exocyclicmethylene group. The presence of doublet signal (δ 2.77, *J* = 10.0) of H-18 supports the presence of C-29 secondary methyl group, intact. Consequently, the exocyclicdouble bond is placed between C-20 and C-30 in VA-10. As expected, the HMBC (Fig. 5.20) spectrum showed correlations between exocyclicmethylene protons and C-19 (δ 39.3) and C-21 (δ 35.2).

Based on the above the structure of VA-10 was established as 2α,3α,24-trihydroxyurs-12,20(30)-dien-28-oic acid (**5.10**), which was isolated earlier from *Prunella vulgaris*[27] as a methyl ester.




**5.10**

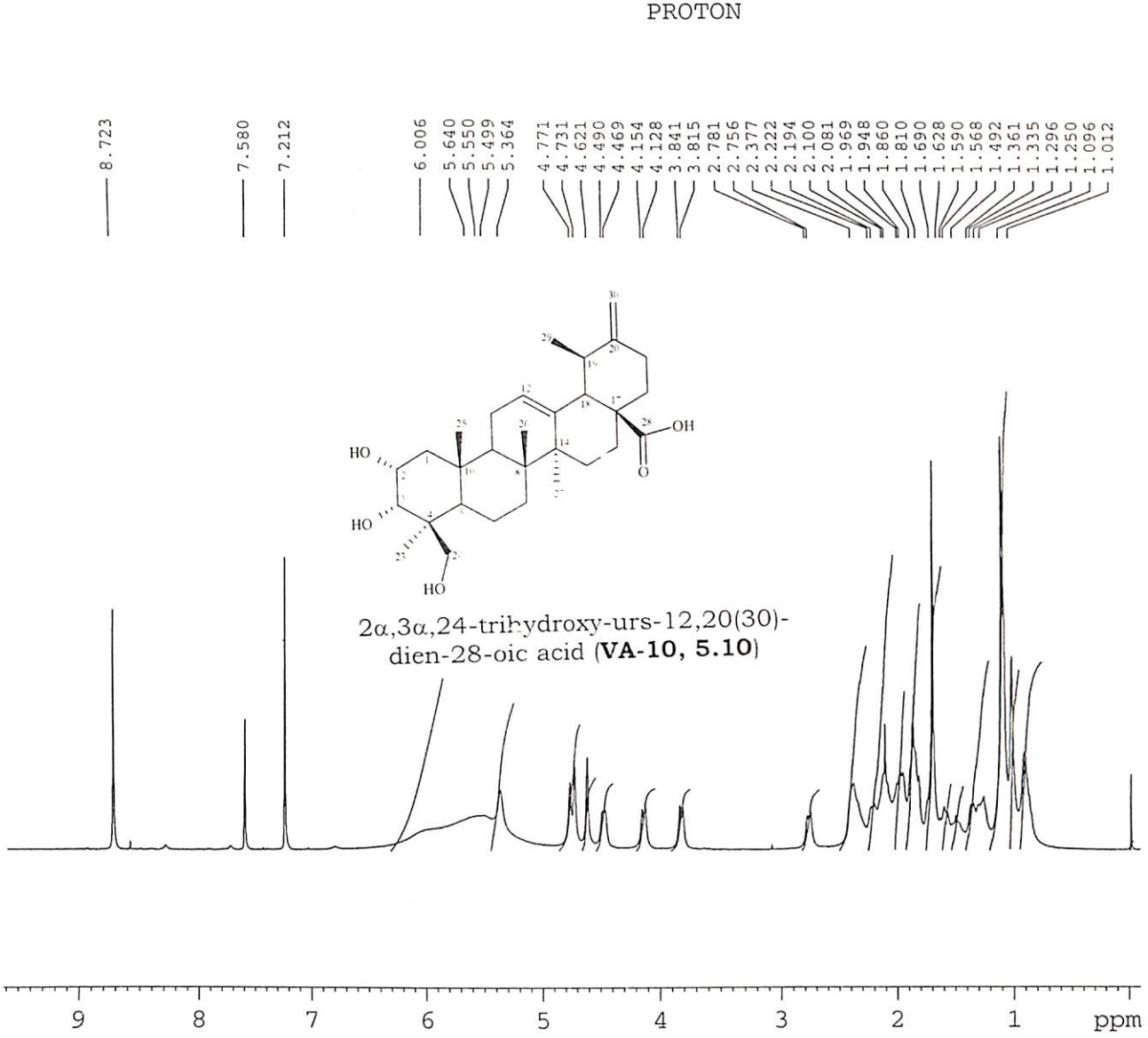



Fig. 5.16: $^1$H NMR spectrum (400 MHz, $d_5$-Pyridine) of compound **VA-10** (**5.10**)

## TABLE 5.14

**$^1$H NMR spectral data of VA-10 (2α,3α,24-trihydroxyurs-12,20(30)-dien-28-oic acid, 5.10)**
**(Fig. 5.16, 400 MHz spectrum, $d_5$-Pyridine)**

| **Chemical shift (δ)** | **Proton integration** | **Multiplicity (*J* in Hz)** | **Assignment** |
|---|---|---|---|
| 4.48 | 1H | m | H-2 |
| 4.62 | 1H | br s | H-3 |
| 5.36 | 1H | br s | H-12 |
| 2.77 | 1H | d (10.0) | H-18 |
| 4.14 | 1H | d (10.4) | $H_a$-24 |
| 3.83 | 1H | d (10.4) | $H_b$-24 |
| 4.77 | 1H | br s | $H_a$-30 |
| 4.75 | 1H | br s | $H_b$-30 |
| 1.63 | 3H | s | H-23 |
| 0.91-1.10 | 12H | m | H-25-H-29 |

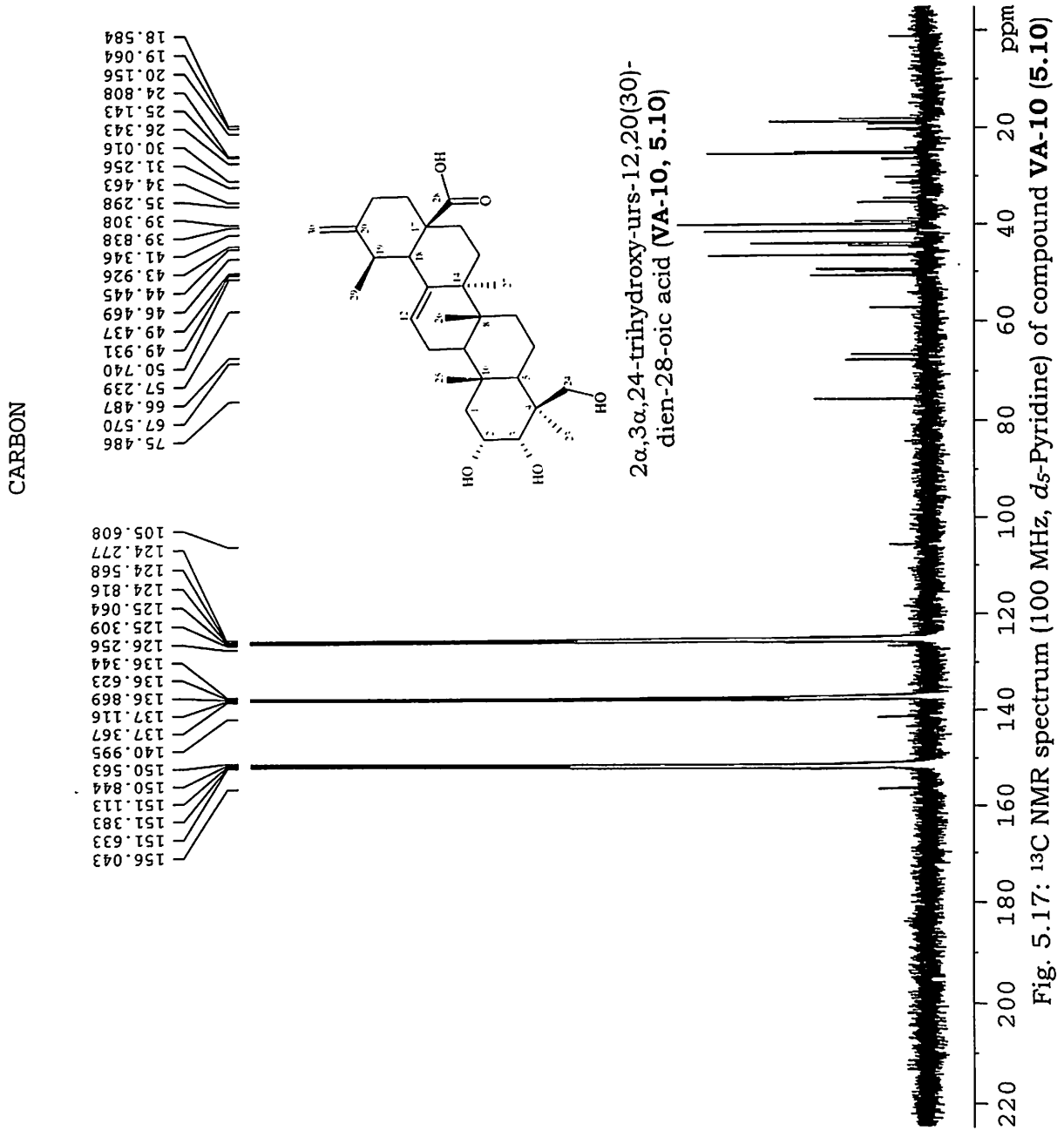
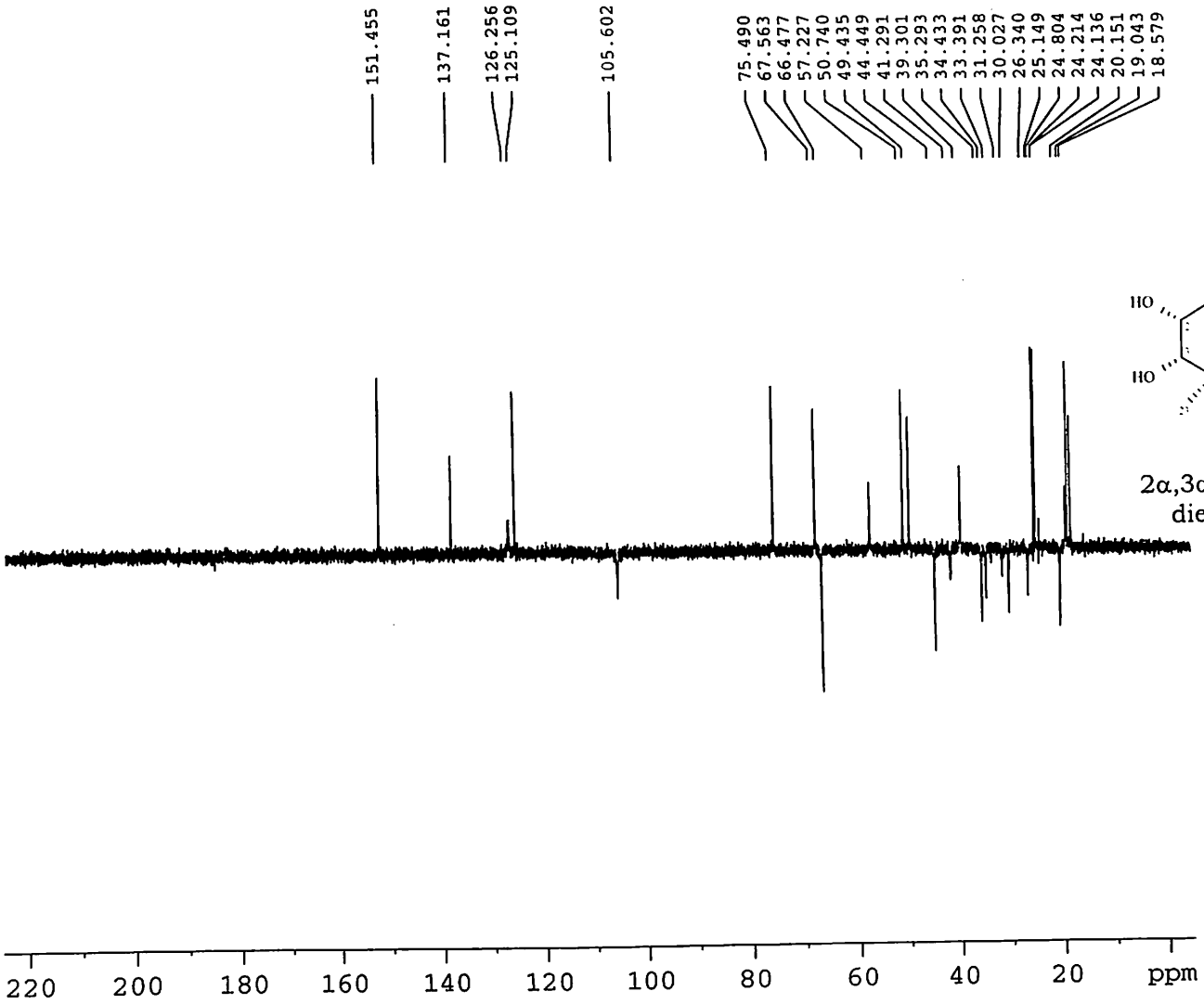
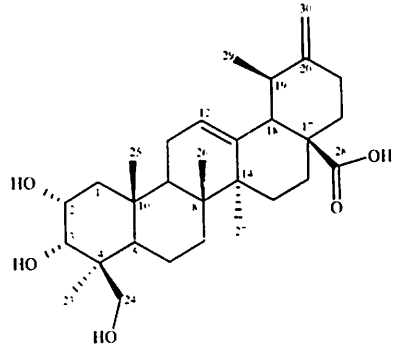



Fig. 5.17: $^{13}C$ NMR spectrum (100 MHz, $d_5$-Pyridine) of compound **VA-10 (5.10)**




2α,3α,24-trihydroxy-urs-12,20(30)-dien-28-oic acid (**VA-10, 5.10**)

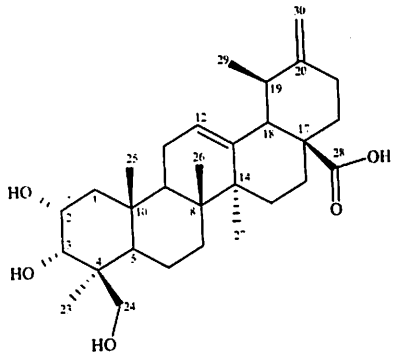
Fig. 5.18: DEPT spectrum ($d_5$-Pyridine) of compound **VA-10,( 5.10)**

## TABLE 5.15

**$^{13}C$ NMR spectral data of VA-11 (2α,3α,24-trihydroxyurs-12,20(30)-dien-28-oic acid, 5.10)**
**(Fig. 5.17, 100 MHz spectrum, $d_5$-Pyridine)**

| Chemical shift (δ) | DEPT | Assignment | Chemical shift (δ) | DEPT | Assignment |
|---|---|---|---|---|---|
| 44.4 | $CH_2$ | C-1 | 26.3 | $CH_2$ | C-16 |
| 67.6 | CH | C-2 | 49.9 | C | C-17 |
| 75.5 | CH | C-3 | 57.2 | CH | C-18 |
| 46.4 | C | C-4 | 39.3 | CH | C-19 |
| 50.7 | CH | C-5 | 156.0 | C | C-20 |
| 20.1 | $CH_2$ | C-6 | 35.2 | $CH_2$ | C-21 |
| 34.4 | $CH_2$ | C-7 | 41.2 | $CH_2$ | C-22 |
| 41.3 | C | C-8 | 25.1 | $CH_3$ | C-23 |
| 49.4 | CH | C-9 | 66.5 | $CH_2$ | C-24 |
| 39.8 | C | C-10 | 18.5 | $CH_3$ | C-25 |
| 24.2 | $CH_2$ | C-11 | 18.0 | $CH_3$ | C-26 |
| 126.2 | CH | C-12 | 24.8 | $CH_3$ | C-27 |
| 140.9 | C | C-13 | [a] | C | C-28 |
| 43.9 | C | C-14 | 19.0 | $CH_3$ | C-29 |
| 30.0 | $CH_2$ | C-15 | 105.6 | $CH_2$ | C-30 |

[a] signal not observed




2α,3α,24-trihydroxy-urs-12,20(30)-dien-28-oic acid (**VA-10, 5.10**)

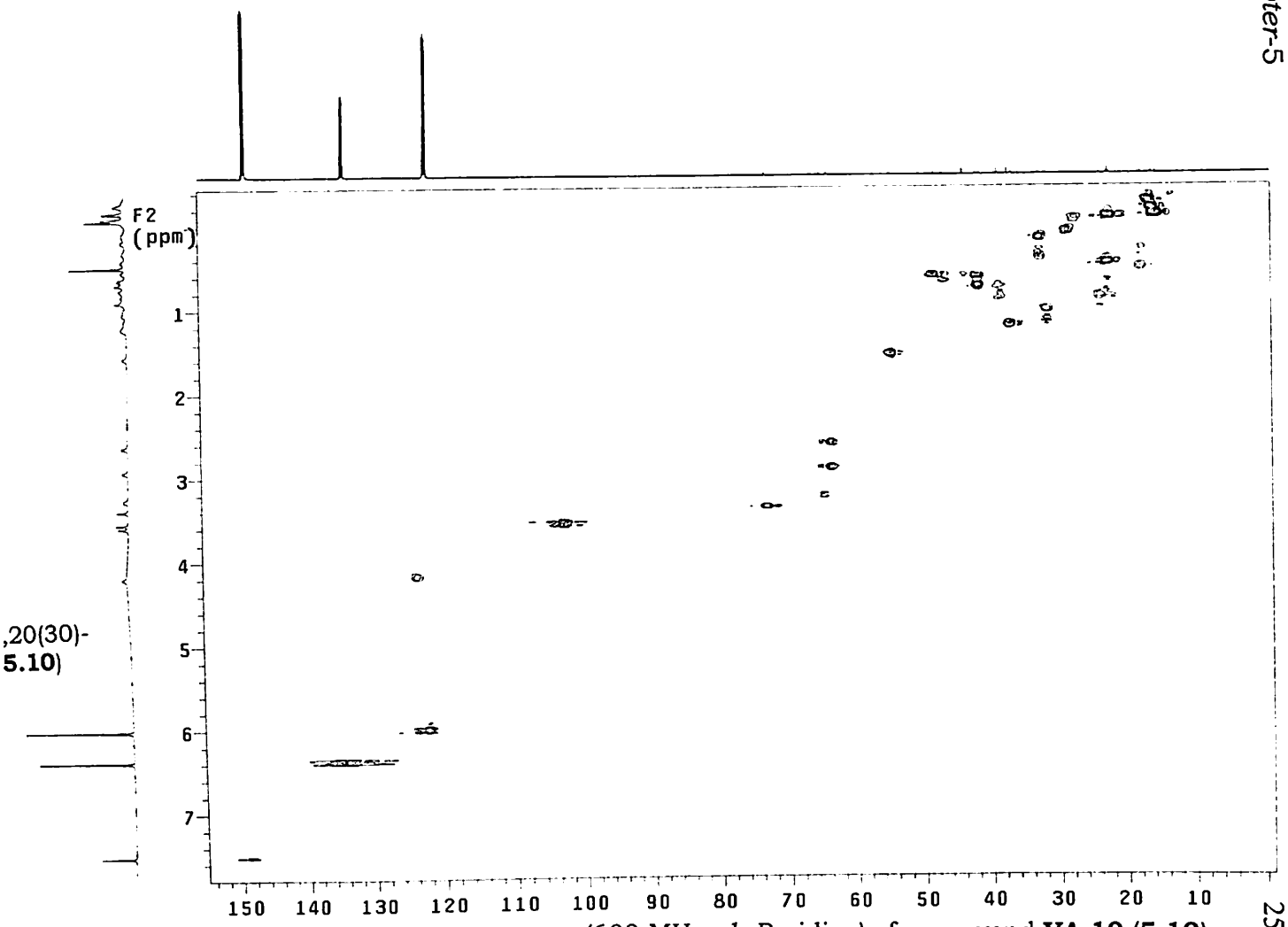
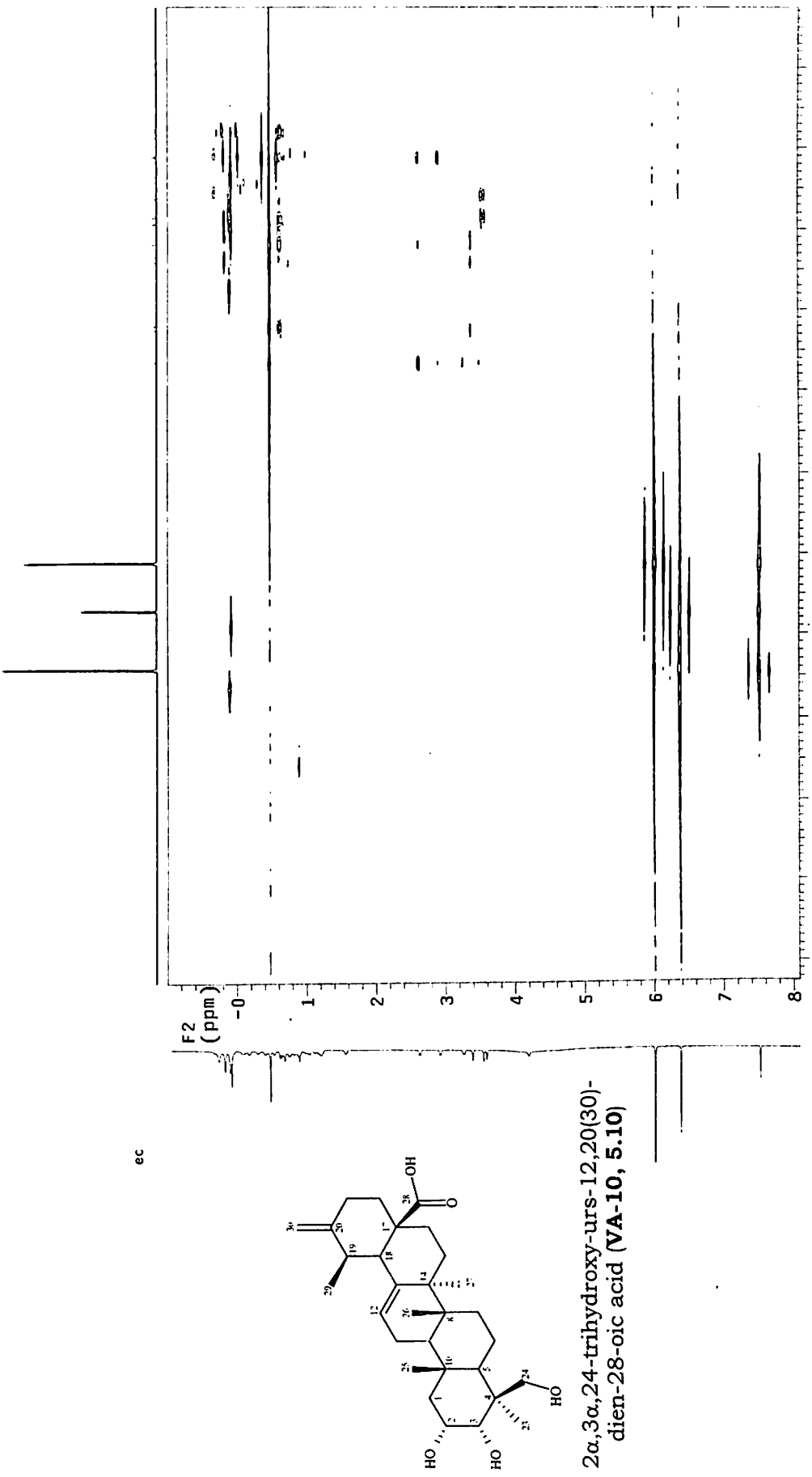



Fig. 5.19: HMQC spectrum (600 MHz, $d_5$-Pyridine) of compound **VA-10 (5.10)**




2α,3α,24-trihydroxy-urs-12,20(30)-dien-28-oic acid (**VA-10, 5.10**)

Fig. 5.20: HMBC spectrum (600 MHz, $d_5$-Pyridine) of compound **VA-10 (5.10)**

## SECTION-II
## (Oleanane derivatives)

### VA-08

It was obtained as a white amorphous powder, m.p. 252-254 °C. The molecular formula, $C_{30}H_{48}O_4$, was deduced from elemental analysis and LC-MS [m/z 471 $(M - H)^-$ data. It showed positive LB test for triterpenoids.

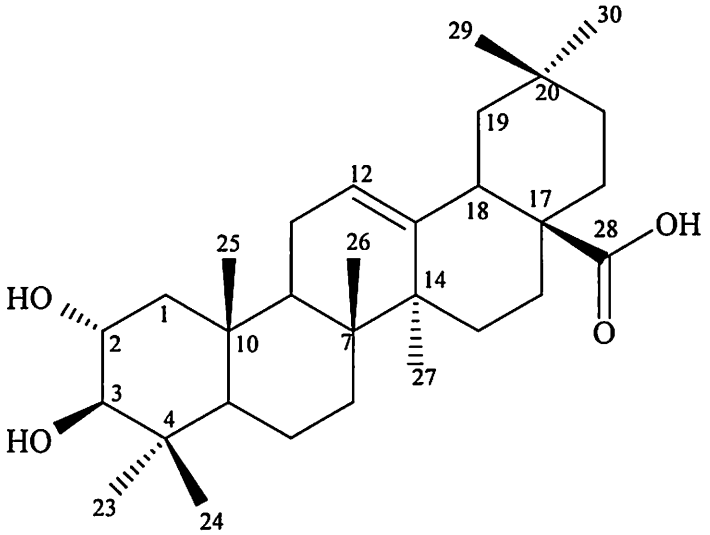
The IR (KBr) spectrum of VA-08 showed the presence of bands at $\nu_{max}$ 3435 (hydroxyl) and 1695 $cm^{-1}$ (COOH). The $^1H$ NMR spectral (Fig. 5. 21, Table 5.16) data showed the presence of two hydroxymethine protons [δ 4.08, br s, H-2 and δ 3.39, br s, H-3), an olefinic proton (δ 5.45, br s, H-12) and a downfield methine proton (δ 3.29, m,) characteristic of H-18 of oleanane derivatives.[1] The $^1H$ NMR spectrum also showed the presence of seven tertiary methyls (but poorly resolved) in the region δ 1.25-0.97 indicative of an oleanane skeleton[2] in VA-08.

A study of the above spectral data in comparison with those of corosolic acid (**5.02**) revealed that VA-08 is a 2,3-dihydroxyolean-12-en-28-oic acid. The relative stereochemistry at C-2 and C-3 was deduced from the spectral data of the diacetyl derivative (VA-08a). The $^1H$ NMR spectral (Fig. 5.22, Table 5.17) data of VA-08a showed the presence of two acetoxymethine protons [δ 5.09 (1H, ddd, $J$ = 11.6,10.3,4.2 Hz, H-2) and δ 4.74 (1H, d, $J$ = 10.3 Hz, H-3)]. The coupling constant $^3J_{H\text{-}2,H\text{-}3}$ (10.3 Hz) is consistent with the trans diaxial orientation[13] of H-2 and H-3 protons and acetoxyls could be assigned as 2-α and 3-β and so were the hydroxyls in VA-08.

Literature survey on oleanane derivatives revealed that the above physical and spectral data of VA-08 were in good agreement with those recorded for maslinic acid (2α,3β-dihydroxyolean-12-en-28-oic acid,

**5.11**) isolated earlier from *Lagerstroemia speciosa*[14] and *Eriobotrya japonica* calli.[30]

Based on the foregoing the structure of VA-08 was deduced as maslinic acid (**5.11**).




**5.11**

Maslinic acid has been reported to possess hypoglycemic,[14] anti-inflammatory[31] and anti-oxidant activities.[32]

## TABLE 5.16

**$^1$H NMR spectral data of VA-08 (Maslinic acid, 5.11) (Fig. 5.21, 400 MHz spectrum, $d_5$-Pyridine)**

| **Chemical shift (δ)** | **Proton integration** | **Multiplicity (*J* in Hz)** | **Assignment** |
|---|---|---|---|
| 4.08 | 1H | br s | H-2 |
| 3.39 | 1H | br s | H-3 |
| 5.45 | 1H | br s | H-12 |
| 3.29 | 1H | m | H-18 |
| 1.25 | 3H | s | H-23 |
| 1.19 | 3H | s | H-27 |
| 1.06-0.93 | 15H | m | H-24-H-26, H-29 and H-30 |

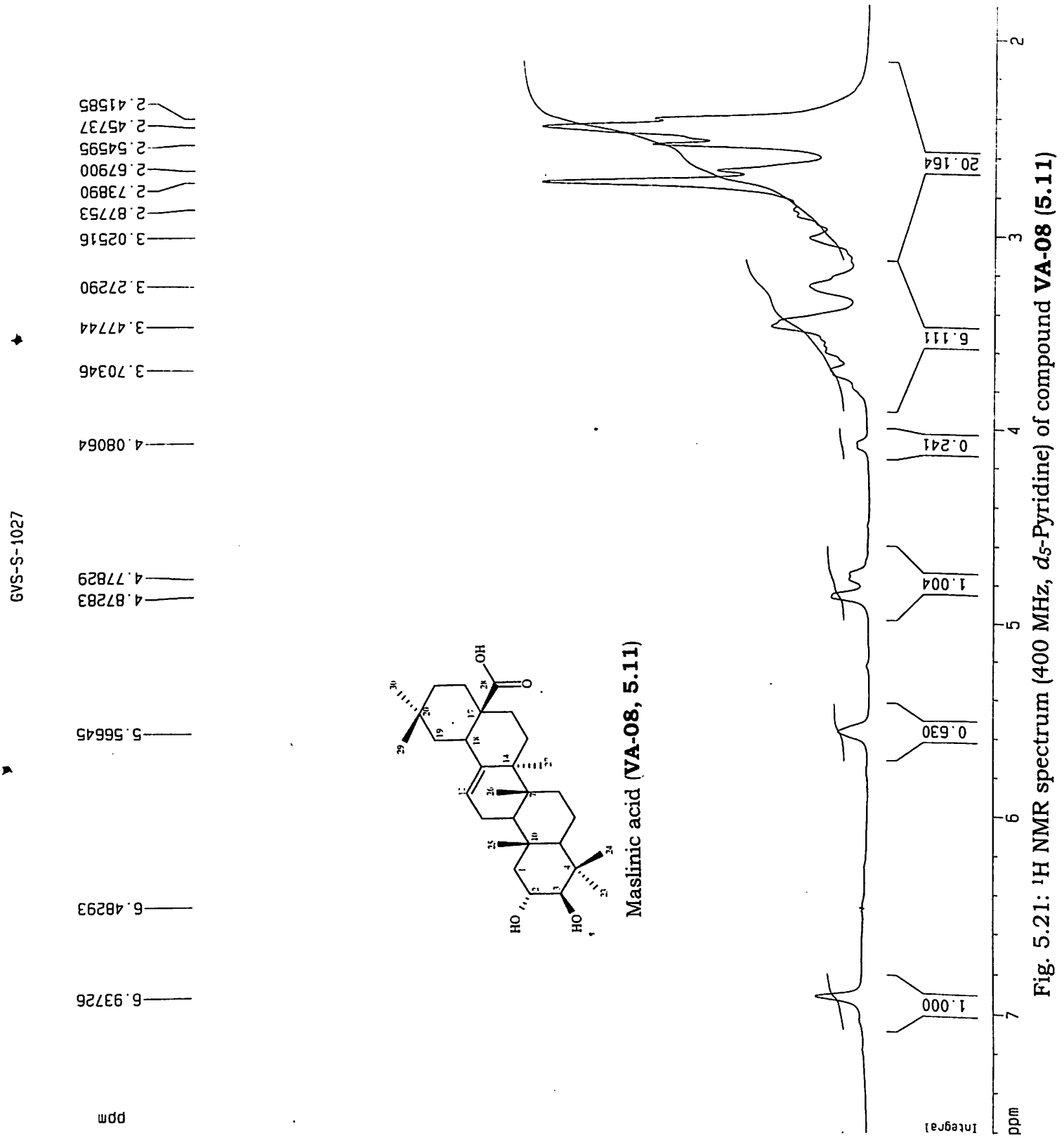



Fig. 5.21: $^1$H NMR spectrum (400 MHz, $d_5$-Pyridine) of compound **VA-08 (5.11)**

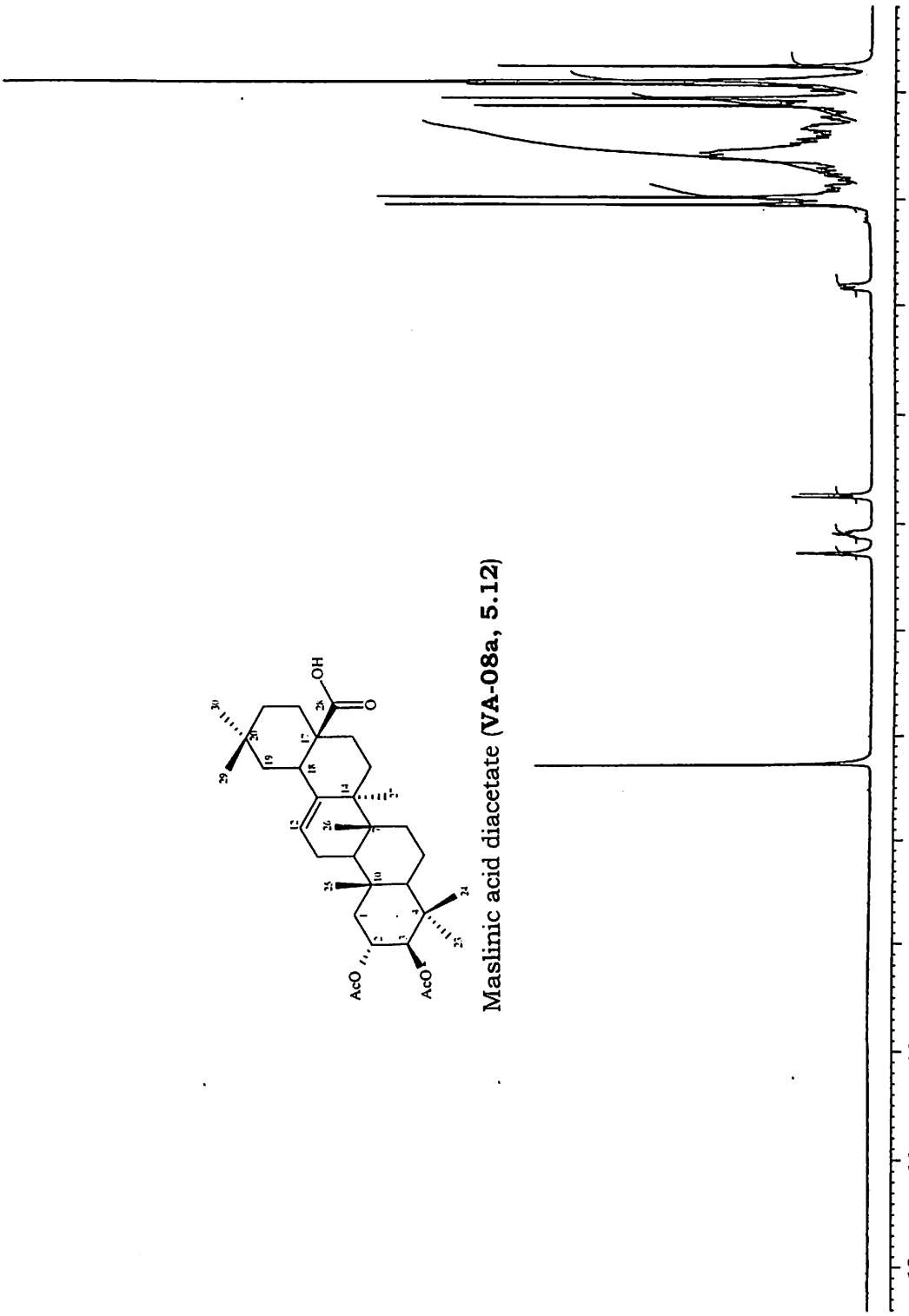
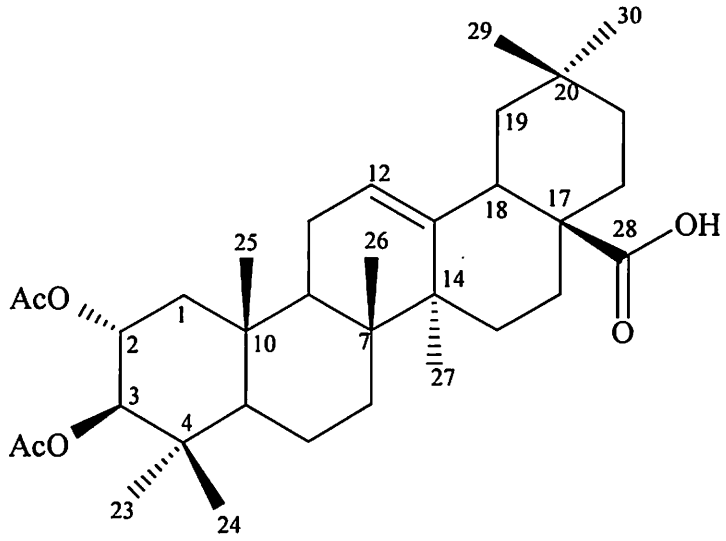



Fig. 5.22: $^1$H NMR spectrum (400 MHz, $CDCl_3$) of compound **VA-08a (5.12)**




**5.12**

## TABLE 5.17

**$^1$H NMR spectral data of VA-08a (Maslinic acid diacetate, 5.12)**

**(Fig. 5.22, 400 MHz spectrum, $CDCl_3$)**

| Chemical shift (δ) | Proton integration | Multiplicity (*J* in Hz) | Assignment |
|---|---|---|---|
| 5.09 | 1H | ddd (11.6, 10.3, 4.2) | H-2 |
| 4.74 | 1H | d (10.3) | H-3 |
| 5.28 | 1H | br s | H-12 |
| 2.83 | 1H | dd (9.8,3.0) | H-18 |
| 0.90 | 3H | s | H-23 |
| 0.98 | 3H | s | H-24 |
| 1.06 | 3H | s | H-25 |
| 0.76 | 3H | s | H-26 |
| 1.13 | 3H | s | H-27 |
| 0.93 | 3H | s | H-29 |
| 0.93 | 3H | s | H-30 |
| 1.97 | 3H | s | -$OCOCH_3$ |
| 2.05 | 3H | s | -$OCOCH_3$ |

## VA-03

It was obtained as a white amorphous powder, m.p. 108-110 °C. The molecular formula, $C_{34}H_{52}O_6$, was deduced from elemental analysis and LC-MS [m/z 555 $(M - H)^-$] data.

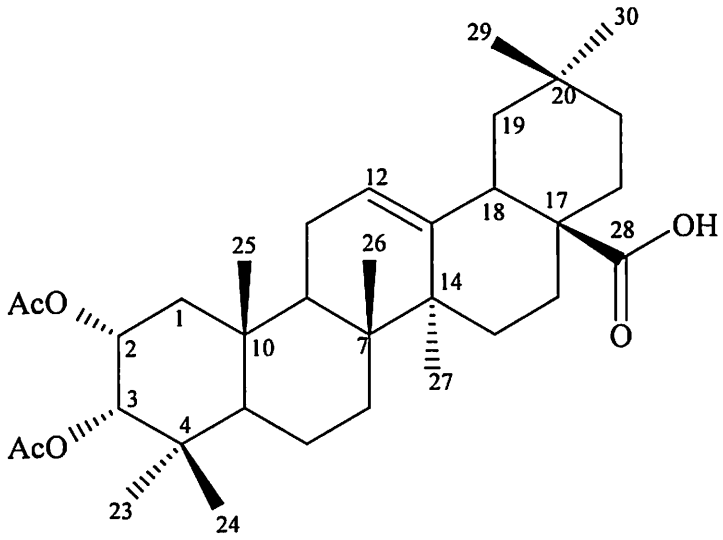
The IR (KBr) spectrum showed bands at $\nu_{max}$ 1742 (ester carbonyl) and 1697 $cm^{-1}$ (COOH). The $^1H$ NMR spectral data (Fig. 5.23, Table 5.18) showed the presence of two acetoxyl groups (δ 2.11, 3H, s and δ 1.96, 3H, s), two acetoxymethine protons [δ 5.24 (1H, m, H-2) and δ 4.96 (1H, d, $J$ = 2.0 Hz, H-3)], a trisubstituted olefinic proton (δ 5.28, br s, H-12) and a methine proton signal at δ 2.83 (1H, dd, $J$ = 13.5,3.6 Hz) characteristic of H-18 of oleanane derivatives.[1] The presence of oleanane skeleton was further ascertained by the presence of seven tertiary methyl signals (δ 1.18, 1.03, 0.97, 0.93, 0.91, 0.87 and 0.76 each 3H, s).

The $^{13}C$ NMR spectral data (Fig. 5.24, Table 5.19) showed the presence of a carboxylic acid (δ 183.5, C-28), two acetoxymethine carbons (δ 77.0, C-3 and δ 68.2, C-2) and two olefinic carbons (δ 122.3 and 143.6) attributable to C-12 and C-13 of oleanane derivatives.[11,33]

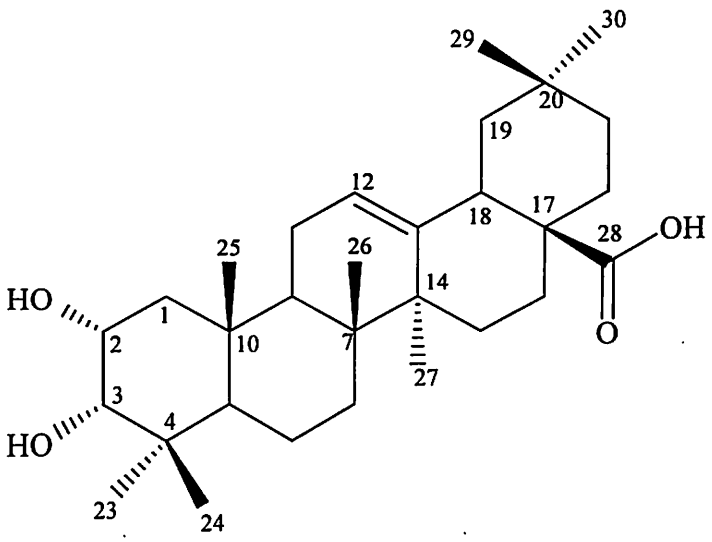
The above spectral data of **VA-03** in comparison with those of maslinic acid (**5.11**) and maslinic acid diacetate (**5.12**) revealed that VA-03 was also a 2,3-dihydroxyolean-12-en-28-oic acid derivative. The coupling constant $^3J_{H\text{-}2,H\text{-}3}$ (2.0 Hz) is consistent with the cis orientation of H-2β and H-3β protons and acetoxyls could be assigned as 2α-OAc and 3α-OAc.

Based on the above the structure of VA-03 could be deduced as 2α,3α-diacetoxyolean-12-en-28-oic acid (epimaslinic acid diacetate, **5.13**). From the foregoing, natural triterpenoid in the present extract could be derived as 2α,3α-dihydroxyolean-12-en-28-oic acid

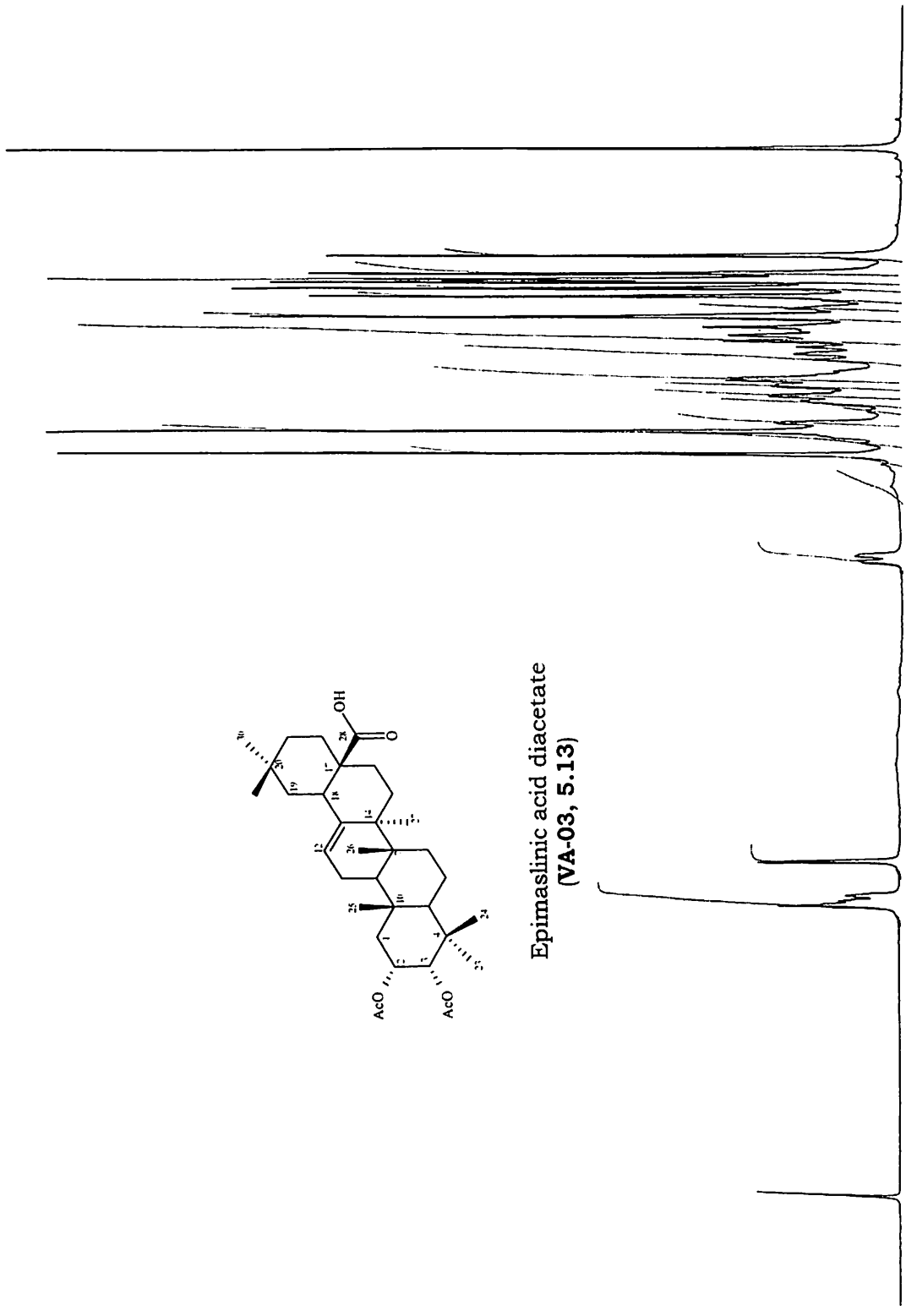
(epimaslinic acid, **5.14**), a triterpenoid isolated earlier from *Shorea acuminata*[34] and *Euonymus revolutus*.[35]




**5.13**




**5.14**



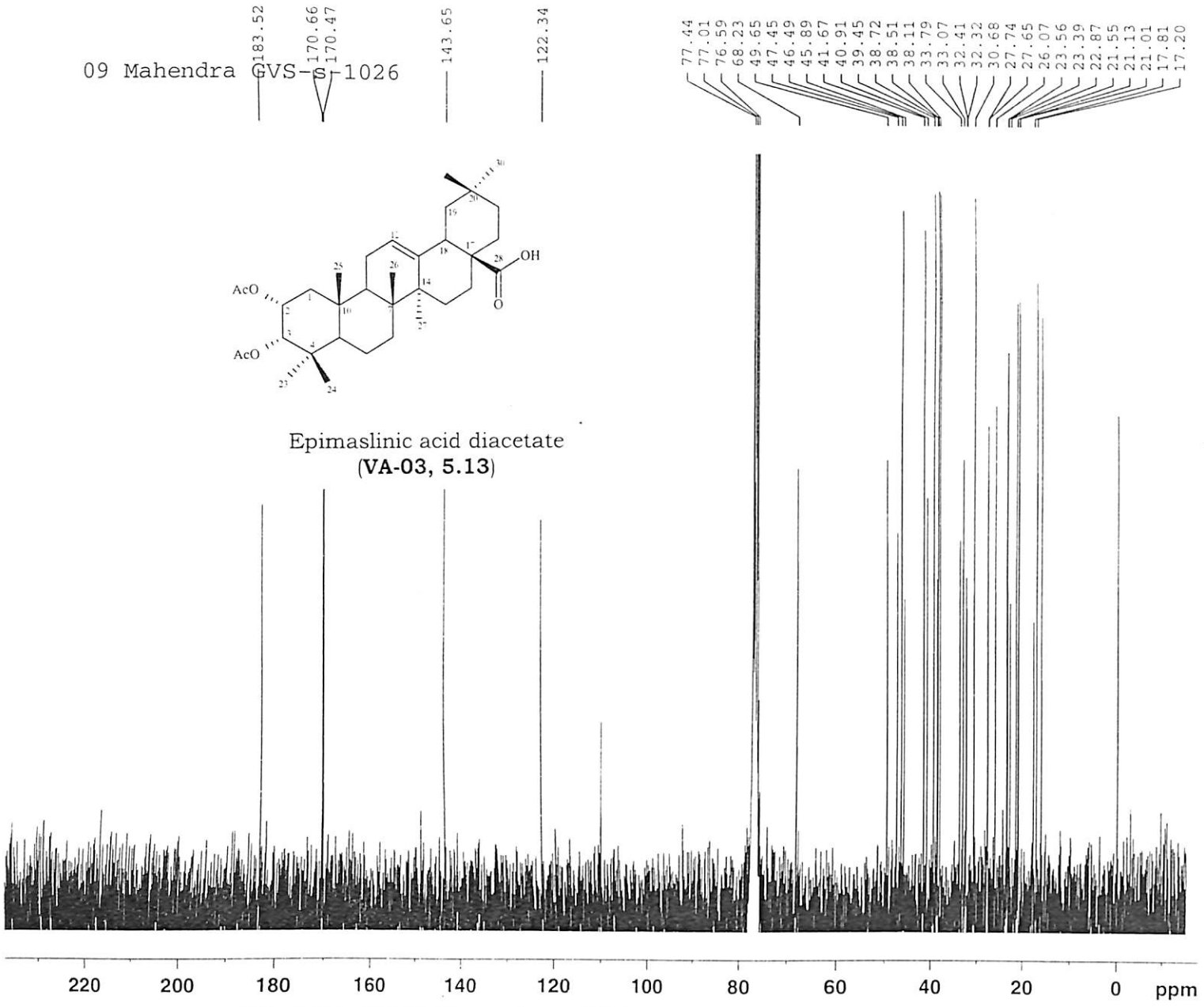

Epimaslinic acid diacetate
**(VA-03, 5.13)**

Fig. 5.23: $^1$H NMR spectrum (300 MHz, $CDCl_3$) of compound **VA-03 (5.13)**

## TABLE 5.18

**$^1$H NMR spectral data of VA-03 (epimaslinic acid diacetate, 5.13) (Fig. 5.23, 300 MHz spectrum, $CDCl_3$)**

| **Chemical shift (δ)** | **Proton integration** | **Multiplicity (*J* in Hz)** | **Assignment** |
|---|---|---|---|
| 5.24 | 1H | m | H-2 |
| 4.96 | 1H | d (2.0) | H-3 |
| 5.28 | 1H | br s | H-12 |
| 2.83 | 1H | dd (13.5, 3.6) | H-18 |
| 0.87 | 3H | s | H-23 |
| 0.97 | 3H | s | H-24 |
| 1.03 | 3H | s | H-25 |
| 0.76 | 3H | s | H-26 |
| 1.18 | 3H | s | H-27 |
| 0.91 | 3H | s | H-29 |
| 0.93 | 3H | s | H-30 |
| 2.11 | 3H | s | -$OCOCH_3$ |
| 1.96 | 3H | s | -$OCOCH_3$ |




Epimaslinic acid diacetate
(**VA-03, 5.13**)

Fig. 5.24: $^{13}C$ NMR spectrum (75 MHz, $CDCl_3$) of compound **VA-03** (**5.13**)

## TABLE 5.19

**$^{13}C$ NMR spectral data of VA-03 (epimaslinic acid diacetate, 5.13) (Fig. 5.24, 75 MHz spectrum, $CDCl_3$)**

| Chemical shift (δ) | DEPT | Assignment | Chemical shift (δ) | DEPT | Assignment |
|---|---|---|---|---|---|
| 38.7 | $CH_2$ | C-1 | 40.9 | CH | C-18 |
| 68.2 | CH | C-2 | 45.9 | $CH_2$ | C-19 |
| 77.0 | CH | C-3 | 30.7 | C | C-20 |
| 38.5 | C | C-4 | 33.8 | $CH_2$ | C-21 |
| 49.6 | CH | C-5 | 33.1 | $CH_2$ | C-22 |
| 17.8 | $CH_2$ | C-6 | 27.6 | $CH_3$ | C-23 |
| 32.4 | $CH_2$ | C-7 | 21.5 | $CH_3$ | C-24 |
| 39.4 | C | C-8 | 16.1 | $CH_3$ | C-25 |
| 47.4 | CH | C-9 | 17.2 | $CH_3$ | C-26 |
| 38.1 | C | C-10 | 26.0 | $CH_3$ | C-27 |
| 22.9 | $CH_2$ | C-11 | 183.5 | C | C-28 |
| 122.3 | CH | C-12 | 32.3 | $CH_3$ | C-29 |
| 143.6 | C | C-13 | 23.6 | $CH_3$ | C-30 |
| 41.7 | C | C-14 | 170.6 | C | -O<u>C</u>O$CH_3$ |
| 27.7 | $CH_2$ | C-15 | 170.5 | C | -O<u>C</u>O$CH_3$ |
| 23.4 | $CH_2$ | C-16 | 21.1 | $CH_3$ | -OCO$CH_3$ |
| 46.5 | C | C-17 | 21.0 | $CH_3$ | -OCO$CH_3$ |

## EXPERIMENTAL

**Ursolic acid (VA-01, 5.01, 45 mg)**

It was obtained as a white amorphous powder from methanol, m.p. 285-287 °C.

Found : C, 79.10%; H, 10.72%

$C_{30}H_{48}O_3$ requires : C, 78.94%; H, 10.52%

IR (KBr) : $\nu_{max}$ 3430, 2927, 2859, 1691, 1459, 1381, and 1281 $cm^{-1}$

$^1H$ NMR : Fig. 5.01, Table 5.01

**Corosolic acid (VA-07, 5.02, 800 mg)**

It was obtained as a white amorphous powder from methanol, m.p. 243-245 °C.

$[\alpha]_D^{25}$ : +40° (*c* 0.4, MeOH)

Found : C, 76.42%; H, 10.32%

$C_{30}H_{48}O_4$ requires : C, 76.27%; H, 10.16%

IR (KBr) : $\nu_{max}$ 3424, 2972, 1693, 1457, 1377, 1235 and 1048 $cm^{-1}$

$^1H$ NMR : Fig. 5.02, Table 5.02

$^{13}C$ NMR : Fig. 5.03, Table 5.03

**Epicorosolic acid (VA-02, 5.03, 25 mg)**

It was obtained as a white amorphous powder from methanol; m.p. 195-197 °C.

Found : C, 76.53%; H, 10.25%

$C_{30}H_{48}O_4$ requires : C, 76.27%; H, 10.16%

IR (KBr) : $\nu_{max}$ 3433, 2929, 1693, 1457, 1378, 1235, 1036 and 826 $cm^{-1}$

$^1H$ NMR : Fig. 5.04, Table 5.04

**Epicorosolic acid diacetate (VA-02a, 5.04, 75 mg)**

It was obtained as a white amorphous powder from methanol, m.p. 102-104 °C.

| | |
|---|---|
| Found | : C, 73.45%; H, 9.42% |
| $C_{34}H_{52}O_6$ requires | : C, 73.38%; H, 9.35% |
| IR (KBr) | : $\nu_{max}$ 3434, 2929, 1744, 1698, 1634, 1456, 1371, 1249 and 1035 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 5.05, Table 5.05 |
| $^{13}C$ NMR | : Fig. 5.06, Table 5.06 |

**Euscaphic acid (VA-06, 5.05, 375 mg)**

It was obtained as a white amorphous powder from methanol, m.p. 268-270 °C.

| | |
|---|---|
| $[\alpha]_D^{25}$ | : +25° (*c* 0.4, MeOH) |
| Found | : C, 73.57%; H, 10.00% |
| $C_{30}H_{48}O_5$ requires | : C, 73.77%; H, 9.83% |
| IR (KBr) | : $\nu_{max}$ 3440, 2930, 1689, 1458, 1382, 1235 and 1040 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 5.07, Table 5.07 |
| $^{13}C$ NMR | : Fig. 5.08, Table 5.08 |
| DEPT | : Fig. 5.09 |

**Acetylation of Euscaphic acid**

A mixture of **5.05** (30 mg), pyridine (2.0 mL) and acetic anhydride (1.5 mL) was stirred at room temperature for 24 h. Usual work up followed by chromatography over silica gel using mixtures of hexane and ethyl acetate in increasing polarity as eluent gave euscaphic acid diacetate (**VA-06a, 5.06**, 20 mg), m.p. 185-187 °C. The IR (KBr) spectrum showed bands at $\nu_{max}$ 3462, 2969, 1741, 1455, 1371, 1253 and 1037 $cm^{-1}$. The $^1H$ NMR spectral data is presented in Fig. 5.10, Table 5.09.

**Euscaphic acid ester glucoside hexaacetate (VA-11a, 5.07)**

It was obtained as a white amorphous powder from methanol, m.p. 182-184 °C.

| | |
|---|---|
| Found | : C, 63.57%; H, 7.87% |
| $C_{48}H_{70}O_{16}$ requires | : C, 63.85%; H, 7.76% |
| IR (KBr) | : $\nu_{max}$ 3449, 2937, 1748, 1636, 1371, 1231 and 1037 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 5.11, Table 5.10 |
| $^{13}C$ NMR | : Fig. 5.12, Table 5.11 |
| DEPT | : Fig. 5.13 |

**2α,3α,24-Trihydroxyurs-12-en-28-oic acid (VA-09, 5.09)**

It was obtained as a white amorphous powder from methanol, m.p. 278-281 °C.

| | |
|---|---|
| Found | : C, 74.10%; H, 10.07% |
| $C_{30}H_{48}O_5$ requires | : C, 73.77%; H, 9.83% |
| IR (KBr) | : $\nu_{max}$ 3430, 2932, 1692, 1646, 1380, 1245 and 1035 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 5.14, Table 5.12 |
| $^{13}C$ NMR | : Fig. 5.15, Table 5.13 |

**2α,3α,24-Trihydroxyurs-12,20(30)-dien-28-oic acid (VA-10, 5.10)**

It was obtained as a white amorphous powder from methanol, m.p. 258-260 °C.

| | |
|---|---|
| Found | : C, 74.10%; H, 9.60% |
| $C_{30}H_{46}O_5$ requires | : C, 74.17%; H, 9.46% |
| IR (KBr) | : $\nu_{max}$ 3429, 2932, 1684, 1639, 1543, 1454, 1036 and 888 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 5.16, Table 5.14 |
| $^{13}C$ NMR | : Fig. 5.17, Table 5.15 |
| DEPT | : Fig. 5.18 |
| HMQC | : Fig. 5.19 |
| HMBC | : Fig. 5.20 |

**Maslinic acid (VA-08, 5.11)**

It was obtained as a white amorphous powder from methanol, m.p. 252-254 °C.

| | |
|---|---|
| Found | : C, 76.37%; H, 10.30% |
| $C_{30}H_{48}O_4$ requires | : C, 76.27%; H, 10.16% |
| IR (KBr) | : $\nu_{max}$ 3424, 2972, 1694, 1454, 1048 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 5.21, Table 5.16 |

**Acetylation of maslinic acid**

A mixture of **5.11** (10 mg), pyridine (1.0 mL) and acetic anhydride (1.0 mL) was stirred at room temperature for 3 h. Usual work up followed by chromatography over silica gel using mixtures of hexane and ethyl acetate in increasing polarity as eluent gave maslinic acid diacetate (**VA-08a, 5.12**, 7.0 mg). The IR (KBr) spectrum showed bands at $\nu_{max}$ 2969, 1741, 1696, 1634, 1253 and 1035 $cm^{-1}$. The $^1H$ NMR spectral data is presented in Fig. 5.22, Table 5.17.

**Epimaslinic acid diacetate (VA-03, 5.13)**

It was obtained as a white amorphous powder from methanol, m.p. 108-110 °C.

| | |
|---|---|
| Found | : C, 73.52%; H, 9.50% |
| $C_{34}H_{52}O_6$ requires | : C, 73.38%; H, 9.35% |
| IR (KBr) | : $\nu_{max}$ 3424, 2972, 1694, 1454, 1048 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 5.23, Table 5.18 |
| $^{13}C$ NMR | : Fig. 5.24, Table 5.19 |

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# STRUCTURE ELUCIDATION OF FLAVONOIDS ISOLATED FROM *V. ALTISSIMA*

The details of isolation of three flavonoids (**VA-15**, **VA-19** and **VA-20**) have been described in **chapter-2.** The details of structure elucidation of these flavonoids are presented in the following pages.

## VA-15

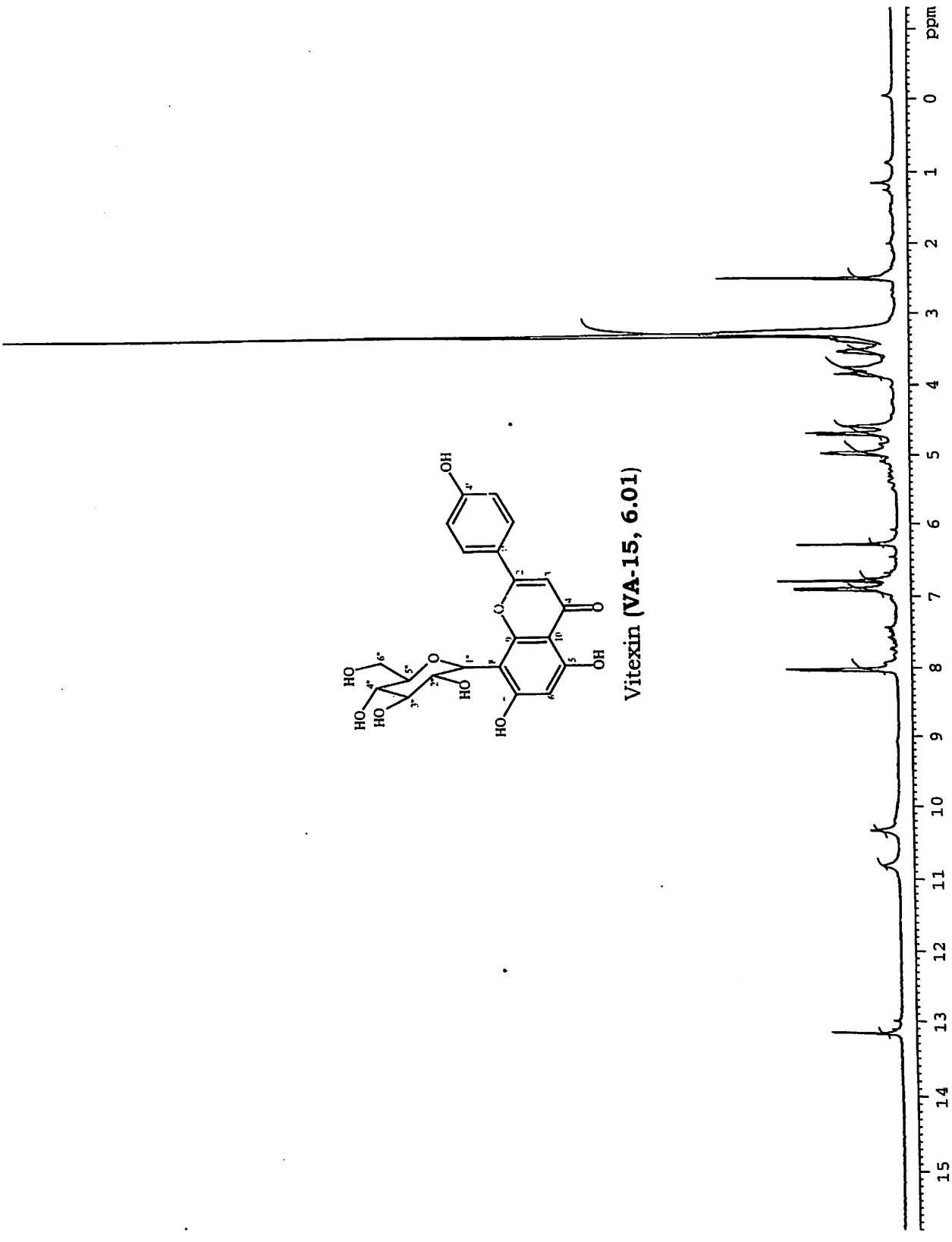
It was obtained as a yellow amorphous powder from methanol, m.p. 275-277 °C. The molecular formula, $C_{21}H_{20}O_{10}$, was deduced from elemental analysis and LC-MS [*m/z* 431 $(M - H)^-$] data.

VA-15 exhibited bands in its UV (MeOH) spectrum at $\lambda_{max}$ 269 and 332 nm. Its IR (KBr) spectrum showed bands at $\nu_{max}$ 3381 (hydroxyl), 1652 (α,β-unsaturated carbonyl), 1568 and 1501 $cm^{-1}$ (aromatic). The $^1H$ NMR (Fig. 6.01, Table 6.01) spectral data showed the presence of a chelated hydroxyl (δ 13.11, 1H, s), an aromatic singlet proton (δ 6.28, 1H, s, H-6) and a downfield olefinic proton (δ 6.78, 1H, s), characteristic of H-3 of a flavonoid nucleus.[1] It also showed the presence of two doublet signals at δ 8.04 (2H, d, *J* = 8.5 Hz) and δ 6.90 (2H, d, *J* = 8.5 Hz) attributable to a para disubstituted phenyl unit.[2]

In addition, the $^1H$ NMR spectrum showed a series of signals between δ 4.69 and 3.19, characteristic of a sugar unit.[3] The absence of usual O-glycosidic anomeric proton signal and the presence of signal at δ 4.69 (1H, d, *J* = 9.7 Hz), attributable to H-1 of the sugar unit, indicated the presence of a C-glycoside.[3]

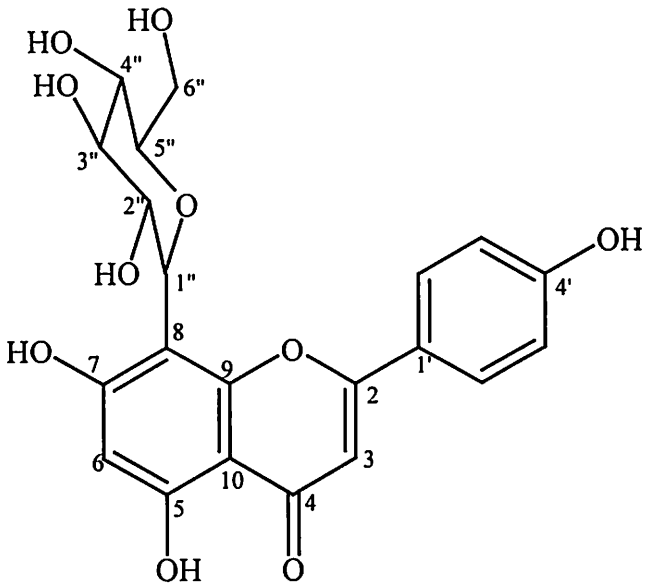
Literature survey on C-glucosylflavonoids, revealed that the physical and spectral data of VA-15 were in good agreement with those recorded for vitexin[4] (**6.01**), isolated earlier from this species[5] and also from *Vitex agnus-castus*[6] and *Vitex peduncularis*.[7,8]

Based on the foregoing, the structure of VA-15 was established as vitexin (**6.01**)




Vitexin (**VA-15, 6.01**)

Fig. 6.01: $^1$H NMR spectrum (400 MHz, $d_6$-DMSO) of compound **VA-15 (6.01)**




**6.01**

## TABLE 6.01

**$^1$H NMR spectral data of VA-15 (Vitexin, 6.01)**

**(Fig. 6.01, 400 MHz spectrum, $d_6$-DMSO)**

| Chemical shift (δ) | Proton integration | Multiplicity (*J* in Hz) | Assignment |
|---|---|---|---|
| 13.11 | 1H | s | 5-OH |
| 6.78 | 1H | s | H-3 |
| 6.28 | 1H | s | H-6 |
| 8.04 | 2H | d (8.5) | H-2′ and H-6′ |
| 6.90 | 2H | d (8.5) | H-3′ and H-5′ |
| 4.69 | 1H | d (9.7) | H-1″ |
| 3.78-3.19 | 6H | m | H-2″-H-6″ |

## VA-19

It was obtained as a yellow amorphous powder. The molecular formula, $C_{28}H_{24}O_{13}$, was deduced from elemental analysis and LC-MS [*m/z* 567 $(M - H)^-$] data.

VA-19 exhibited bands in its UV (MeOH) spectrum at $\lambda_{max}$ 267 and 342 nm. The IR (KBr) spectrum showed bands at 3345 (hydroxyl), 1645 (α,β-unsaturated ketone) and 1565 and 1468 $cm^{-1}$ (aromatic). The $^1H$ NMR spectral (Fig. 6.02, Table 6.02) data showed the presence of a chelated hydroxyl (δ 13.11, 1H, s) characteristic of 5-OH of flavonoids,[9] four phenolic hydroxyls (δ 11.11, 10.26, 10.11 and 9.16 each 1H, s), an olefinic proton signal at δ 6.69 (1H, s) assignable to H-3 of flavonoids[1] and an aromatic proton signal at δ 6.08 (1H, s) revealed the presence of a trisubstituted A ring in VA-19. The $^1H$ NMR spectrum showed the presence of an ABX system [δ 7.65 (1H, br d, *J* = 8.5 Hz), δ 7.57 (1H, br s) and δ 6.92 (1H, d, *J* = 8.5 Hz)] characteristic of a 1,2,4-trisubstituted phenyl unit.[10] In addition, the $^1H$ NMR spectrum showed a series of signals between δ 4.99 and 3.40 indicative of a sugar moiety.[3] The presence of a signal at δ 4.99 (1H, d, *J* = 10.0 Hz), attributable to H-1″ of the sugar unit, revealed the presence of a β-*C*-glycoside[3] in VA-19. A fact supported further by the correlation observed between anomeric proton δ 4.99 (1H, d, *J* = 10.0 Hz) and C-1″ ($\delta_C$ 71.6) in the HMQC spectrum.

The $^1H$ NMR spectrum also showed the presence of four aromatic protons constituted by two AB doublets [δ 7.59 (2H, d, *J* = 8.5 Hz) and δ 6.75 (2H, d, *J* = 8.5 Hz) attributable to a *p*-hydroxybenzoyl unit.[11]

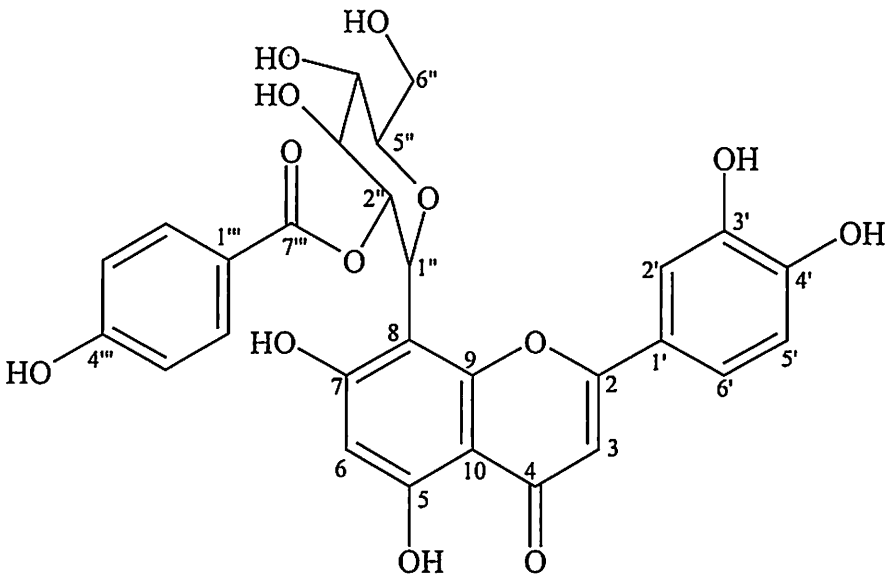
The above spectral data indicated the presence of luteolin,[11] C-glucoside and *p*-hydroxybenzoyl units in VA-19.

The $^{13}C$ NMR spectral data (Fig. 6.03, Table 6.03) showed the presence of a ketone carbonyl (δ 182.4), ester carbonyl (δ 165.1), two olefinic carbons (δ 164.6 and 102.9) and a series of signals at δ 82.7, 76.5, 72.9, 71.2 and 61.8 attributable to a C-glucoside moiety. These assignments were supported by the DEPT (Fig. 6.04) spectral data and the correlations observed in the HMQC (Fig. 6.05, Table 6.04) spectrum.

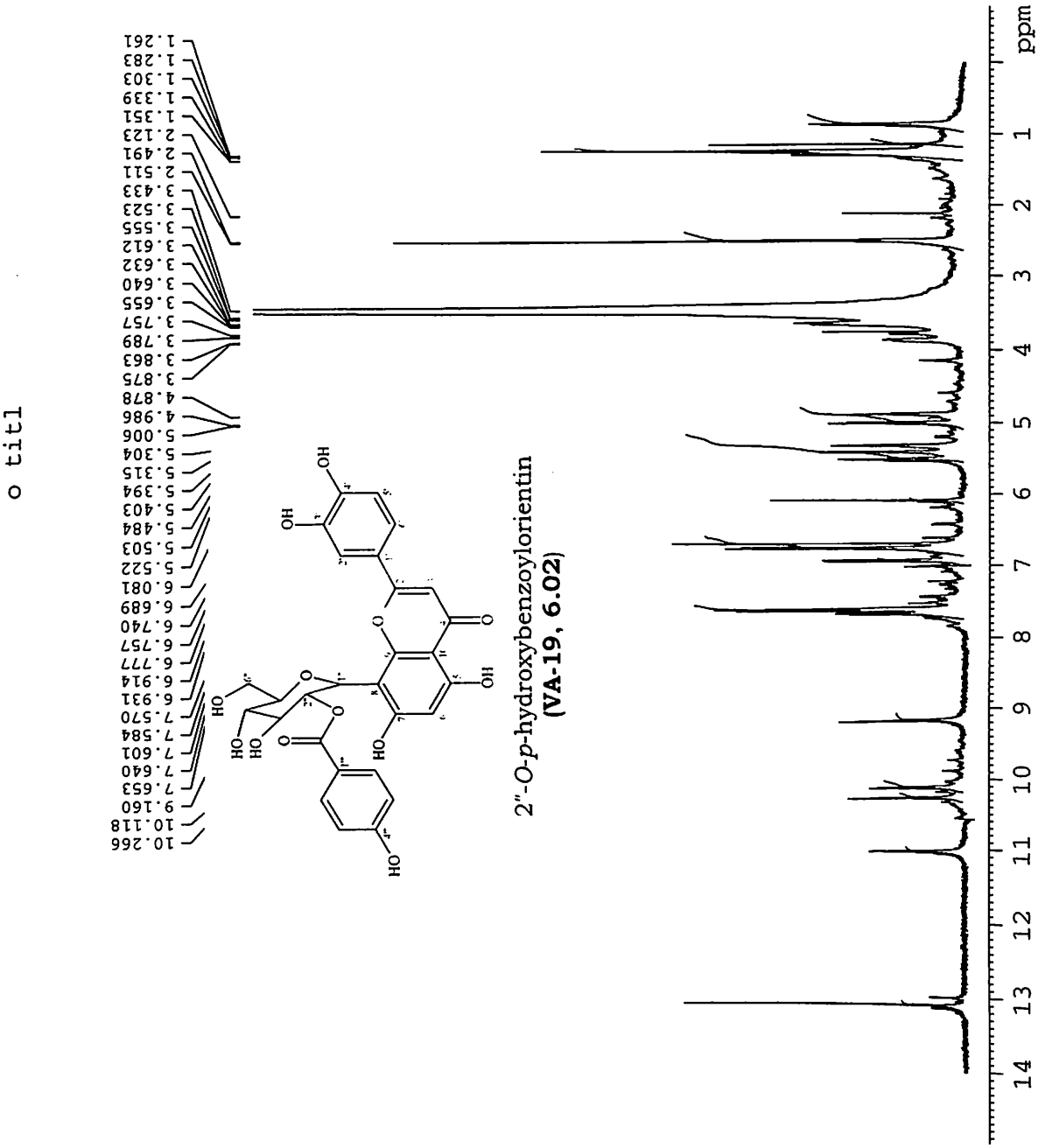
The observed $^{13}C$ NMR chemical shifts of methine (δ 98.1, C-6) and the C-glycosylated carbon (δ 104.3, C-8) of ring-A revealed the presence of C-glucosyl unit on C-8 in VA-19. Because, in orientin derivatives, a C-8 glycosylated flavonoid, the C-8 and C-6 carbons resonate at δ 103-105 and δ 96-98. Whereas in isoorientin (C-6 glycosylated flavonoid) the C-6 and C-8 resonate at δ 107-109 and δ 93-95 respectively.[12] The presence of C-8 glycosyl substitution in VA-19 was supported further by the HMBC correlations (Fig. 6.06, Table 6.05) observed between anomeric proton [4.99 (1H, d, $J$ = 10.0 Hz)] and the quaternary carbons $\delta_C$ 162.5 (C-7) and $\delta_C$ 156.8 (C-9).

The upfield shift[11] to the extent of 2.0 ppm observed for C-1″ ($\delta_C$ 71.6) in comparison with orientin indicated the location of *p*-hydroxybenzoyl unit on C-2″, a fact supported further by the HMBC correlations between H-2″ (δ 5.50, 1H, t, $J$ = 9.5 Hz) and the ester carbonyl ($\delta_C$ 165.1, C-7‴).

Based on the foregoing, the structure of VA-19 was deduced as 2″-*O*-*p*-hydroxybenzoylorientin (**6.03**) a new acylated flavonoid.




**6.02**




2″-*O*-*p*-hydroxybenzoylorientin
**(VA-19, 6.02)**

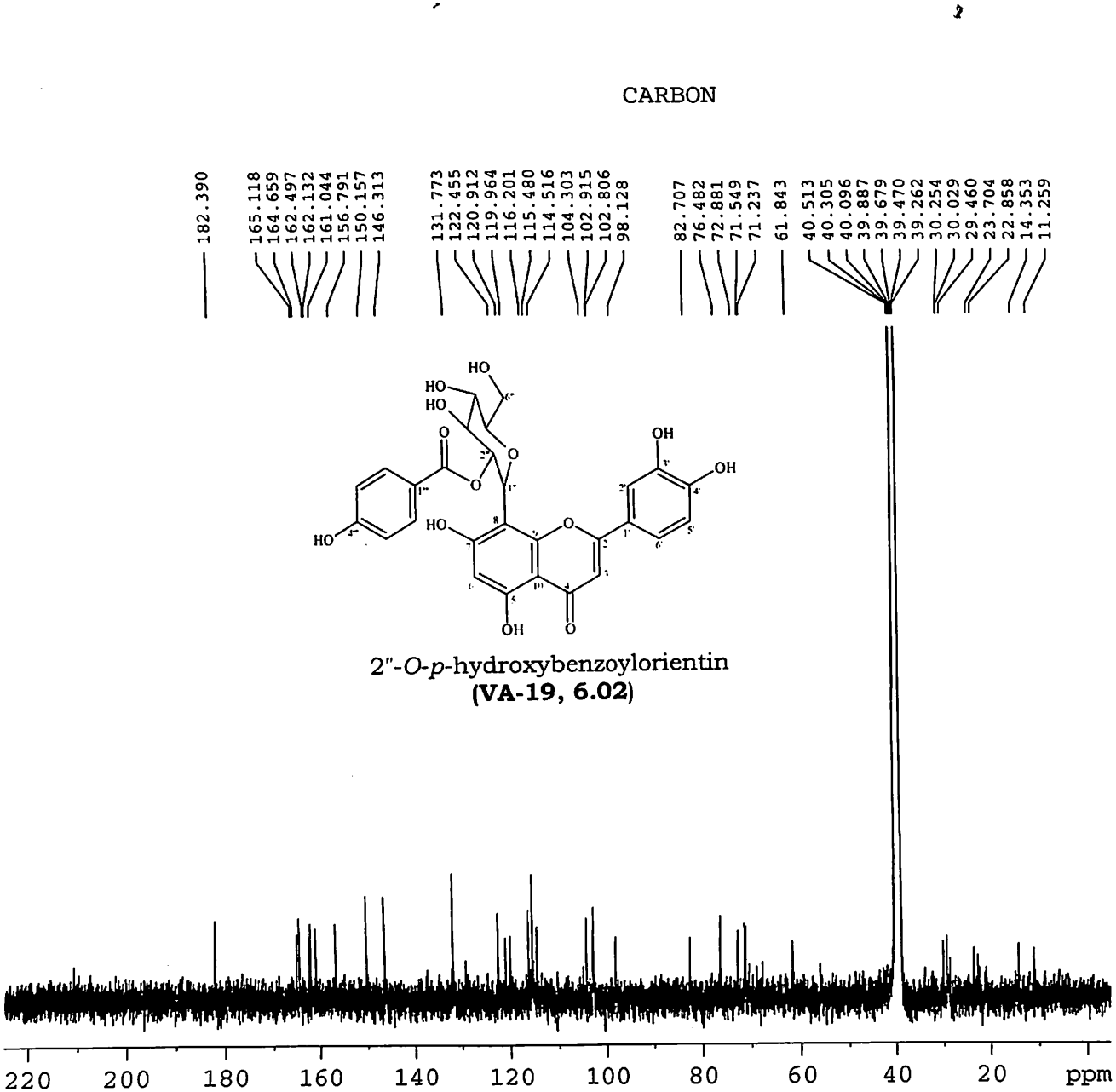
Fig. 6.02: $^1$H NMR spectrum (500 MHz, $d_6$-DMSO) of compound **VA-19 (6.02)**

## TABLE 6.02

**$^1$H NMR spectral data of VA-19**
**(2″-*O*-*p*-hydroxybenzoylorientin, 6.02)**

**(Fig. 6.02, 500 MHz spectrum, $d_6$-DMSO)**

| **Chemical shift (δ)** | **Proton integration** | **Multiplicity (*J* in Hz)** | **Assignment** |
|---|---|---|---|
| 13.11 | 1H | s | 5-OH |
| 11.11 | 1H | s | -OH |
| 10.26 | 1H | s | -OH |
| 10.11 | 1H | s | -OH |
| 9.16 | 1H | s | -OH |
| 6.69 | 1H | s | H-3 |
| 6.08 | 1H | s | H-6 |
| 7.57 | 1H | br s | H-2′ |
| 6.92 | 1H | d (8.5) | H-5′ |
| 7.65 | 1H | br d (8.5) | H-6′ |
| 4.99 | 1H | d (10.0) | H-1″ |
| 5.50 | 1H | t (9.5) | H-2″ |
| 3.87-3.40 | 5H | m | H-3″, H-4″, H-5″ and H-6″ |
| 7.59 | 2H | d (8.5) | H-2‴ and H-6‴ |
| 6.75 | 2H | d (8.5) | H-3‴ and H-5‴ |




2″-*O*-*p*-hydroxybenzoylorientin
**(VA-19, 6.02)**

Fig. 6.03: $^{13}C$ NMR spectrum (100 MHz, $d_6$-DMSO) of compound **VA-19 (6.02)**

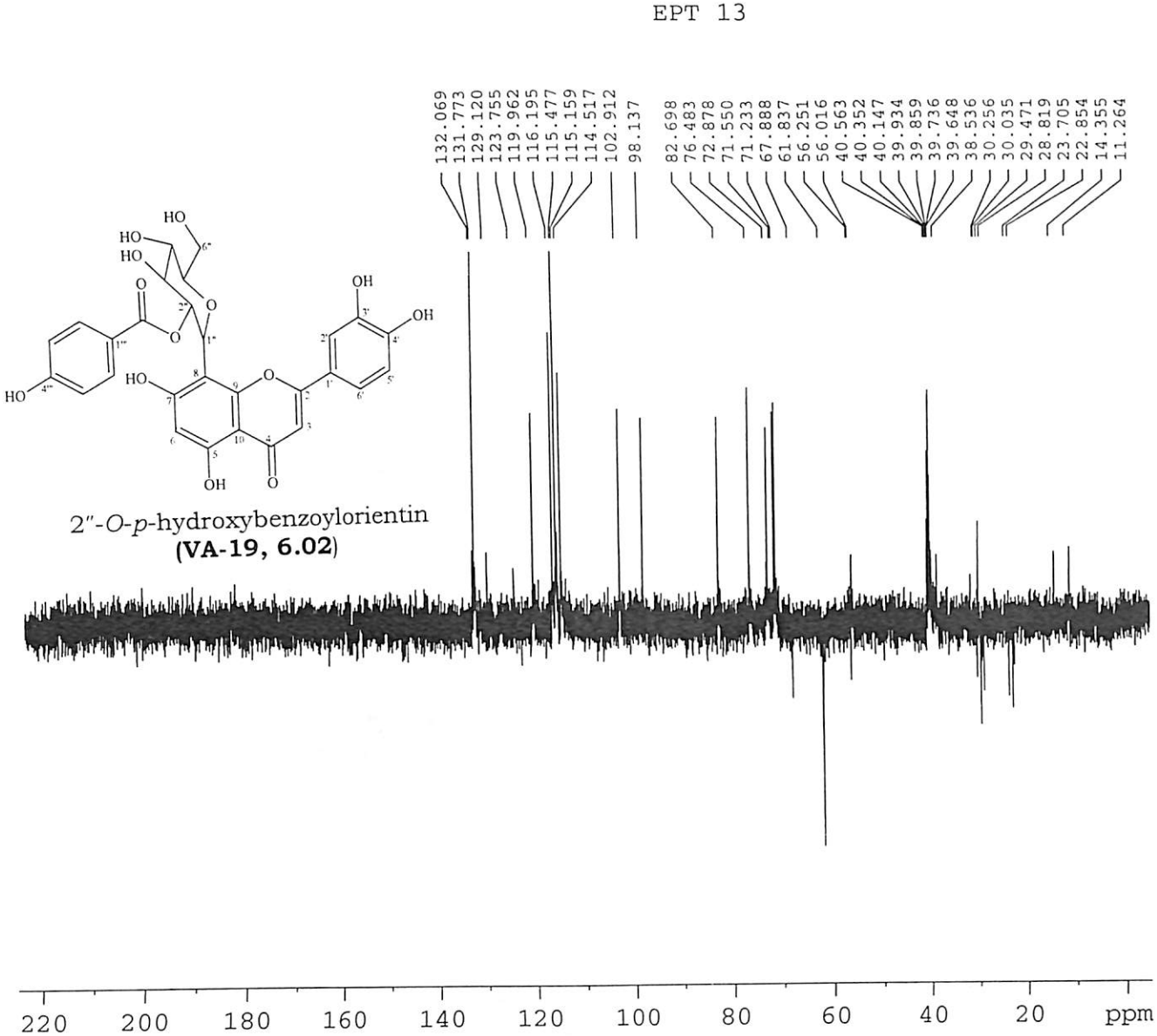


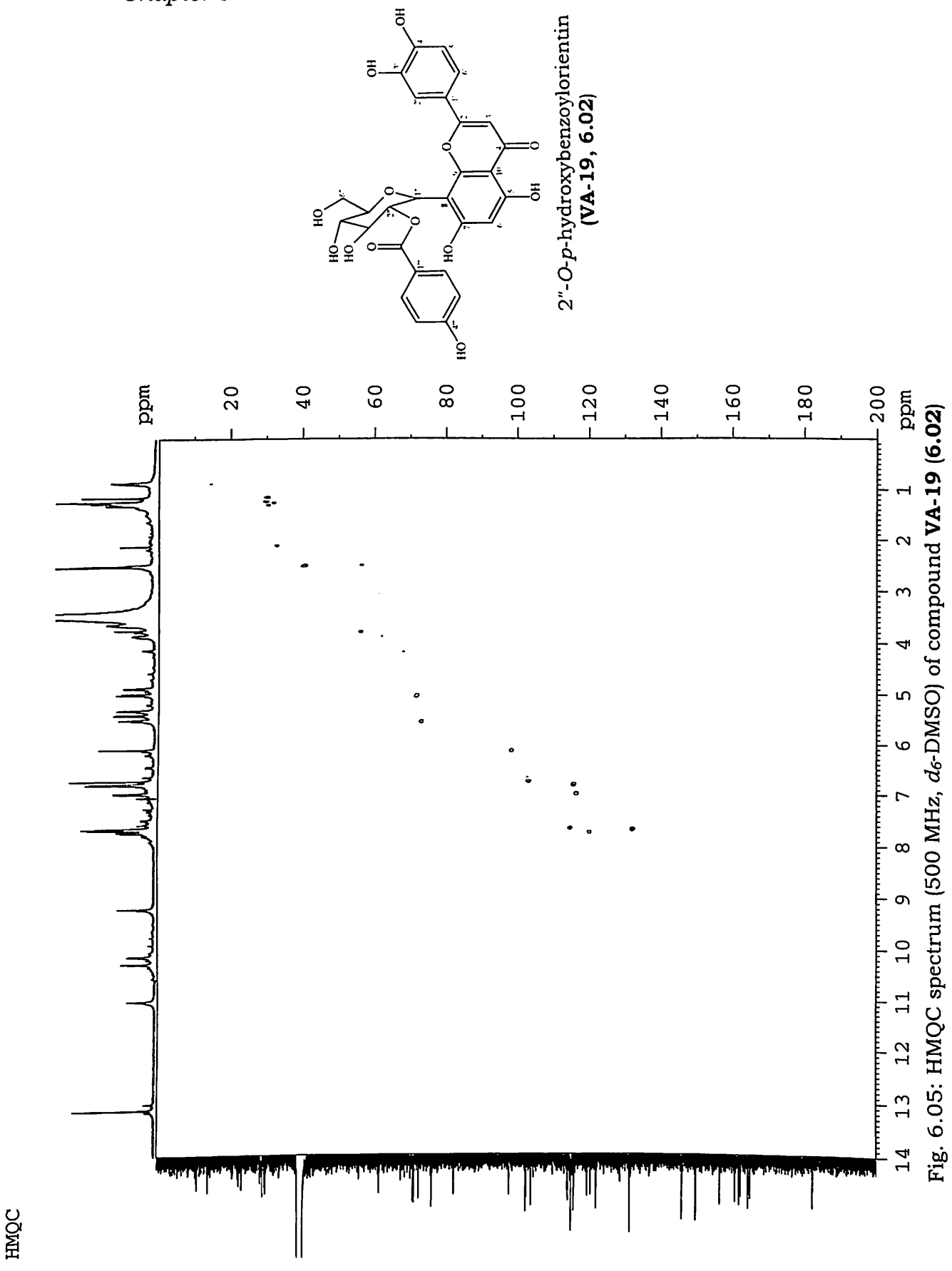

Fig. 6.04: DEPT spectrum ($d_6$-DMSO) of compound **VA-19** (**6.02**)

## TABLE 6.03

**$^{13}$C NMR spectral data of VA-19**
**(2″-*O*-*p*-hydroxybenzoylorientin, 6.02)**

**(Fig. 6.03, 100 MHz spectrum, $d_6$-DMSO)**

| Carbon number | Chemical shift (δ) | Carbon number | Chemical shift (δ) |
|---|---|---|---|
| C-2 | 164.6 | C-6′ | 119.9 |
| C-3 | 102.9 | C-1″ | 71.6 |
| C-4 | 182.4 | C-2″ | 72.9 |
| C-5 | 161.0 | C-3″ | 76.5 |
| C-6 | 98.1 | C-4″ | 71.2 |
| C-7 | 162.5 | C-5″ | 82.7 |
| C-8 | 104.3 | C-6″ | 61.8 |
| C-9 | 156.8 | C-1‴ | 120.9 |
| C-10 | 102.8 | C-2‴ | 131.8 |
| C-1′ | 122.4 | C-3‴ | 115.5 |
| C-2′ | 114.5 | C-4‴ | 162.1 |
| C-3′ | 146.3 | C-5‴ | 115.5 |
| C-4′ | 150.1 | C-6‴ | 131.8 |
| C-5′ | 116.2 | C-7‴ | 165.1 |




2″-*O*-*p*-hydroxybenzoylorientin
**(VA-19, 6.02)**

Fig. 6.05: HMQC spectrum (500 MHz, $d_6$-DMSO) of compound **VA-19 (6.02)**

## TABLE 6.04

**HMQC spectral data of VA-19**
**(2″-*O*-*p*-hydroxybenzoylorientin, 6.02)**

**(Fig. 6.05, solvent, $d_6$-DMSO)**

| **Proton chemical shift (δ)** | **Correlated carbon chemical shift (δ)** | **Assignment** |
|---|---|---|
| 6.69 (H-3) | 102.9 | C-2 |
| 6.08 (H-6) | 98.1 | C-6 |
| 7.57 (H-2′) | 114.5 | C-2′ |
| 6.92 (H-5′) | 116.2 | C-5′ |
| 7.65 (H-6′) | 119.9 | C-6′ |
| 4.99 (H-1″) | 71.6 | C-1″ |
| 5.50 (H-2″) | 72.9 | C-2″ |
| 3.87 (H-6″) | 61.8 | C-6″ |
| 7.59 (H-2‴ and H-6‴) | 131.8 | C-2‴ and C-6‴ |
| 6.75 (H-3‴ and H-5‴) | 115.5 | C-3‴ and C-5‴ |

HMBC

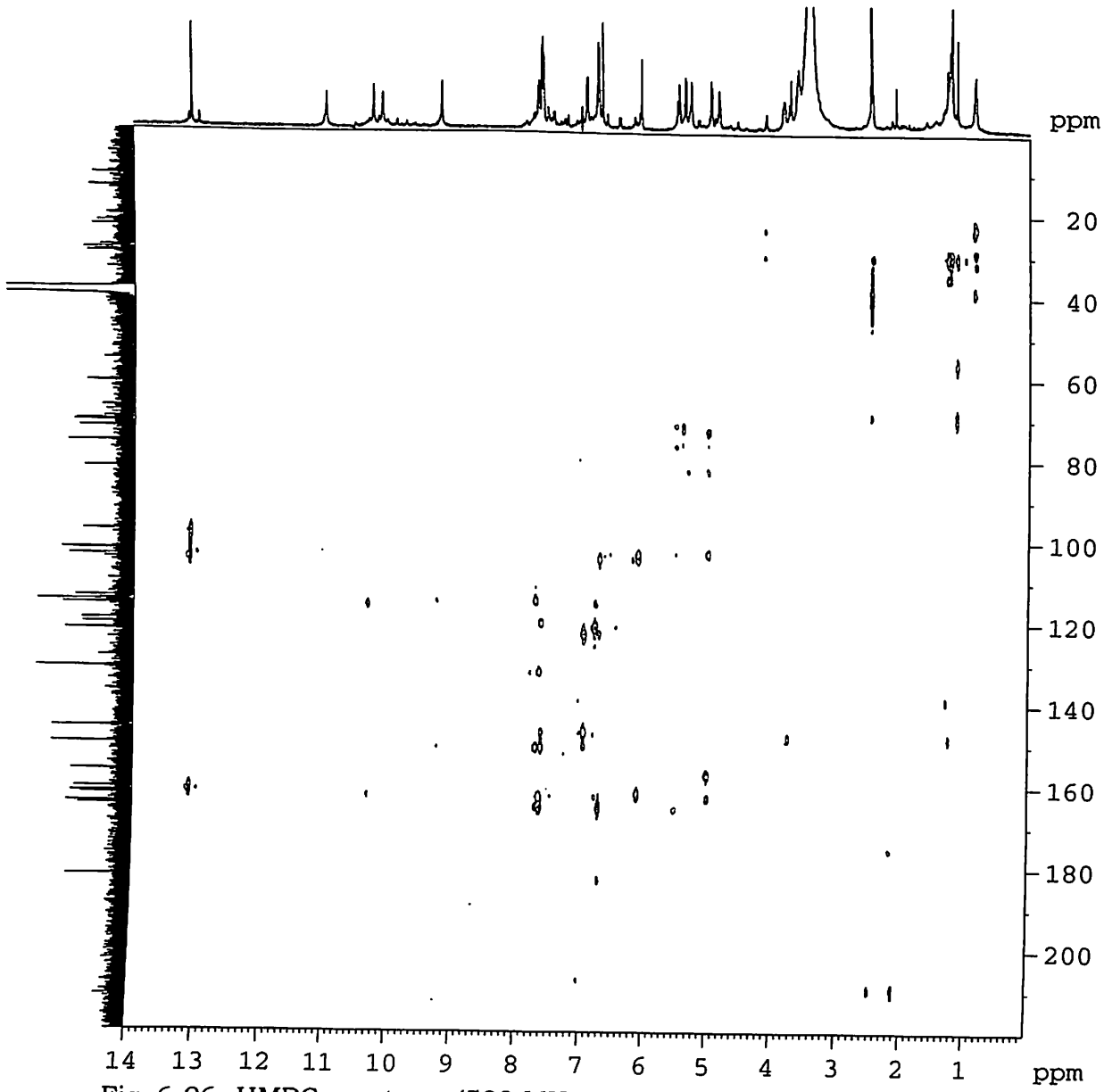
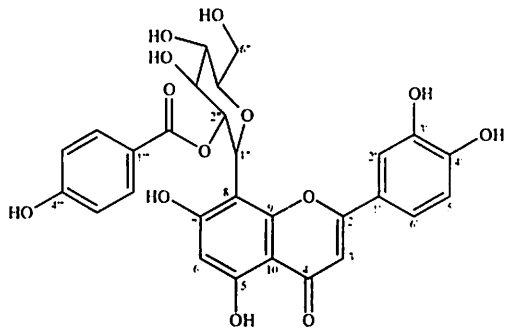



Fig. 6.06: HMBC spectrum (500 MHz, $d_6$-DMSO) of compound **VA-19 (6.02)**

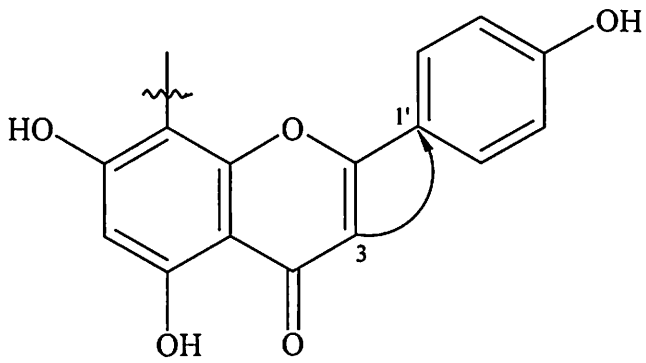
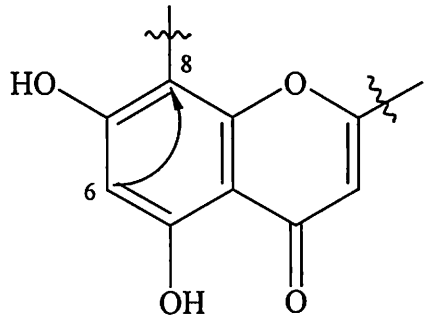
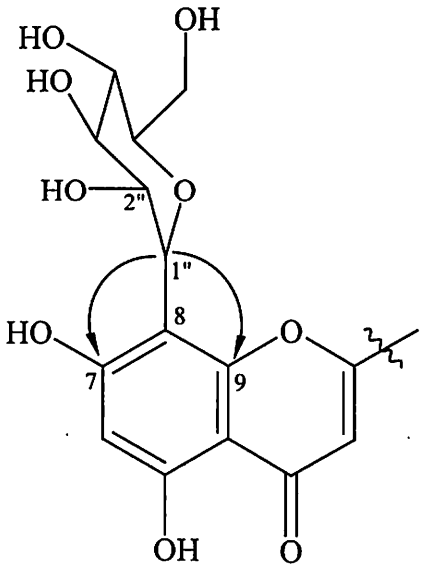


2″-*O*-*p*-hydroxybenzoylorientin
**(VA-19, 6.02)**

**TABLE 6.05**

**HMBC spectral data of VA-19**
**(2″-*O*-*p*-hydroxybenzoylorientin, 6.02)**

**(Fig. 6.06, solvent, $d_6$-DMSO)**

| Chemical shift (δ) | Correlated carbons | Structural units derived |
|---|---|---|
| 6.69 (H-3) | C-1′ |  |
| 6.08 (H-6) | C-8 |  |
| 4.99 (H-1″) | C-7 and C-9 |  |

## VA-20

It was obtained as a yellow amorphous powder from methanol, m.p. 253-255 °C. The molecular formula, $C_{21}H_{20}O_{11}$ was deduced from elemental analysis and LC-MS [*m/z* 447 $(M - H)^-$] data.

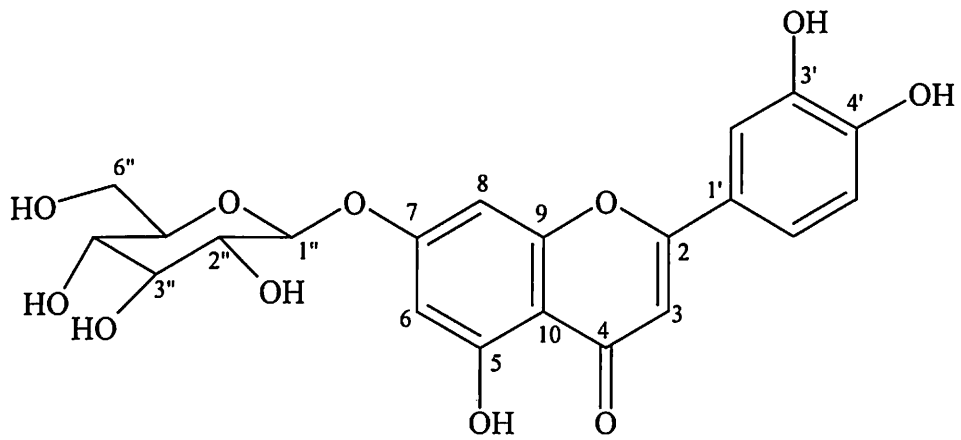
VA-20 exhibited bands in its UV (MeOH) spectrum at $\lambda_{max}$ 262 and 350 nm. Its IR (KBr) spectrum showed bands at $\nu_{max}$ 3474 (hydroxyl), 1657 (α,β-unsaturated ketone), 1604 (C=C), 1500 and 1444 $cm^-$ (aromatic). The $^1H$ NMR spectral data (Fig. 6.07, Table 6.06) showed the presence of a chelated hydroxyl (δ 12.98, 1H, s), a downfield methine proton (δ 6.75, 1H, s) characteristic of H-3 of flavonoids,[1] two meta coupled protons [δ 6.79 (1H, d, *J* = 1.9 Hz) and δ 6.45 (1H, d, *J* = 1.9 Hz) assignable to H-8 and H-6 of ring A of 5,7-dihydroxy flavonoids.[13] The $^1H$ NMR spectrum also showed the presence of an ABX system [δ 7.46 (1H, dd, *J* = 8.3, 2.0 Hz), δ 7.43 (1H, d, *J* = 2.0 Hz) and δ 6.92 (1H, d, *J* = 8.3 Hz)] characteristic of 1,2,4-trisubstituted phenyl unit.[10] The above spectral data revealed the presence of a luteolin skeleton[11] in VA-20.

In addition, the $^1H$ NMR spectrum showed a series of signals between δ 3.73-3.15, attributable to a sugar moiety. The coupling constant (*J* = 7.3 Hz) of the anomeric proton located at δ 5.09 and the $^{13}C$ NMR chemical shifts of the sugar carbons[11] (δ 99.7, 77.0, 76.3, 73.0, 69.4 and 60.5) revealed the presence of β-O-glucoside unit in VA-20.

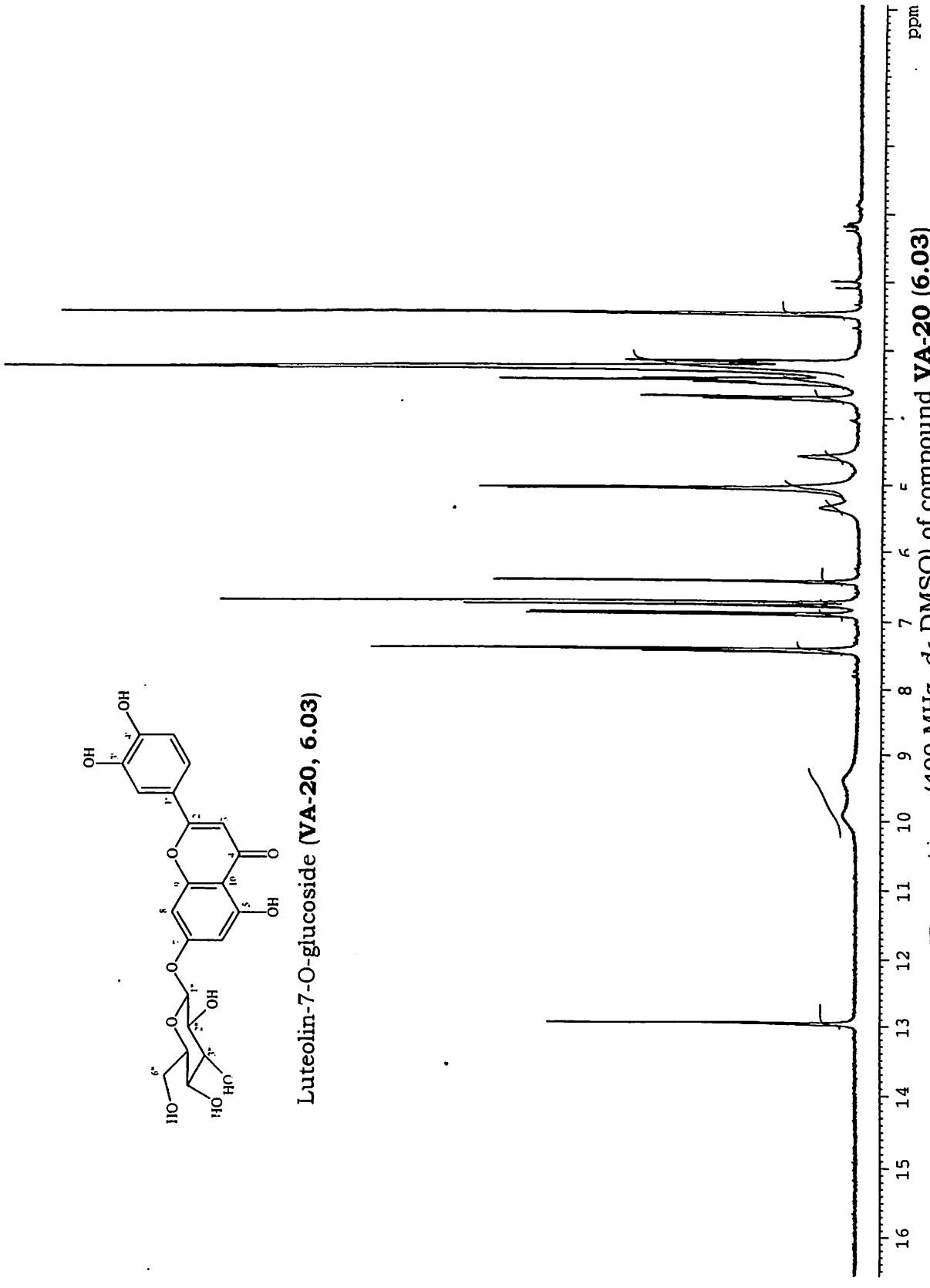
The $^{13}C$ NMR spectral (Fig. 6.08, Table 6.07) data showed the presence of a ketone carbonyl (δ 181.8), two olefinic carbons (δ 164.3 and 103.0), four hydroxyl carbons (δ 162.8, 161.0, 149.8 and 145.7).

Literature survey on flavonoids of luteolin nucleus revealed that the physical and spectral data of VA-20 were in good agreement with those recorded for luteolin-7-*O*-glucoside[14] (**6.03**), isolated earlier from *Vitex agnus castus*[15] and *Vernonia cinerea.*[16]

Based on the foregoing, the structure of VA-20 was established as luteolin-7-*O*-glucoside (**6.03**).




**6.03**




Luteolin-7-O-glucoside (**VA-20, 6.03**)

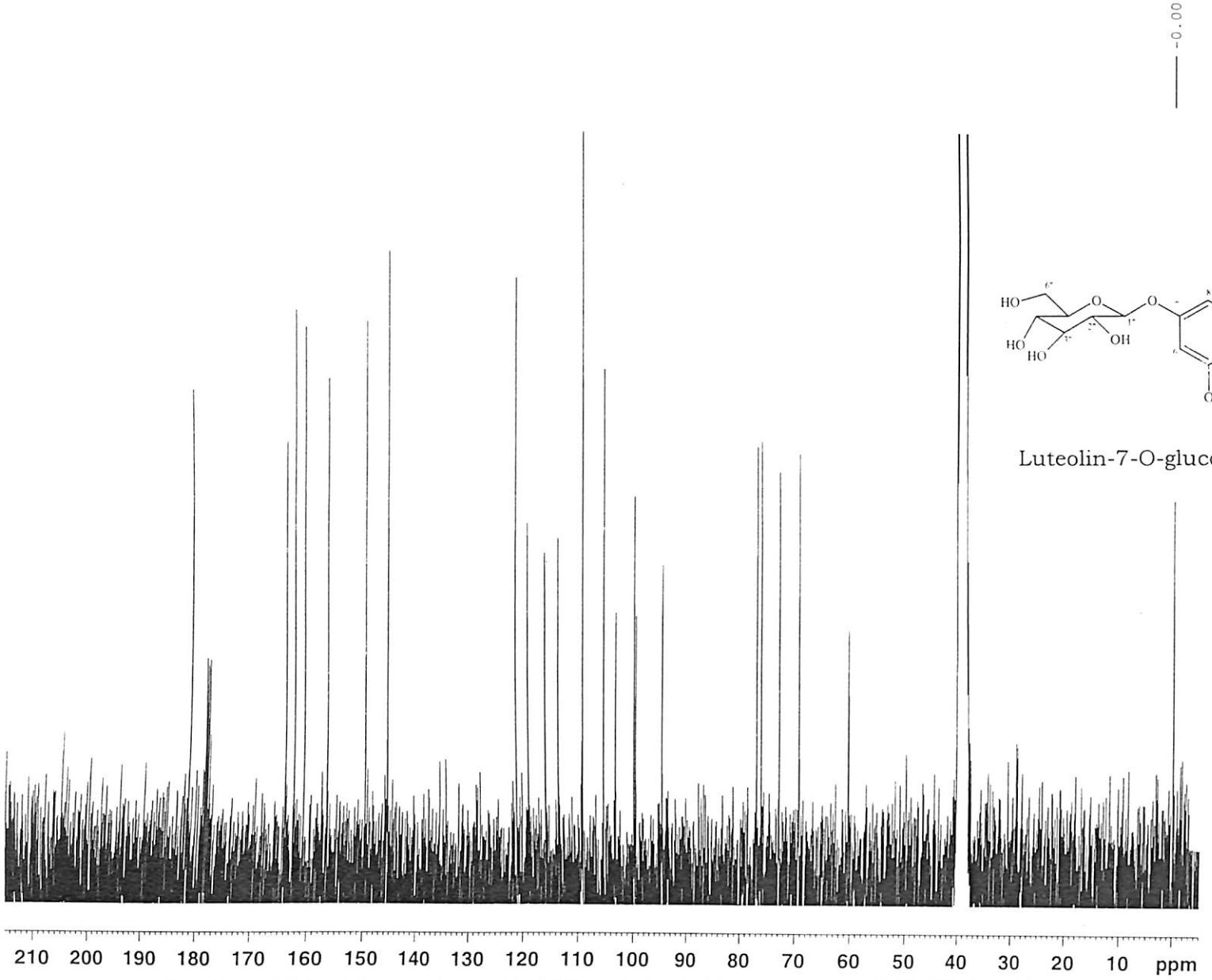
Fig. 6.07: $^1$H NMR spectrum (400 MHz, $d_6$-DMSO) of compound **VA-20 (6.03)**

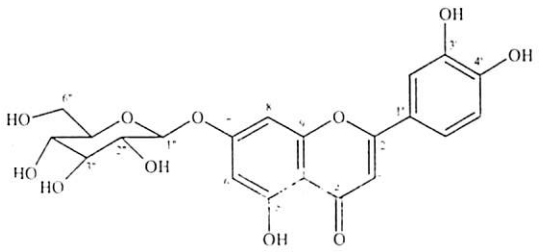
## TABLE 6.06

**$^1$H NMR spectral data of VA-20 (Luteolin-7-*O*-glucoside, 6.03)**

**(Fig. 6.07, 400 MHz spectrum, $d_6$-DMSO)**

| Chemical shift (δ) | Proton integration | Multiplicity (*J* in Hz) | Assignment |
|---|---|---|---|
| 12.98 | 1H | s | 5-OH |
| 6.75 | 1H | s | H-3 |
| 6.45 | 1H | d (1.9) | H-6 |
| 6.79 | 1H | d (1.9) | H-8 |
| 7.43 | 1H | d (2.0) | H-2′ |
| 6.92 | 1H | d (8.3) | H-5′ |
| 7.46 | 1H | dd (8.3,2.0) | H-6′ |
| 5.09 | 1H | d (7.3) | H-1″ |
| 3.73-3.17 | 6H | m | H-2″- H-6″ |




Luteolin-7-O-glucoside (**VA-20, 6.03**)

Fig. 6.08: $^{13}C$ NMR spectrum (75 MHz, $d_6$-DMSO) of compound **VA-20** (**6.03**)

## TABLE 6.07

**$^{13}$C NMR spectral data of VA-20 (Luteolin-7-*O*-glucoside, 6.03)**

**(Fig. 6.08, 75 MHz spectrum, $d_6$-DMSO)**

| Carbon number | Chemical shift (δ) | Carbon number | Chemical shift (δ) |
|---|---|---|---|
| C-2 | 164.3 | C-3′ | 145.7 |
| C-3 | 103.0 | C-4′ | 149.8 |
| C-4 | 181.8 | C-5′ | 115.8 |
| C-5 | 161.0 | C-6′ | 119.0 |
| C-6 | 99.4 | C-1″ | 99.7 |
| C-7 | 162.8 | C-2″ | 73.0 |
| C-8 | 94.6 | C-3″ | 76.3 |
| C-9 | 156.8 | C-4″ | 69.4 |
| C-10 | 105.2 | C-5″ | 77.0 |
| C-1′ | 121.2 | C-6″ | 60.5 |
| C-2′ | 113.4 | | |

## EXPERIMENTAL

### Vitexin (VA-15, 6.01, 11 mg)

It was obtained as yellow amorphous powder from methanol, m.p. 275-277 °C

| | |
|---|---|
| Found | : C, 58.25%; H, 4.86% |
| $C_{21}H_{20}O_{10}$ requires | : C, 58.33%; H, 4.62% |
| UV (MeOH) | : $\lambda_{max}$ 269, 332 nm |
| IR (KBr) | : $\nu_{max}$ 3381. 1652, 1568 and 1501 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 6.01, Table 6.01 |

### 2″-*O*-*p*-Hydroxybenzoylorientin (VA-19, 6.01, 9 mg)

It was obtained as yellow amorphous powder from methanol.

| | |
|---|---|
| Found | : C, 60.12%; H, 5.03% |
| $C_{28}H_{24}O_{13}$ requires | : C, 59.14%; H, 4.22% |
| UV (MeOH) | : $\lambda_{max}$ 267, 342 nm |
| IR (KBr) | : $\nu_{max}$ 3345. 1645, 1565, and 1468 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 6.02, Table 6.02 |
| $^{13}C$ NMR | : Fig. 6.03, Table 6.03 |
| DEPT | : Fig. 6.04 |
| HMQC | : Fig. 6.05, Table 6.04 |
| HMBC | : Fig. 6.06, Table 6.05 |

**Luteolin-7-*O*-glucoside (VA-20, 6.03, 140 mg)**

It was obtained as yellow amorphous powder from methanol, m.p. 253-255 °C

| | |
|---|---|
| Found | : C, 56.45%; H, 4.62% |
| $C_{21}H_{20}O_{11}$ requires | : C, 56.25%; H, 4.46% |
| UV (MeOH) | : $\lambda_{max}$ 262, 350 nm |
| IR (KBr) | : $\nu_{max}$ 3474. 1657, 1604, 1500 and 1444 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 6.07, Table 6.06 |
| $^{13}C$ NMR | : Fig. 6.08, Table 6.07 |

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# STRUCTURE ELUCIDATION OF THE CHEMICAL CONSTITUENTS FROM *T. LABIALIS*

The details of isolation of the chemical constituents from the methanolic extractives of *T. labialis* were described in **chapter-2**. Structure elucidation of these constituents, namely, **TL-01 – TL-04** is presented in this chapter.

## TL-02

It was obtained as colorless crystals from aqueous methanol, m.p. 140-142 °C. Its molecular formula, $C_{14}H_{16}O_9$, was deduced from elemental analysis and LC-MS [*m/z* 327 $(M-H)^-$] data.

The UV (MeOH) spectrum of TL-02 showed absorption maxima at 277 nm. Its IR (KBr) spectrum showed bands at 3390 (hydroxyl), 1702 (carbonyl), 1612 and 1528 $cm^{-1}$ (aromatic). The $^1H$ NMR spectral data (Fig. 7.01, Table 7.01) showed the presence of two aromatic hydroxyl groups (δ 9.75, s and δ 8.43, s), an aromatic singlet proton at δ 6.98 (1H, s, H-7), methoxyl group (δ 3.76 , 3H, s) and a series of signals between δ 3.19 and 3.98 characteristic of a sugar moiety. The absence of usual O-glycosidic anomeric proton signal in the region 5.00-5.20 ppm and the presence of signal at δ 4.95 (1H, d, $J$ = 10.4 Hz, H-$10_b$) was consistent with the presence of a C-glycoside.[1] This was supported by the $^{13}C$ NMR signal at δ 72.1 (C-$10_b$) corresponding to the anomeric carbon.

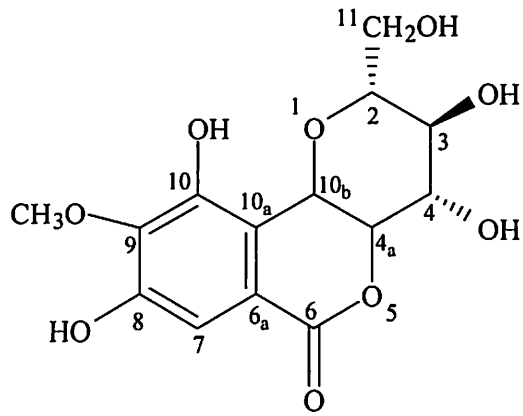
The $^{13}C$ NMR spectrum (Fig. 7.02, Table 7.02) showed the presence of a lactone carbonyl (δ 163.4), three oxygenated aromatic carbons (δ 140.6, 148.0, and 151.0), two quaternary carbons (δ 116.0 and 118.1), an aromatic methine carbon (δ 109.5), and a methoxyl group (δ 59.8) and six signals located at δ 61.1, 70.7, 72.1, 73.7, 79.8 and 81.7, attributable to a C-glucose unit. These assignments were supported by the DEPT (Fig. 7.03) spectral data and expected correlations were observed in the HMQC spectrum (Fig. 7.04, Table 7.03). The above spectral data indicated the presence of a glucoisocoumarin skeleton[2,3] in TL-02.

In the HMBC spectrum (Fig. 7.05, Table 7.04) the lone aromatic proton (δ 6.98, 1H, s, H-7) showed correlation with the lactone carbonyl

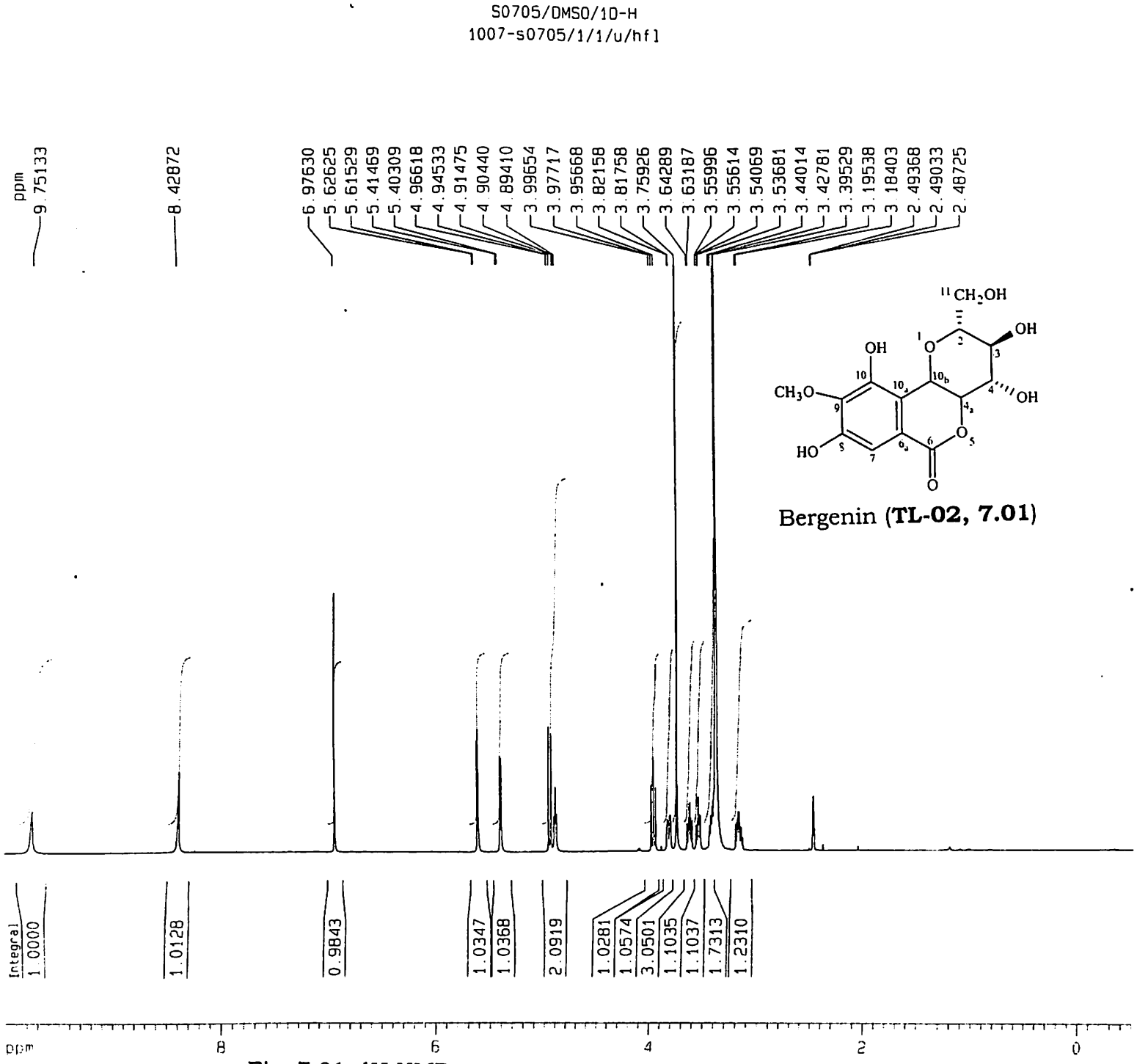
(δ 163.4, C-6). The anomeric proton (δ 4.95, H-$10_b$) showed correlations with aromatic quaternary carbons (δ 116.0, C-$10_a$ and δ 118.1, C-$6_a$).

The above physical and spectral data of TL-02 were found to be corroborative with those reported for bergenin (**7.01**), a glucoisocoumarin derivative, isolated earlier from *Macaranga peltata*[2] and *Mallotus japonicus*[3]

Based on the foregoing, the structure of TL-02 was established as bergenin (**7.01**)




**7.01**




Bergenin (**TL-02, 7.01**)

Fig. 7.01: $^1$H NMR spectrum (500 MHz, $d_6$-DMSO) of compound **TL-02 (7.01)**

## TABLE 7.01

**$^1$H NMR spectral data of TL-02 (Bergenin, 7.01)**

**(Fig. 7.01, 500 MHz spectrum, $d_6$-DMSO)**

| Chemical shift (δ) | Proton integration | Multiplicity (*J* in Hz) | Assignment |
|---|---|---|---|
| 9.75 | 1H | s | -OH |
| 8.43 | 1H | s | -OH |
| 3.57 | 1H | m | H-2 |
| 3.19 | 1H | m | H-3 |
| 3.98 | 1H | t (9.9) | H-4 |
| 3.66 | 1H | m | $H\text{-}4_a$ |
| 6.98 | 1H | s | H-7 |
| 4.95 | 1H | d (10.4) | $H\text{-}10_b$ |
| 3.84 | 1H | m | $H\text{-}11_a$ |
| 3.45 | 1H | m | $H\text{-}11_b$ |
| 3.76 | 3H | s | $OCH_3$ |

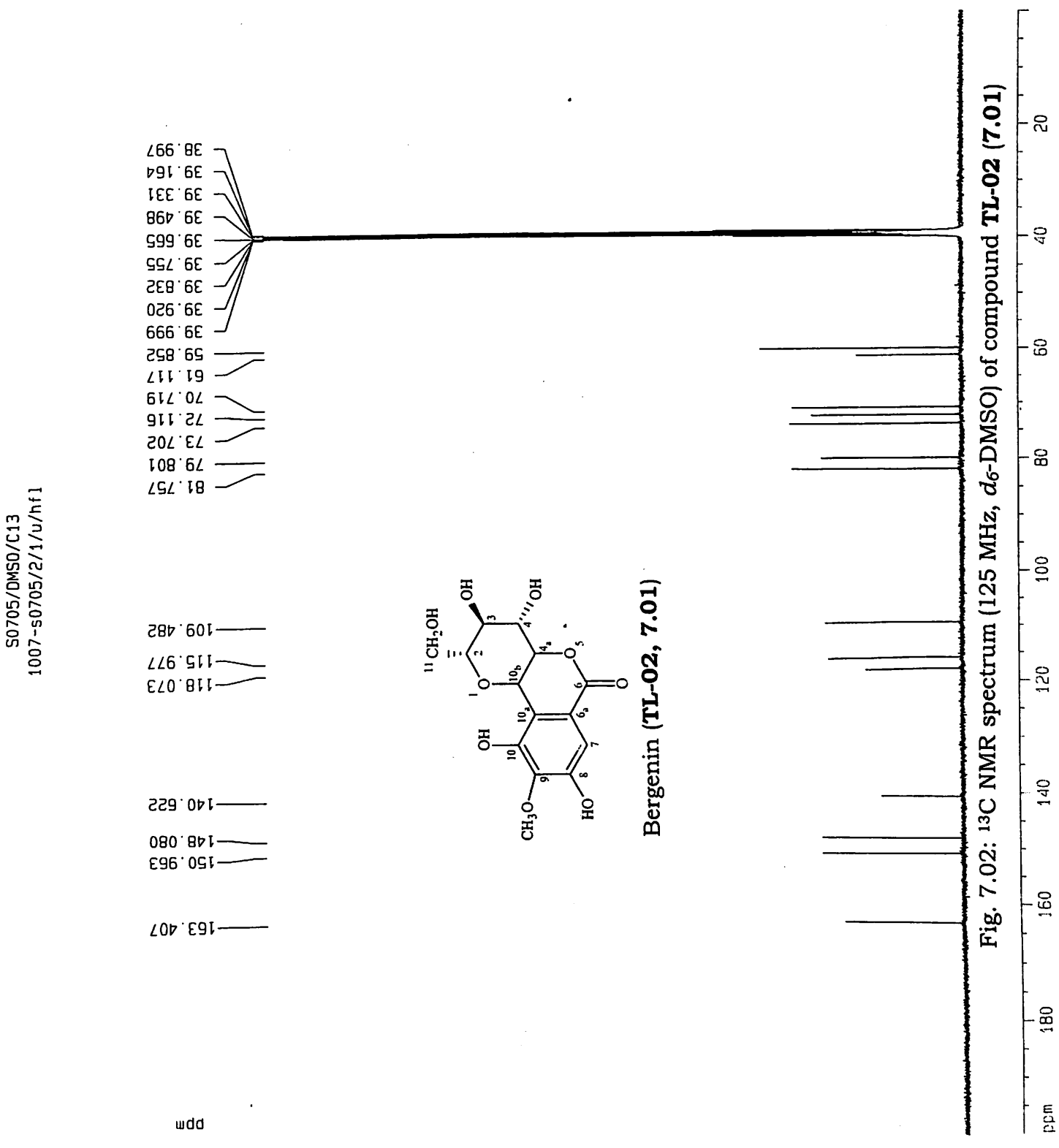
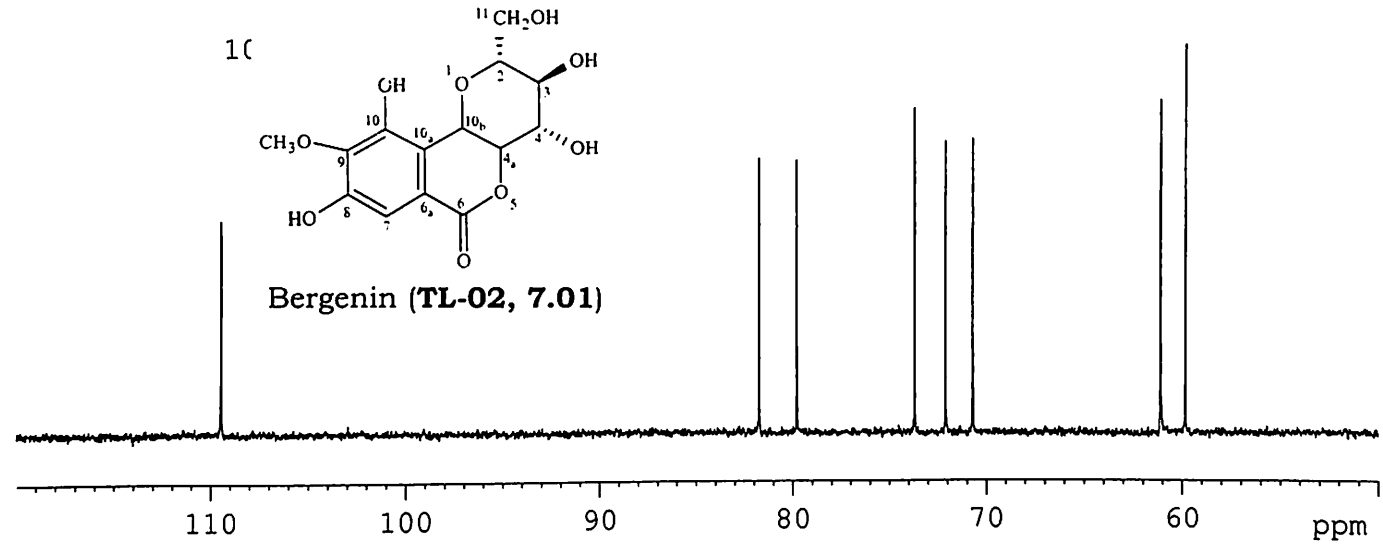
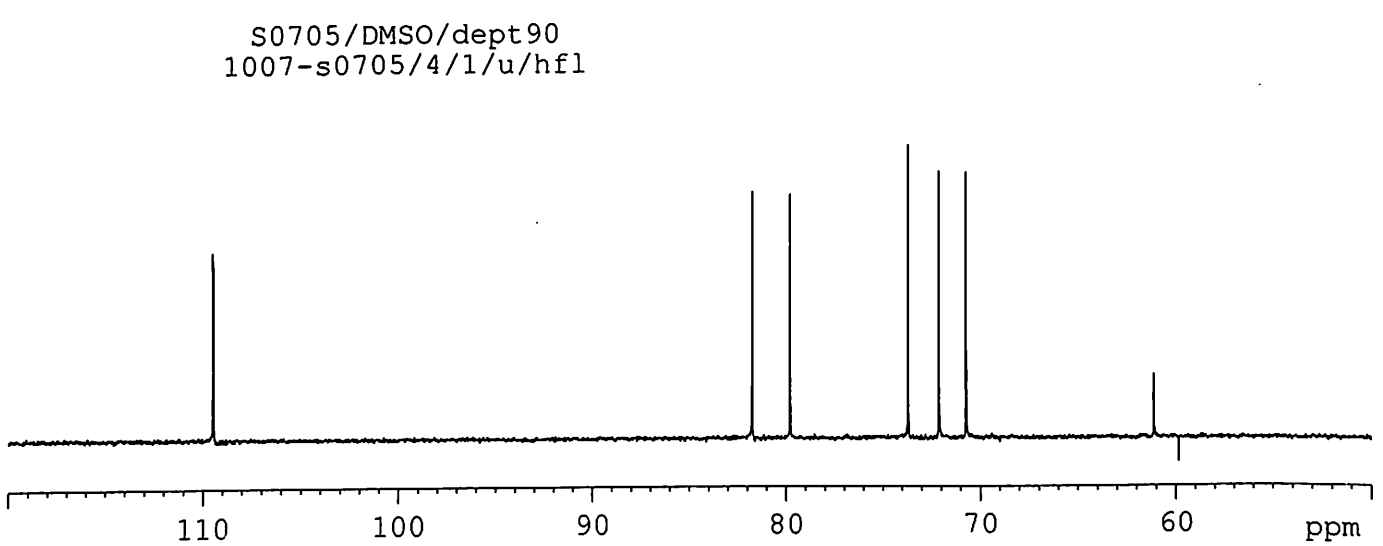



Fig. 7.02: $^{13}C$ NMR spectrum (125 MHz, $d_6$-DMSO) of compound **TL-02 (7.01)**



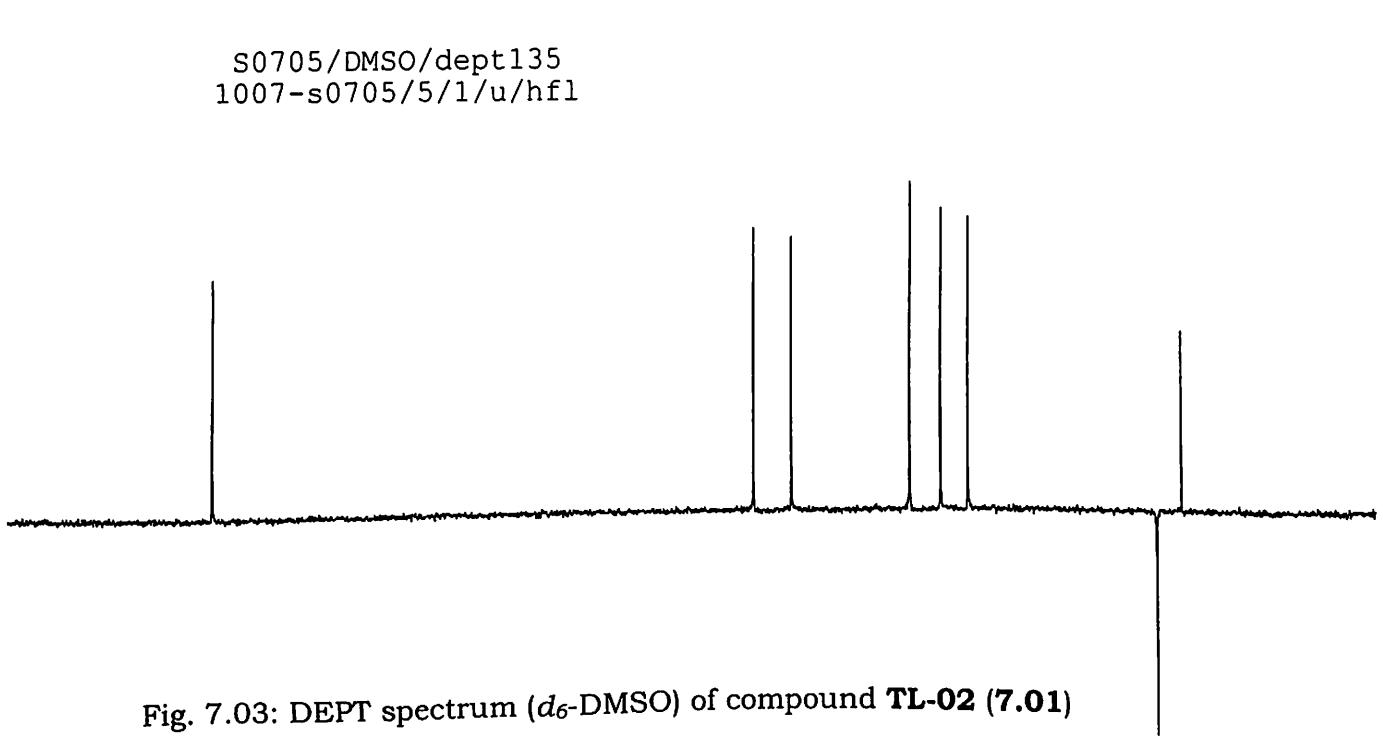




Fig. 7.03: DEPT spectrum ($d_6$-DMSO) of compound **TL-02 (7.01)**

## TABLE 7.02

**$^{13}$C NMR spectral data of TL-02 (Bergenin, 7.01)**

**(Fig. 7.02, 125 MHz spectrum, $d_6$-DMSO)**

| Chemical shift (δ) | DEPT | Assignment |
|---|---|---|
| 81.7 | CH | C-2 |
| 70.7 | CH | C-3 |
| 79.8 | CH | C-4 |
| 73.7 | CH | $C_a$-4 |
| 163.4 | C | C-6 |
| 118.1 | C | $C_a$-6 |
| 109.5 | CH | C-7 |
| 151.0 | C | C-8 |
| 140.6 | C | C-9 |
| 148.0 | C | C-10 |
| 116.0 | C | C-$10_a$ |
| 72.1 | CH | C-$10_b$ |
| 61.1 | $CH_2$ | C-11 |
| 59.8 | $CH_3$ | $OCH_3$ |

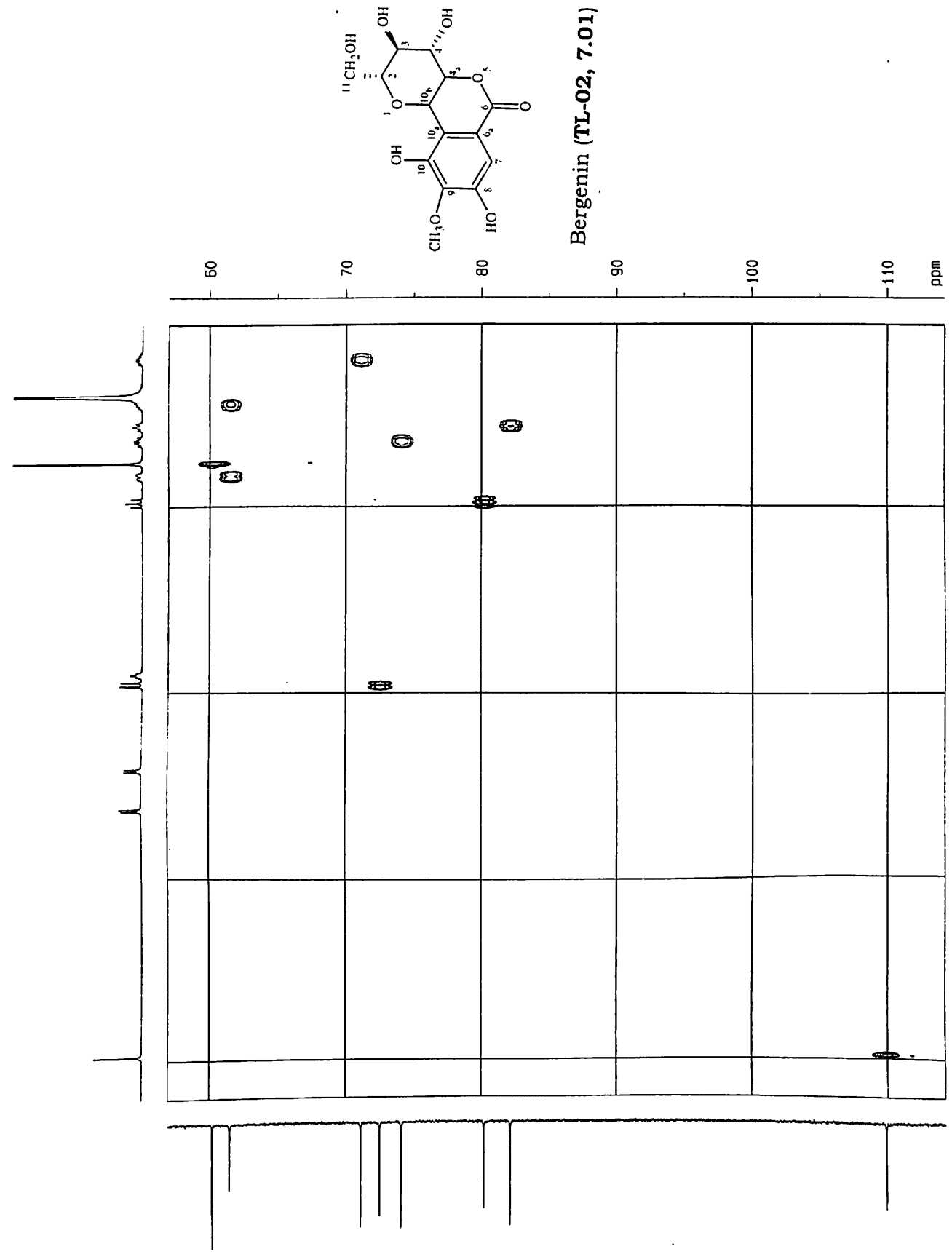



Fig. 7.04: HMQC spectrum (500 MHz, $d_6$-DMSO) of compound **TL-02 (7.01)**

## TABLE 7.03

**HMQC spectral data of TL-02 (Bergenin, 7.01)**

**(Fig. 7.04, solvent: $d_6$-DMSO)**

| **Proton chemical shift (δ)** | **Correlated carbon chemical shift (δ)** | **Assignment** |
|---|---|---|
| 3.57 (H-2) | 81.7 | C-2 |
| 3.19 (H-3) | 70.7 | C-3 |
| 3.98 (H-4) | 79.8 | C-4 |
| 3.66 ($H\text{-}4_a$) | 73.7 | $C\text{-}4_a$ |
| 6.98 (H-7) | 109.5 | C-7 |
| 4.95 ($H\text{-}10_b$) | 72.1 | $C\text{-}10_b$ |
| 3.84 and 3.45(H-11) | 61.1 | C-11 |
| 3.76 (-OMe) | 59.8 | -OMe |

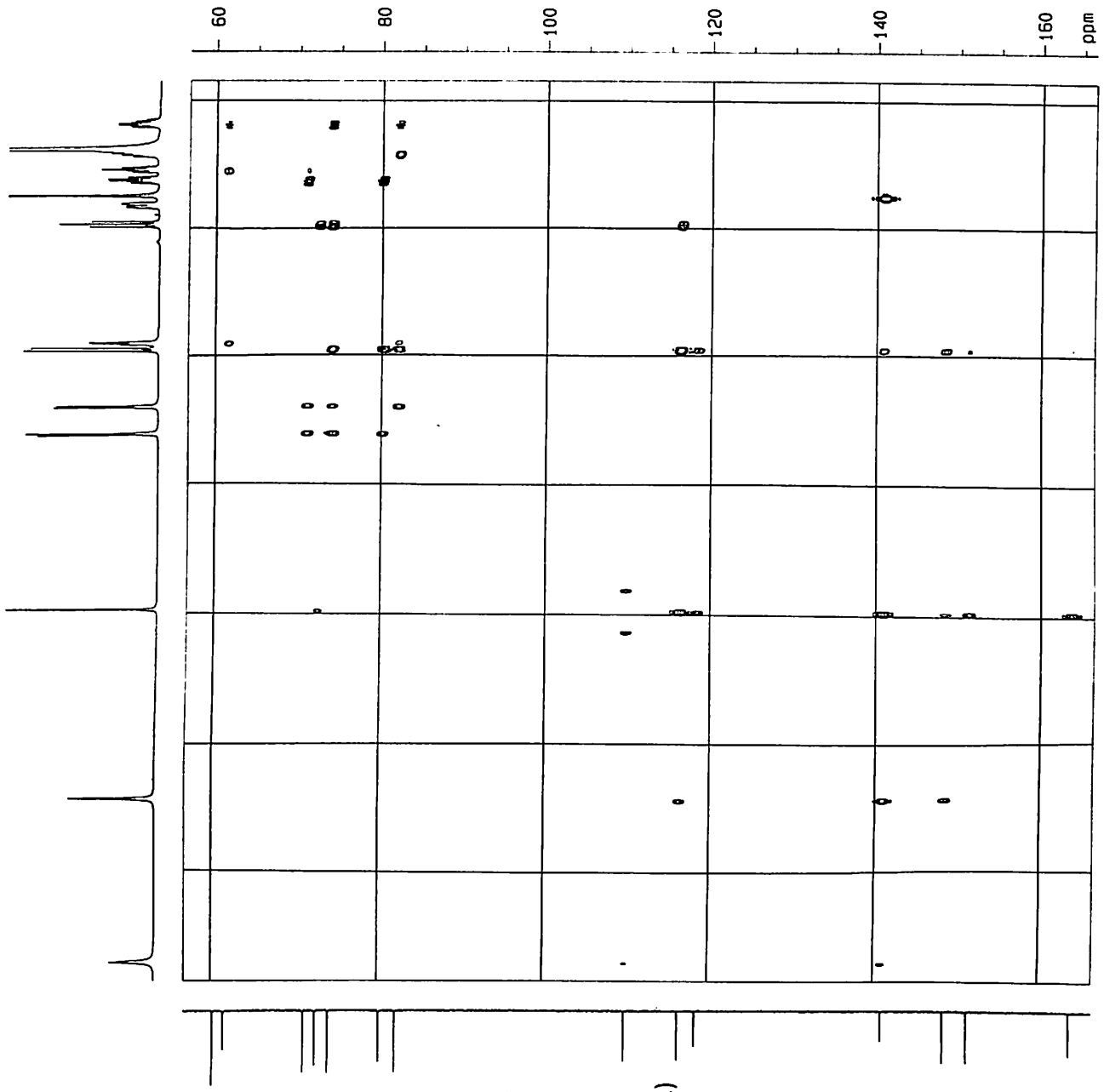
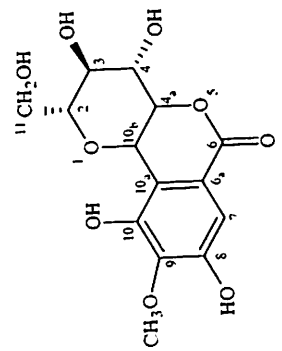


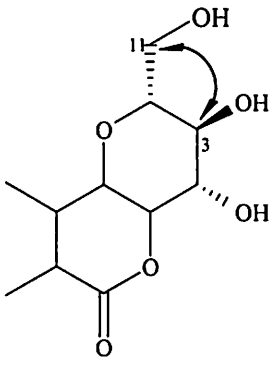
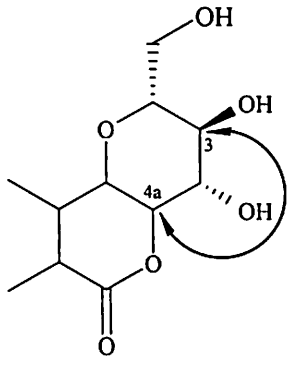
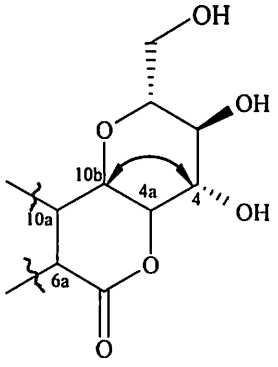
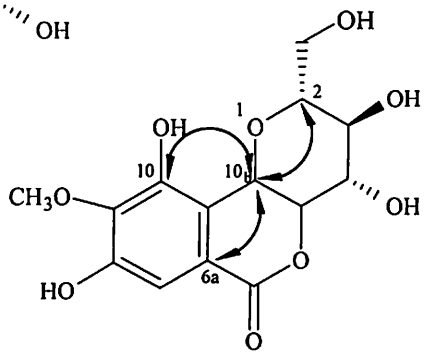
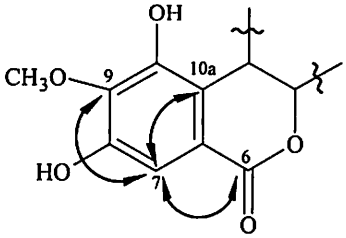
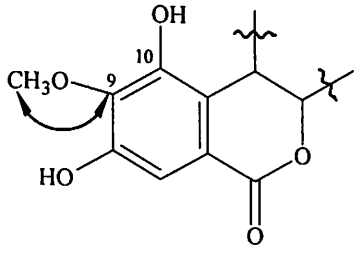

Fig. 7.05: HMBC spectrum (500 MHz, $d_6$-DMSO) of compound **TL-02 (7.01)**



Bergenin (**TL-02, 7.01**)

## TABLE 7.04

**HMBC spectral data of TL-02 (Bergenin, 7.01)**
**(Fig. 7.05, solvent: $d_6$-DMSO)**

| Chemical shift (δ) | Correlated carbons | Structural units derived |
|---|---|---|
| 3.19 (H-3) | C-11 |  |
| 3.66 ($H\text{-}4_a$) | C-3 |  |
| 3.98 (H-4) | $C\text{-}10_b$ |  |
| 4.94 ($H\text{-}10_b$) | C-2, $C\text{-}C\text{-}6_a$, C-10 |  |
| 6.98 (H-7) | C-6, C-9, $C\text{-}10_a$ |  |
| 3.76 (OMe) | C-9 |  |

## TL-03

It was obtained as pale yellow crystalline solid from aqueous methanol, m.p. 232-233° C. Its molecular formula $C_{21}H_{20}O_9$ was established based on elemental analysis and LC-MS [*m/z* 439 $(M+Na)^+$] data.

The UV (MeOH) spectrum showed absorption maxima at 256 nm. Its IR (KBr) spectrum showed bands at 3372 (hydroxyl), 1623 (α,β-unsaturated ketone) 1514 $cm^{-1}$ (aromatic). The $^1H$ NMR spectrum (Fig. 7.06, Table 7.05) showed the presence of five aromatic protons constituted by an ABX spin system, characteristic of a 1,2,4-trisubstituted phenyl unit[4] [δ 8.04 (1H, d, *J* = 8.8 Hz), 7.14 (1H, dd, *J* = 8.8, 2.2 Hz) and δ 7.22 (1H, d *J* = 2.2 Hz)] and an AA' BB' spin system [δ 7.40 (2H, d, *J* = 8.6 Hz) and 6.81 (2H, d, *J* = 8.6 Hz)] attributable to a para disubstituted phenyl unit.[5] It also showed the presence of a highly deshielded proton at δ 8.36, s, characteristic of an isoflavone skeleton.[6] In addition, the $^1H$ NMR spectrum showed the presence of a series of signals between δ 3.17 and 3.70 attributable to a sugar moiety. The coupling constant of the anomeric proton (δ 5.09, 1H, d, *J* = 7.6 Hz) was consistent with the β- configuration of sugar linkage.

The $^{13}C$ NMR spectrum (Fig. 7.07, Table 7.06) showed the presence of a carbonyl carbon (δ 174.8, C-4), a β-olefinic carbon (δ 153.3, C-2) two oxygenated aromatic carbons (δ 161.4 and 157.3) and signals at δ 60.7, 69.7, 73.2, 76.5, 77.2 and 100.0 attributable to a sugar moiety. These assignments were supported by the DEPT (Fig. 7.08) spectral data and the correlations observed in the HMQC (Fig. 7.09, Table 7.07) spectrum.

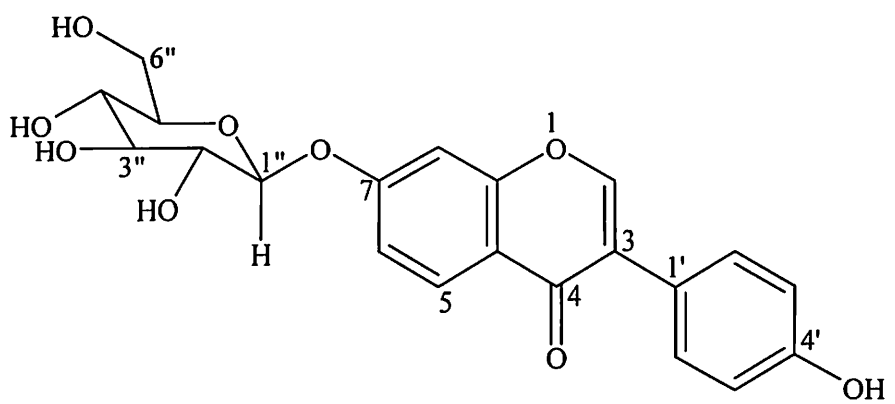
The presence of a glucose unit at C-7 of the isoflavone, was supported by the HMBC correlations (Fig. 7.10, Table 7.08) observed

between the anomeric proton (δ 5.09, H-1") and the C-7 (δ 161.4) of the isoflavone moiety.

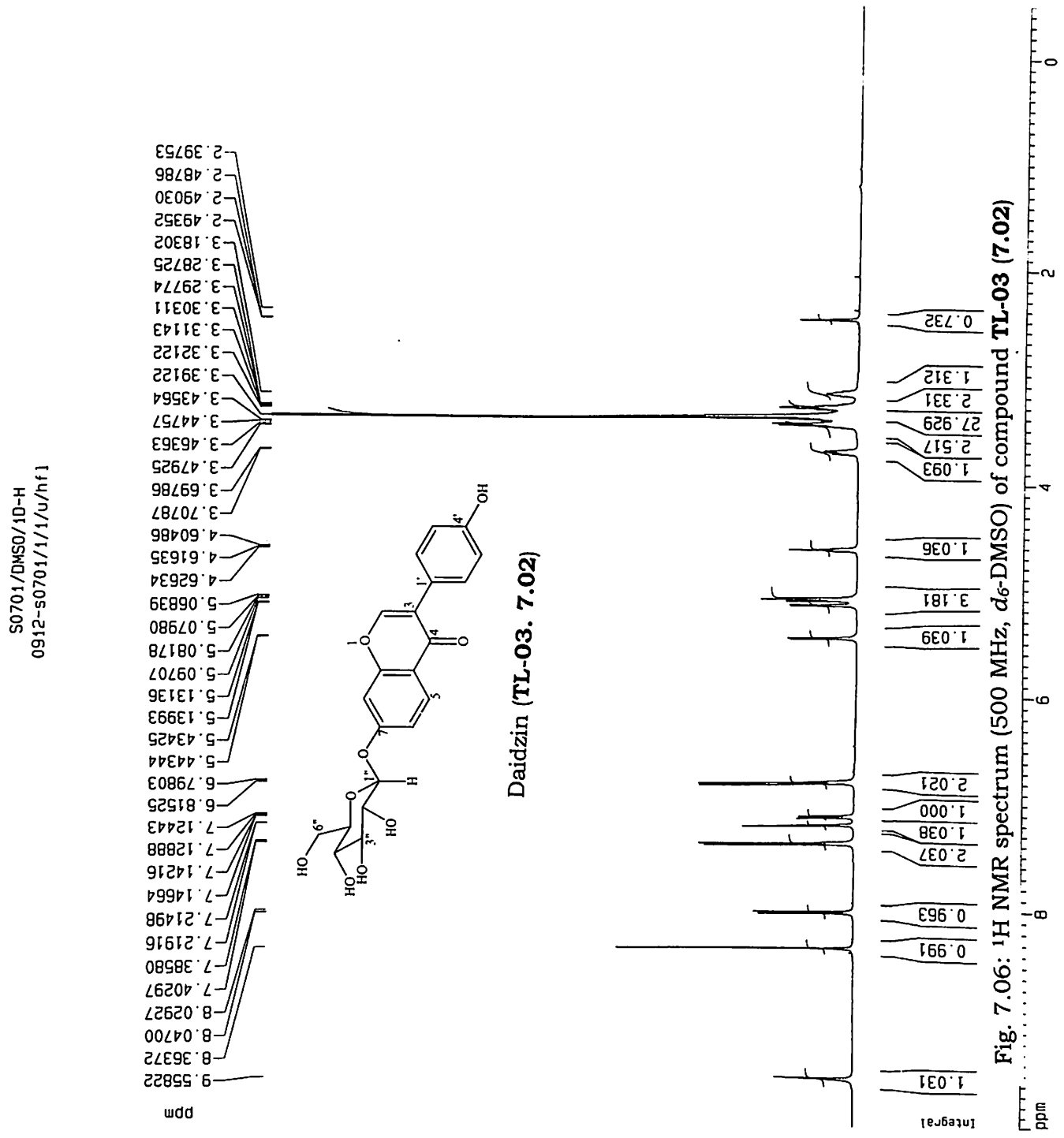
Literature survey on isoflavoneglycosides revealed that the physical and spectral data of TL-03 were in good agreement with those recorded for daidzin[7,8] (**7.02**), isolated earlier from *Glycine max* (soya).

The identity of TL-03 as daidzin was ascertained further by the direct comparison with the authentic sample (co-HPTLC and mmp) supplied by Laila Impex, R & D centre, Vijayawada.

Based on the foregoing, the structure of TL-03 was established as daidzin (**7.02**)




**7.02**




Daidzin (**TL-03. 7.02**)

Fig. 7.06: $^1$H NMR spectrum (500 MHz, $d_6$-DMSO) of compound **TL-03 (7.02)**

## TABLE 7.05

**$^1$H NMR spectral data of TL-03 (Daidzin, 7.02)**

**(Fig. 7.06, 500 MHz spectrum, $d_6$-DMSO)**

| Chemical shift (δ) | Proton integration | Multiplicity (J in Hz) | Assignment |
|---|---|---|---|
| 8.36 | 1H | s | H-2 |
| 8.04 | 1H | d (8.8) | H-5 |
| 7.14 | 1H | dd (8.8, 2.2) | H-6 |
| 7.22 | 1H | d (2.2) | H-8 |
| 7.40 | 2H | d (8.6) | H-2′ and H-6′ |
| 6.81 | 2H | d (8.6) | H-3′ and H-5′ |
| 5.09 | 1H | d (7.6) | H-1″ |
| 3.29 | 1H | m | H-2″ |
| 3.46 | 1H | m | H-3″ |
| 3.17 | 1H | m | H-4″ |
| 3.33 | 1H | m | H-5″ |
| 3.46 | 1H | m | $H_a$-6″ |
| 3.70 | 1H | m | $H_b$-6″ |

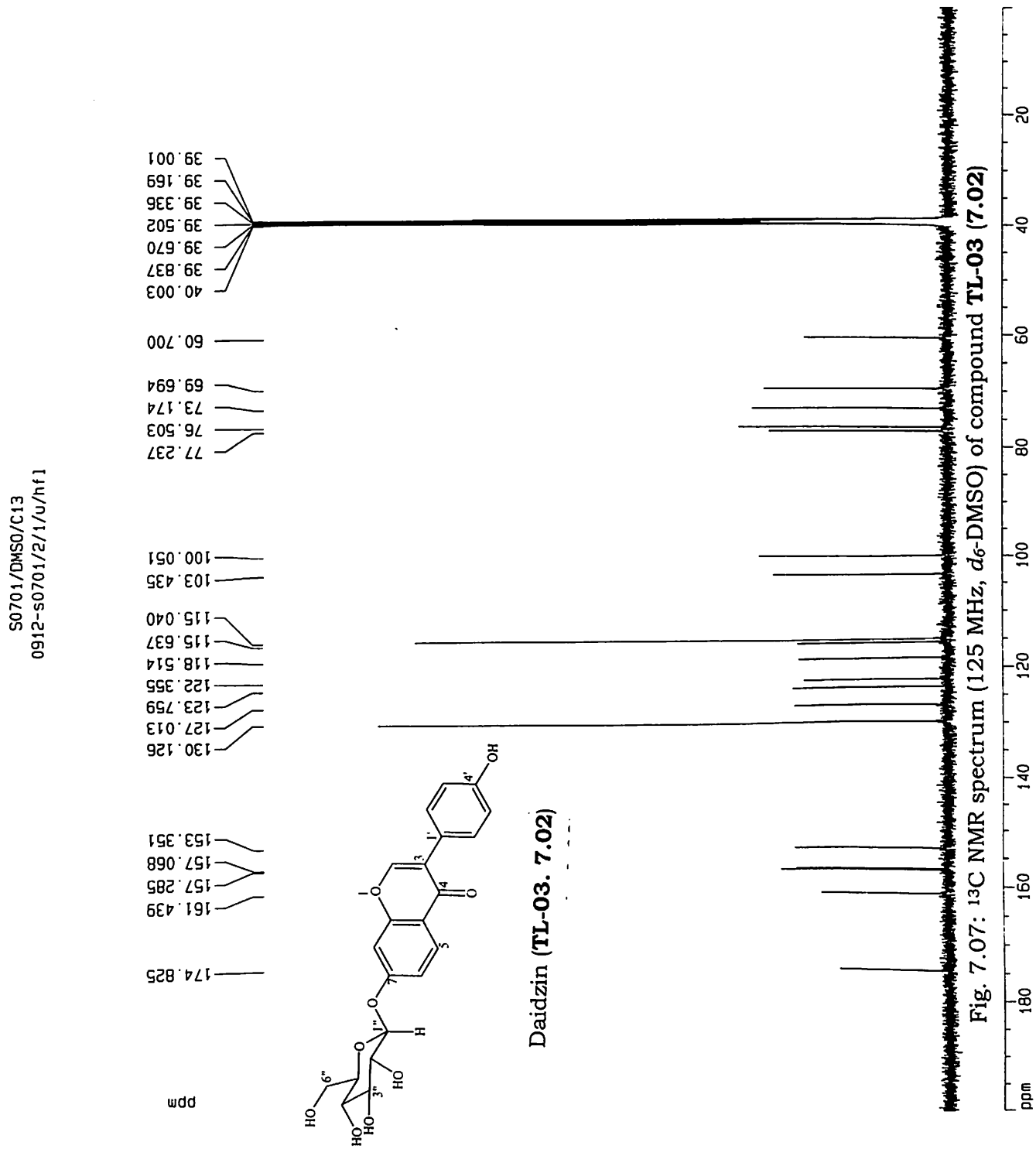
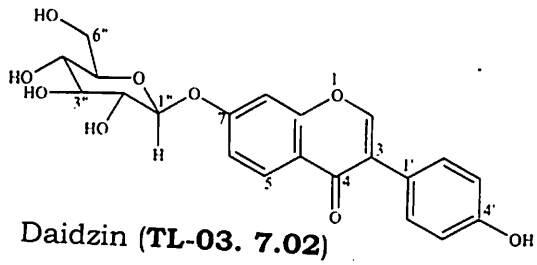
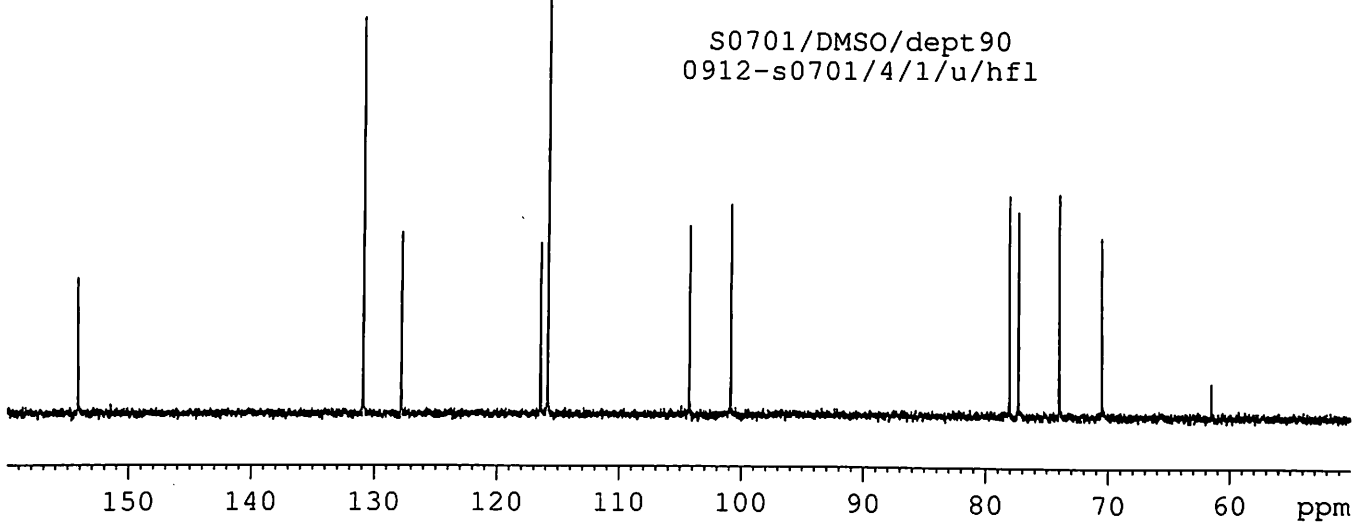



Fig. 7.07: $^{13}C$ NMR spectrum (125 MHz, $d_6$-DMSO) of compound **TL-03 (7.02)**



Daidzin (**TL-03. 7.02**)



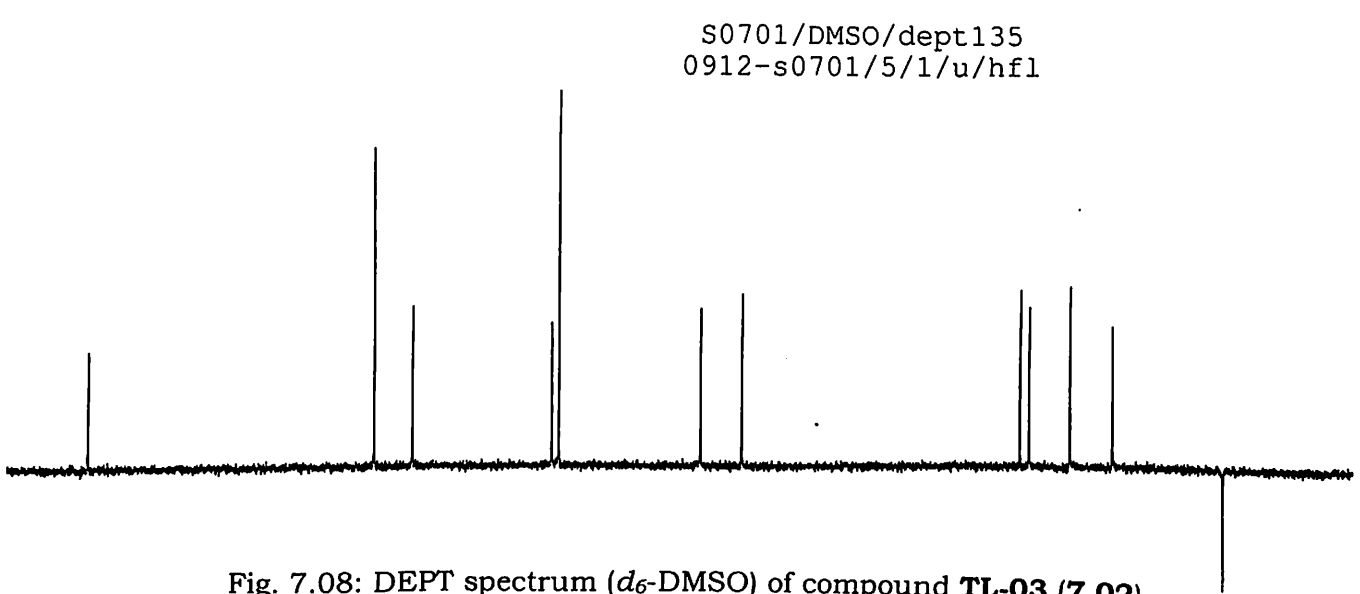
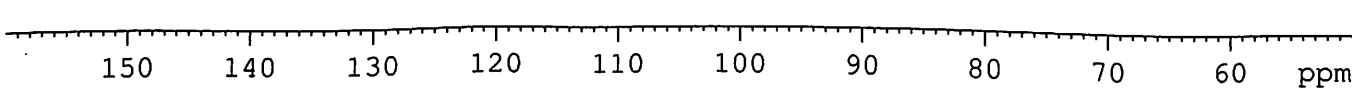




Fig. 7.08: DEPT spectrum ($d_6$-DMSO) of compound **TL-03 (7.02)**



## TABLE 7.06

**$^{13}$C NMR spectral data of TL-03 (Daidzin, 7.02)**

**(Fig. 7.07, 125 MHz spectrum, $d_6$-DMSO)**

| Chemical shift (δ) | DEPT | Assignment |
|---|---|---|
| 153.3 | CH | C-2 |
| 123.7 | C | C-3 |
| 174.8 | C | C-4 |
| 127.0 | CH | C-5 |
| 115.6 | CH | C-6 |
| 161.4 | C | C-7 |
| 103.4 | CH | C-8 |
| 157.1 | C | C-9 |
| 118.5 | C | C-10 |
| 122.3 | C | C-1′ |
| 130.1 | CH | C-2′ and C-6′ |
| 115.0 | CH | C-3′ and C-5′ |
| 157.3 | C | C-4′ |
| 100.0 | CH | C-1″ |
| 73.2 | CH | C-2″ |
| 77.2 | CH | C-3″ |
| 69.7 | CH | C-4″ |
| 76.5 | CH | C-5″ |
| 60.7 | $CH_2$ | C-6″ |

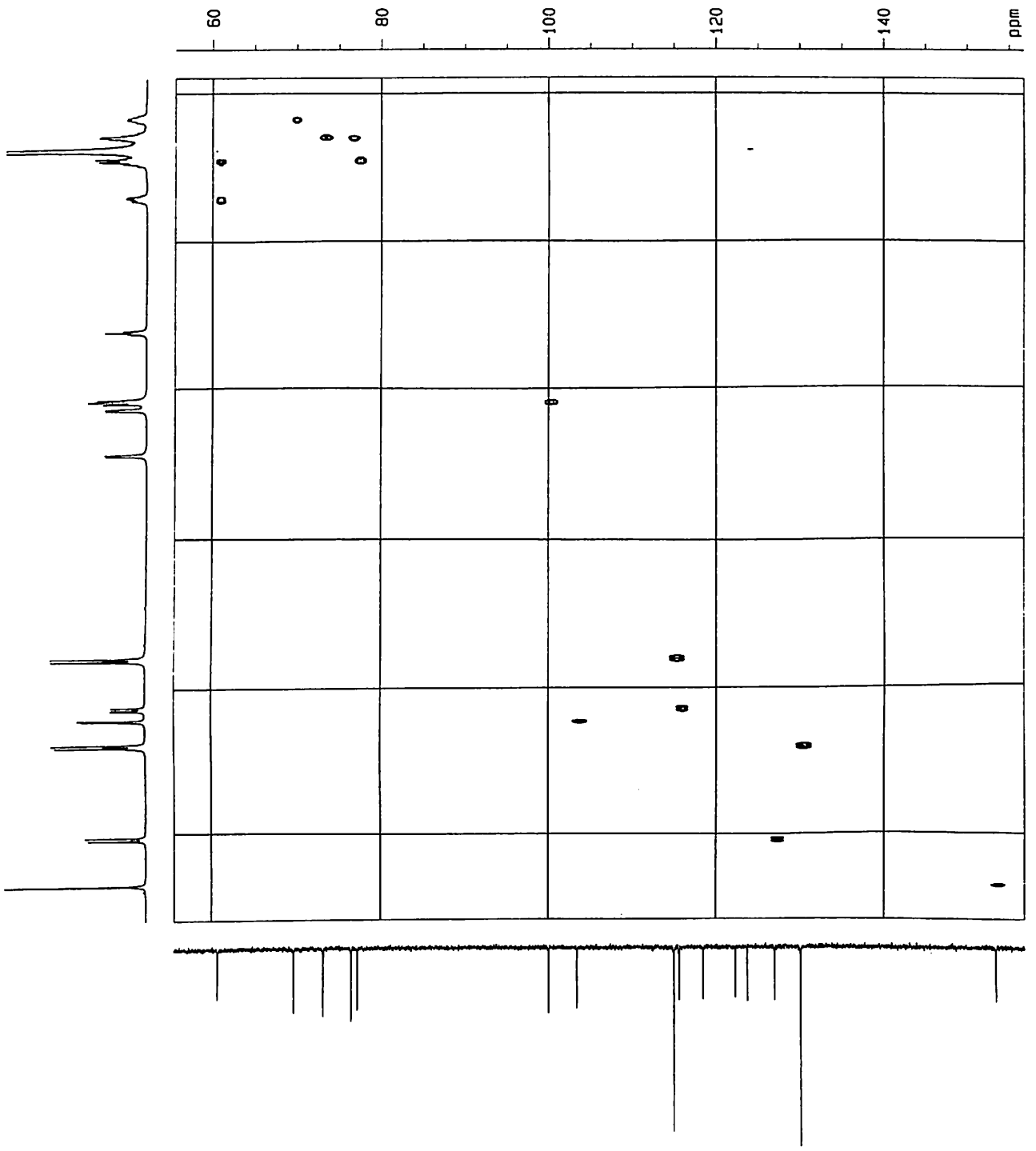


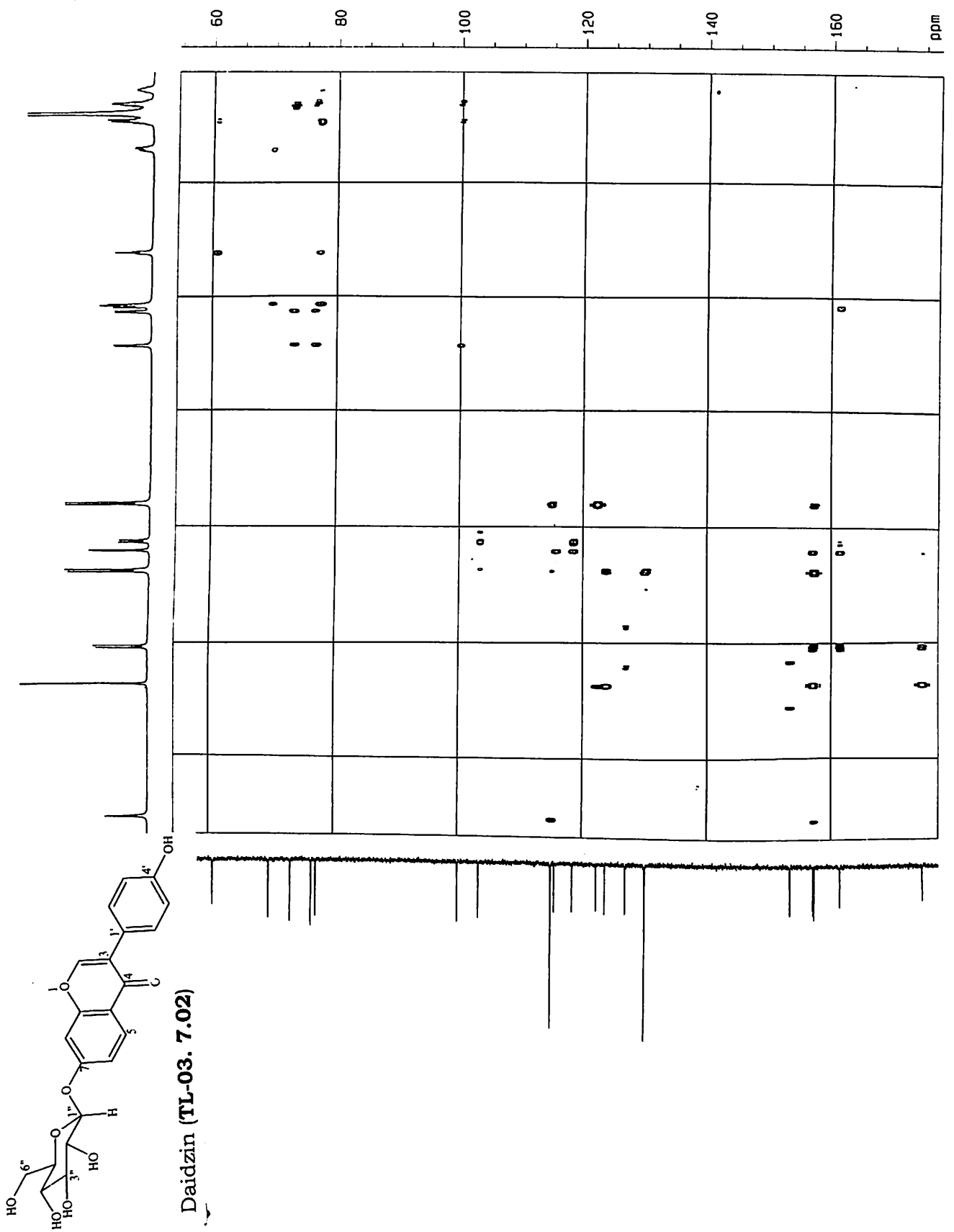

Fig. 7.09: HMQC spectrum (500 MHz, $d_6$-DMSO) of compound **TL-03 (7.02)**

## TABLE 7.07

**HMQC spectral data of TL-03 (Daidzin, 7.02)**

**(Fig. 7.09, solvent: $d_6$-DMSO)**

| Proton chemical shift (δ) | Correlated carbon chemical shift (δ) | Assignment |
|---|---|---|
| 8.36 (H-2) | 153.3 | C-2 |
| 8.04 (H-5) | 127.0 | C-5 |
| 7.14 (H-6) | 115.6 | C-6 |
| 7.22 (H-8) | 103.4 | C-8 |
| 7.40 (H-2′ and H-6′) | 130.1 | C-2′, C-6′ |
| 6.81(H-3′ and H-5′) | 115.0 | C-3′, C-5′ |
| 5.09 (H-1″) | 100.0 | C-1″ |
| 3.29 (H-2″) | 73.2 | C-2″ |
| 3.46 (H-3″) | 77.2 | C-3″ |
| 3.17 (H-4″) | 69.8 | C-4″ |
| 3.33 (H-5″) | 76.5 | C-5″ |
| 3.70 and 3.46 ($H_a$-6″ and $H_b$-6″) | 60.7 | C-6″ |



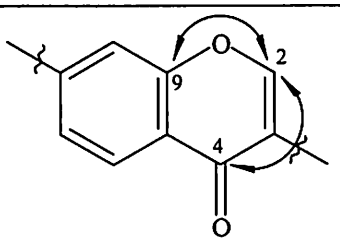
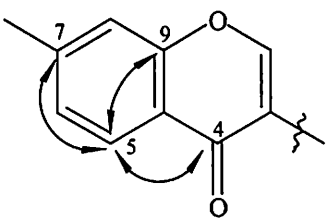
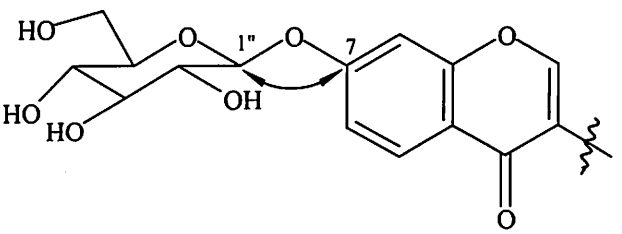
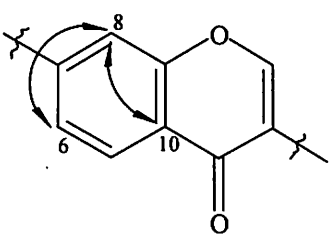

Daidzin (**TL-03. 7.02**)

Fig. 7.10: HMBC spectrum (500 MHz, $d_6$-DMSO) of compound **TL-03 (7.02)**

## TABLE 7.08

**HMBC spectral data of TL-03 (Daidzin, 7.02)**

**(Fig. 7.10, solvent: $d_6$-DMSO)**

| Chemical shift (δ) | Correlated carbons | Structural units derived |
|---|---|---|
| 8.36 (H-2) | C-4, C-9 |  |
| 8.04 (H-5) | C-4, C-7, C-9 |  |
| 5.09 (H-1″) | C-7 |  |
| 7.22 (H-8) | C-6, C-10 |  |

## TL-04

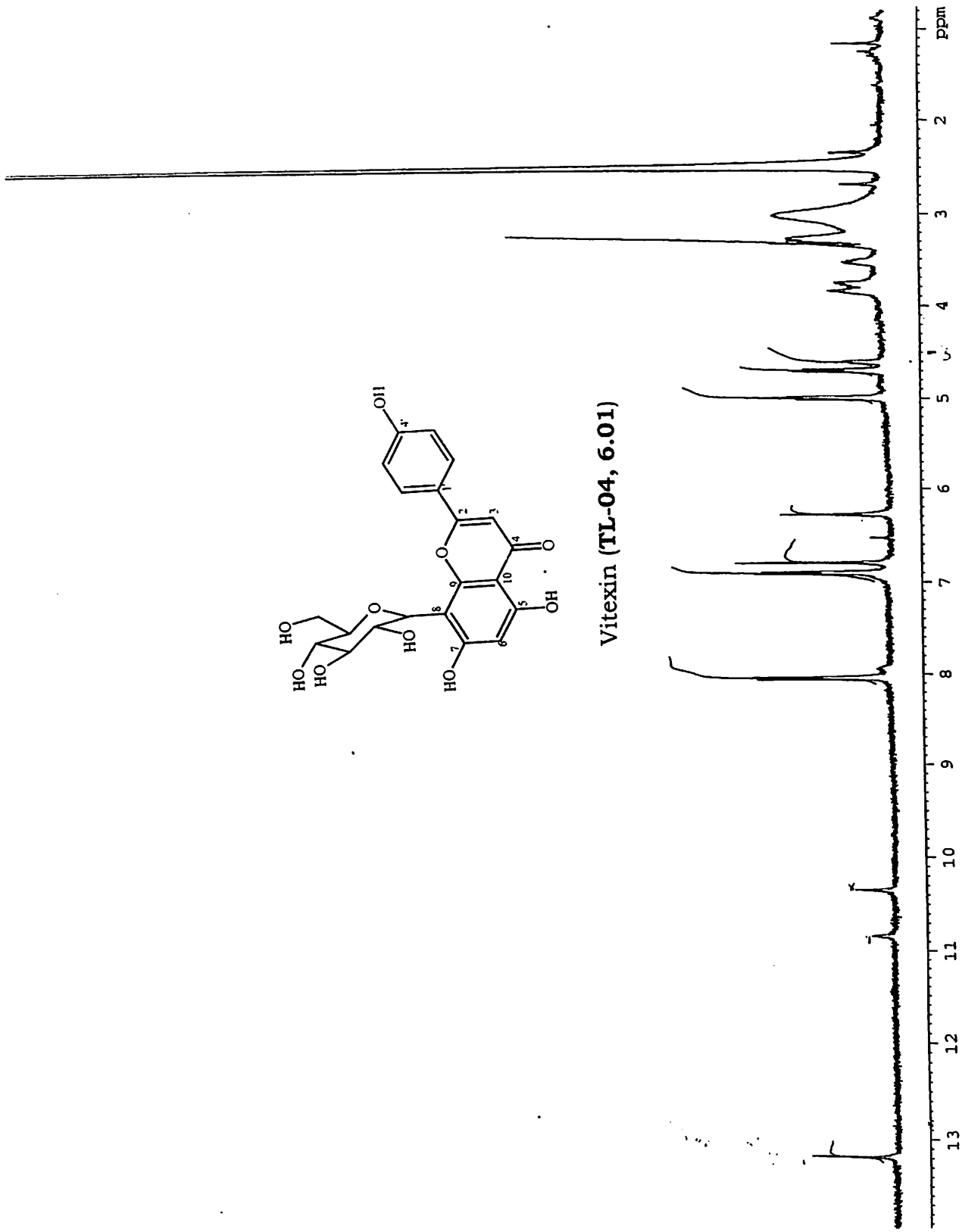
It was obtained as a yellow amorphous powder, m.p. 275-277 °C. The molecular formula, $C_{21}H_{20}O_{10}$, was deduced from elemental analysis and LC-MS [*m/z* 431 $(M-H)^-$] data.

TL-04 exhibited bands in its UV (MeOH) spectrum at $\lambda_{max}$ 270 and 334 nm. Its IR (KBr) spectrum showed bands at $\nu_{max}$ 3381 (hydroxyl), 1652 (α,β-unsaturated carbonyl), 1568 and 1501 $cm^{-1}$ (aromatic). The $^1H$ NMR (Fig. 7.11, Table 7.09) spectrum showed the presence of a chelated hydroxyl (δ 13.17, 1H, s), an aromatic singlet proton (δ 6.28, 1H, s) and a downfield olefinic proton (δ 6.79, 1H, s), suggestive of a flavonoid nucleus.[9] It also showed the presence of an $A_2B_2$ spin system [δ 8.03 (2H, d, *J* = 8.5 Hz) and δ 6.90 (2H, d, *J* = 8.5 Hz)] attributable to a para disubstituted phenyl unit.[5]

In addition, the $^1H$ NMR spectrum showed the presence of a series of signals between δ 3.19 and 4.69 characteristic of a sugar unit. The presence of signal at δ 4.69 (1H, d, *J* = 9.8 Hz), attributable to H-1 of the sugar unit, indicated the presence of a C-glycoside.[1]

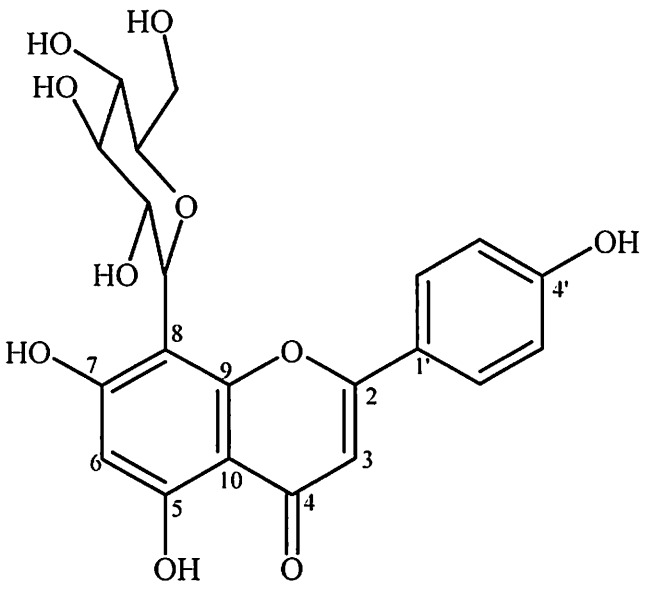
Literarure survey on C-glucosylflavonoids, revealed that the physical and spectral data of TL-04 were in good agreement with those recorded for vitexin[10] (**6.01**), isolated earlier from *Vitex agnus-castus*[11] and *Vitex peduncularis*.[12,13]

Based on the foregoing, the structure of TL-04 was established as vitexin (**6.01**).




Vitexin (**TL-04, 6.01**)

Fig. 7.11: $^1$H NMR spectrum (400 MHz, $d_6$-DMSO) of compound **TL-04 (6.01)**




**6.01**

## TABLE 7.09

**$^1$H NMR spectral data of TL-04 (Vitexin, 6.01)**

**(Fig. 7.11, 400 MHz spectrum, $d_6$-DMSO)**

| Chemical shift (δ) | Proton integration | Multiplicity (*J* in Hz) | Assignment |
|---|---|---|---|
| 13.17 | 1H | s | 5-OH |
| 6.79 | 1H | s | H-3 |
| 6.28 | 1H | s | H-6 |
| 8.03 | 2H | d (8.5) | H-2′ and H-6′ |
| 6.90 | 2H | d (8.5) | H-3′ and H-5′ |
| 4.69 | 1H | d (9.8) | H-1″ |
| 3.78-3.19 | 6H | m | H-2″-H-6″ |

## TL-01

It was obtained as a white crystalline solid from methanol, m.p. 180-182 °C, $[\alpha]_D^{25}$ +51.0° (c 0.75, $H_2O$). The molecular formula, $C_7H_{14}O_6$, was deduced from elemental analysis and LC-MS (*m/z* 193 $(M-H)^-$] data.

The IR (KBr) spectrum showed bands at $\nu_{max}$ 3403 (hydroxyl) and 1072 $cm^{-1}$ (C-O). The $^1H$ NMR (Fig. 7.12, Table 7.10) spectrum showed the presence of a methoxyl group (δ 3.53, 3H, s) and six oxygenated methine protons [δ 3.27 (1H, t, *J* = 9.8 Hz), 3.58 (1H, t, *J* = 9.8 Hz), 3.69 (1H, dd, *J* = 2.8, 9.8 Hz), 3.75 (1H, dd, *J* = 2.8, 9.8 Hz), and δ 3.94 (2H, m)].

The $^{13}C$ NMR spectral data (Fig. 7.13, Table 7.11) showed the presence of a methoxyl group (δ 60.1) and six oxygenated methine carbons (δ 70.2, 70.9, 71.8, 72.0, 72.5, and 83.1). These assignments were supported by the DEPT (Fig. 7.14) spectral data and HMQC (Fig. 7.15, Table 7.12) correlations.

The above spectral data indicated the presence of a cyclitol skeleton[14] in TL-01. In the HMBC spectrum (Fig. 7.16, Table 7.13) correlations were observed between methoxyl group (δ 3.53, s) and downfield oxygenated carbon (δ 83.1, C-3); H-3 methine proton (δ 3.27, t, *J* = 9.8 Hz) and methoxyl carbon (δ 60.1). These correlations and coupling constants indicated the presence of an equatorial methoxyl group[15] in TL-01.

Literature survey on naturally occurring cyclitols revealed that the physical, spectral data and optical rotation of TL-01 were in good agreement with those recorded for 3-*O*-methyl-D-chiroinositol[14,15] (**7.03**).

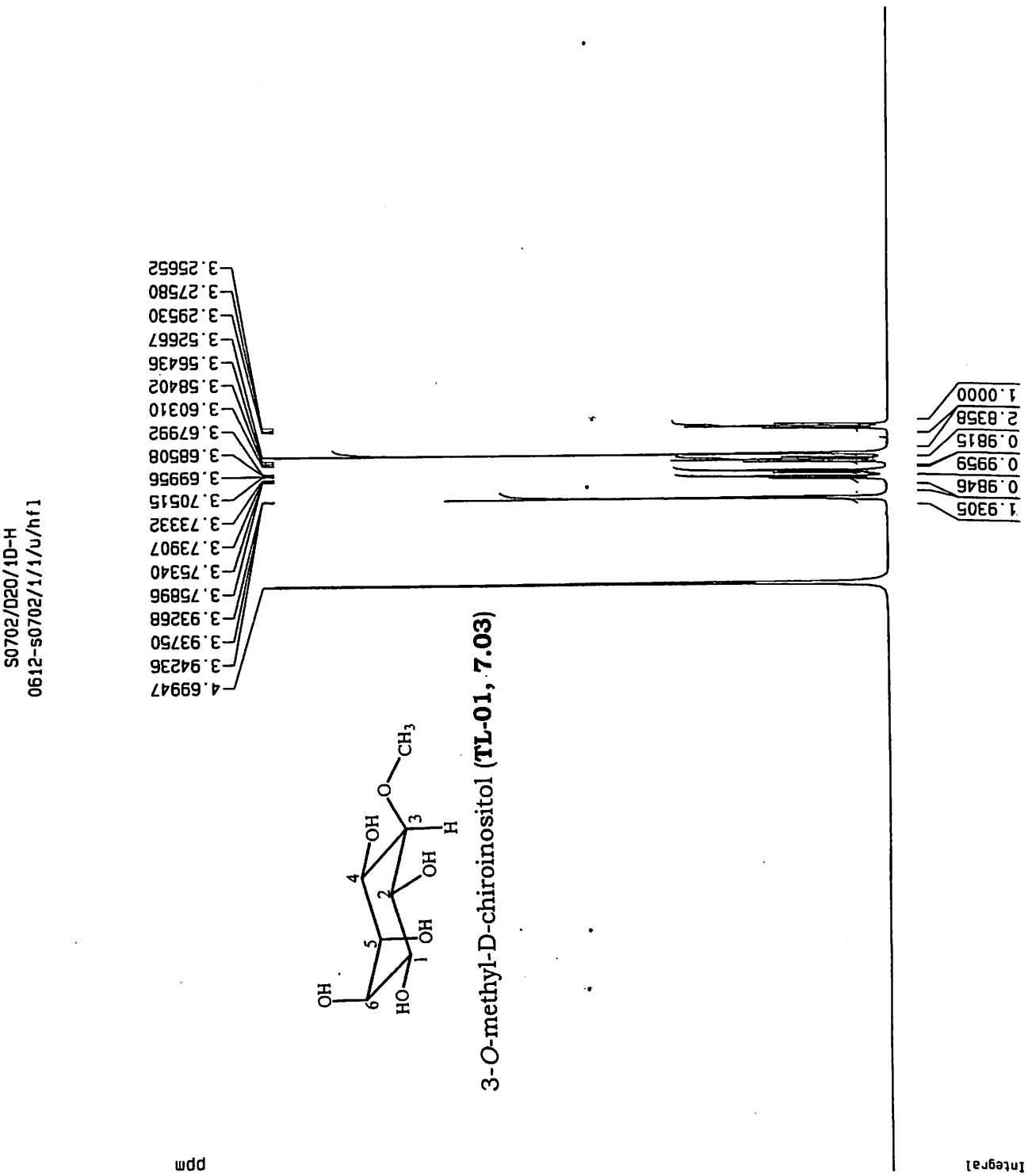
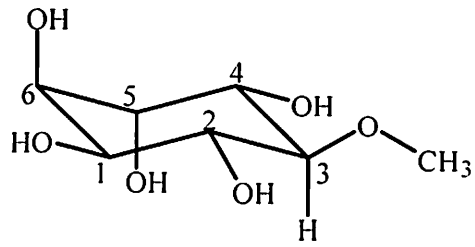



Fig. 7.12: $^1$H NMR spectrum (500 MHz, $D_2O$) of compound **TL-01 (7.03)**

Based on the foregoing, the structure of TL-01 was established as 3-*O*-methyl-D-chiroinositol (**7.03**)

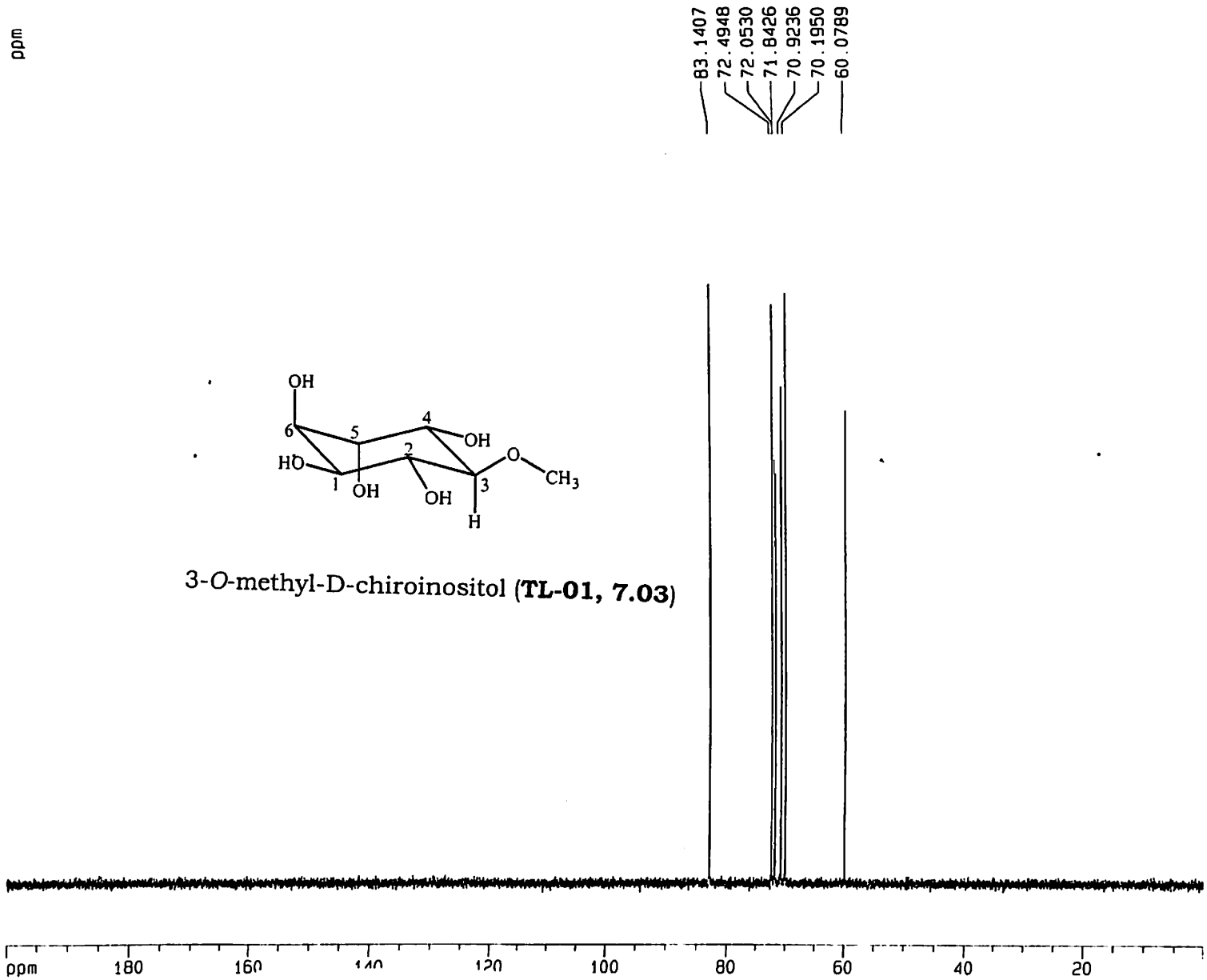



**7.03**

## TABLE 7.10

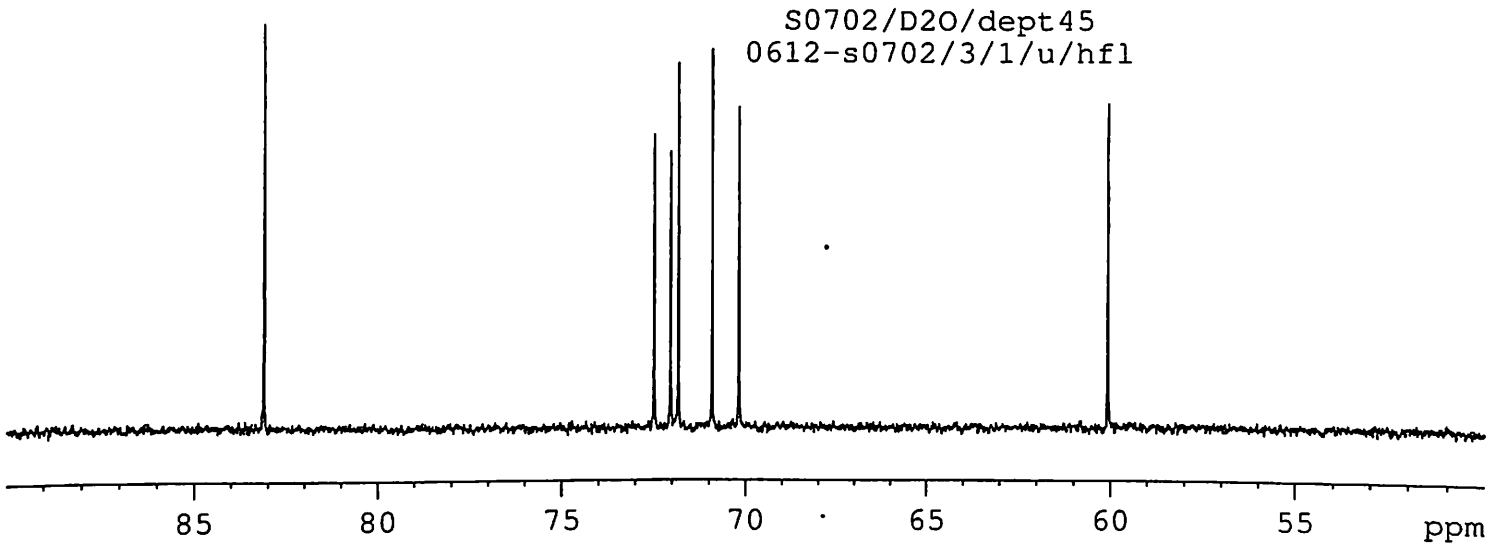
**$^1$H NMR spectral data of TL-01 (3-*O*-methyl-D-chiroinositol, 7.03) (Fig. 7.12, 500 MHz spectrum, $D_2O$)**

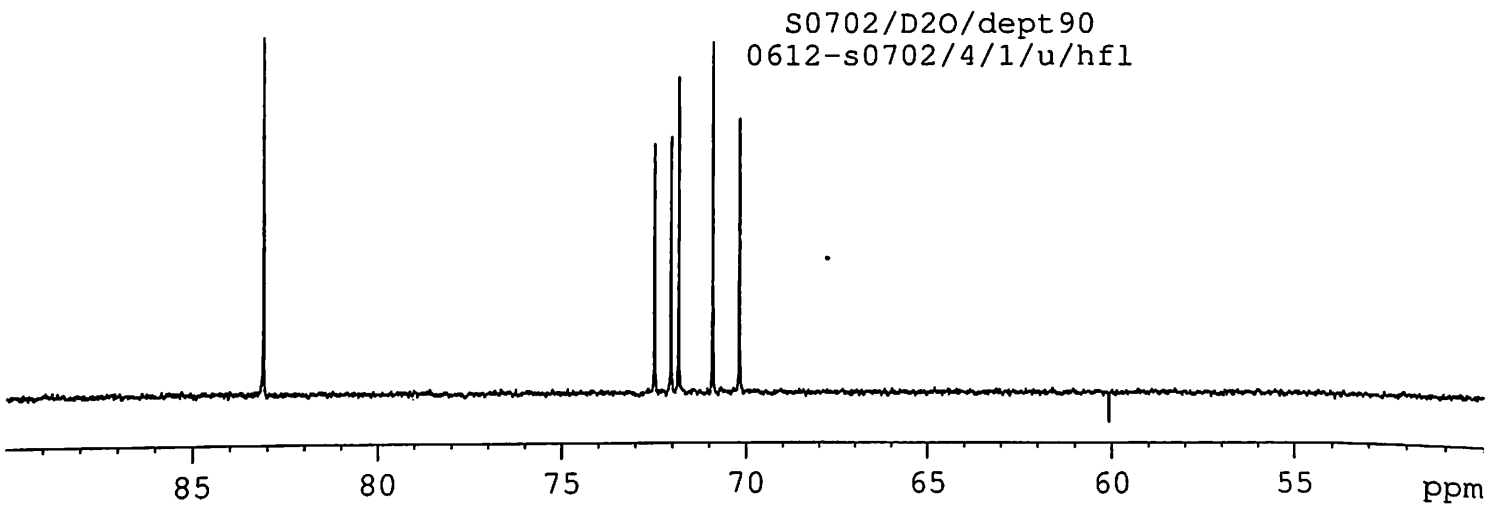
| Chemical shift (δ) | Proton integration | Multiplicity (J in Hz) | Assignment |
|---|---|---|---|
| 3.94 | 2H | m | H-1 and H-6 |
| 3.75 | 1H | dd (9.8, 2.8) | H-2 |
| 3.27 | 1H | t (9.8) | H-3 |
| 3.58 | 1H | t (9.8) | H-4 |
| 3.69 | 1H | dd (9.8, 2.8) | H-5 |
| 3.53 | 3H | s | OMe |

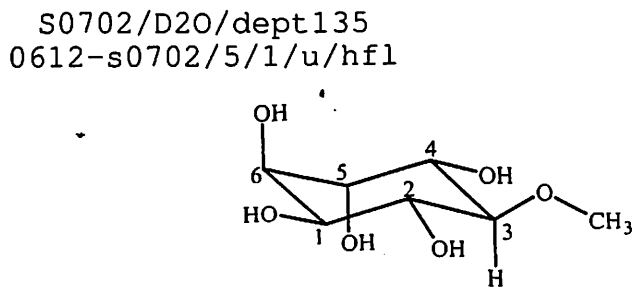



3-*O*-methyl-D-chiroinositol (**TL-01, 7.03**)

Fig. 7.13: $^{13}C$ NMR spectrum (125 MHz, $D_2O$) of compound **TL-01** (**7.03**)




3-*O*-methyl-D-chiroinositol (**TL-01, 7.03**)

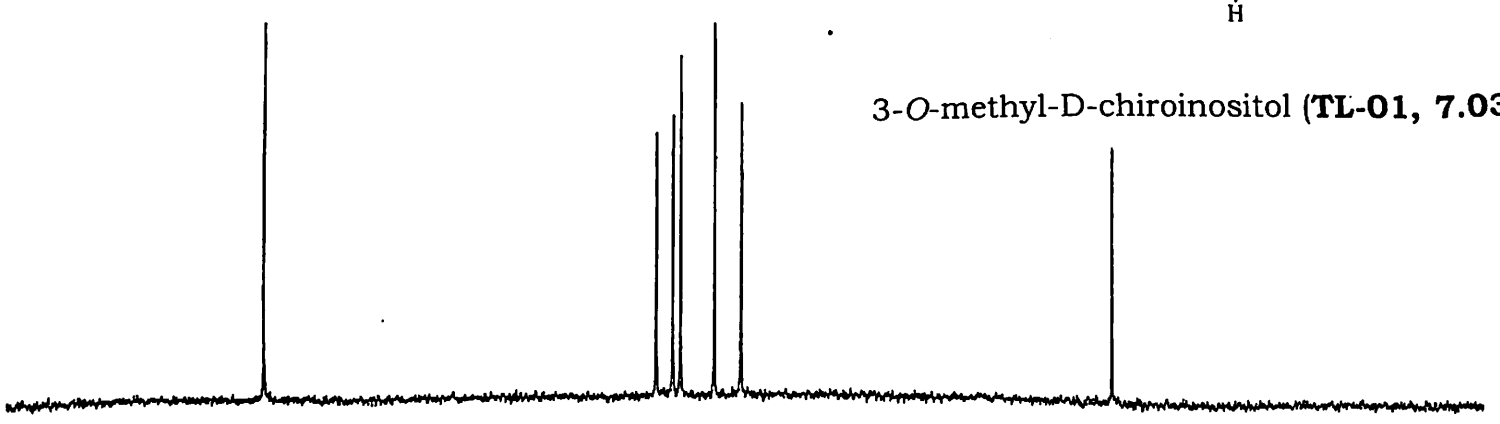
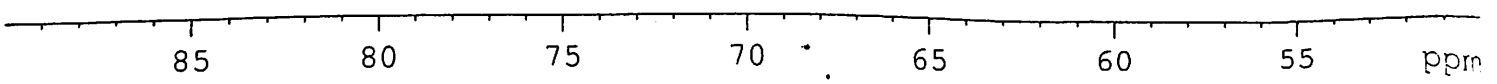


Fig. 7.14: DEPT spectrum ($D_2O$) of compound **TL-01 (7.03)**



## TABLE 7.11

**$^{13}C$ NMR spectral data of TL-01 (3-O-methyl-D-chiroinositol, 7.03)**

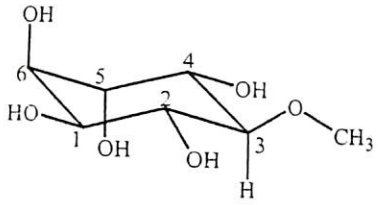
**(Fig. 7.13, 500 MHz spectrum, $D_2O$)**

| Chemical shift (δ) | DEPT | Assignment |
|---|---|---|
| 71.8 | CH | C-1 |
| 70.2 | CH | C-2 |
| 83.1 | CH | C-3 |
| 72.5 | CH | C-4 |
| 70.9 | CH | C-5 |
| 72.0 | CH | C-6 |
| 60.1 | $CH_3$ | OMe |

## TABLE 7.12

**HMQC spectral data of TL-01 (3-O-methyl-D-chiroinositol, 7.03)**
**(Fig. 7.15, solvent: $D_2O$)**

| Proton chemical shift (δ) | Correlated carbon chemical shift (δ) | Assignment |
|---|---|---|
| 3.94 (H-1) | 71.8 | C-1 |
| 3.75 (H-2) | 70.2 | C-2 |
| 3.27 (H-3) | 83.1 | C-3 |
| 3.58 (H-4) | 72.5 | C-4 |
| 3.69 (H-5) | 70.9 | C-5 |
| 3.94 (H-6) | 72.0 | C-6 |
| 3.53 (OMe) | 60.1 | OMe |




3-*O*-methyl-D-chiroinositol (**TL-01, 7.03**)

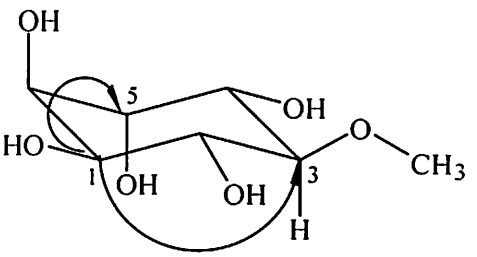
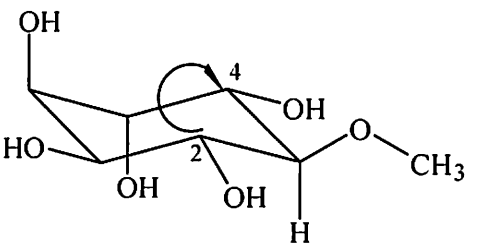
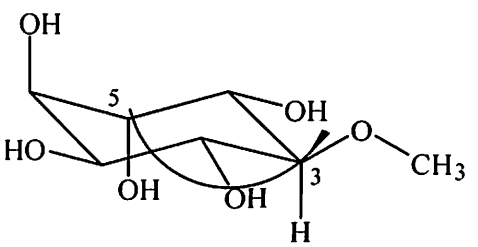
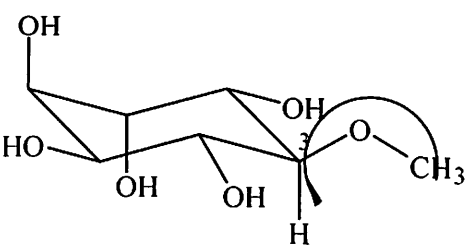



Fig. 7.16: HMBC spectrum (500 MHz, $D_2O$) of compound **TL-01** (**7.03**)

## TABLE 7.13

**HMBC spectral data of TL-01 (3-*O*-methyl-D-chiroinositol, 7.03)**

**(Fig. 7.16, solvent: $D_2O$)**

| Chemical shift (δ) | Correlated carbons | Structural units derived |
|---|---|---|
| 3.94 (H-1) | C-3, C-5 |  |
| 3.75 (H-2) | C-4 |  |
| 3.69 (H-5) | C-3 |  |
| 3.53 (OMe) | C-3 |  |

## EXPERIMENTAL

### Bergenin (TL-02, 7.01, 180 mg)

It was obtained as colorless crystals from aqueous methanol, m.p. 140-142 °C

| | |
|---|---|
| $[\alpha]_D^{25}$ | : –20.0° (*c*, 0.2 MeOH) |
| Found | : C, 51.65%; H, 4.96% |
| $C_{14}H_{16}O_9$ requires | : C, 51.21%; H, 4.87% |
| UV (MeOH) | : $\lambda_{max}$ 277 nm |
| IR (KBr) | : $\nu_{max}$ 3390. 1702, 1612, and 1528 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 7.01, Table 7.01 |
| $^{13}C$ NMR | : Fig. 7.02, Table 7.02 |
| DEPT | : Fig. 7.03 |
| HMQC | : Fig. 7.04, Table 7.03 |
| HMBC | : Fig. 7.05, Table 7.04 |

### Daidzin (TL-03, 7.02, 145 mg)

It was obtained as pale yellow crystalline solid from aqueous methanol, m.p. 232-233 °C

| | |
|---|---|
| Found | : C, 60.25%; H, 4.95% |
| $C_{21}H_{20}O_9$ requires | : C, 60.57%; H, 4.80% |
| UV (MeOH) | : $\lambda_{max}$ 256 nm |
| IR (KBr) | : $\nu_{max}$ 3372. 1623, 1514 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 7.06, Table 7.05 |
| $^{13}C$ NMR | : Fig. 7.07, Table 7.06 |
| DEPT | : Fig. 7.08 |
| HMQC | : Fig. 7.09, Table 7.07 |
| HMBC | : Fig. 7.10, Table 7.08 |

**Vitexin (TL-04, 6.01, 45 mg)**

It was obtained as yellow amorphous powder from methanol, m.p. 275-277 °C

| | |
|---|---|
| Found | : C, 58.17%; H, 4.83% |
| $C_{21}H_{20}O_{10}$ requires | : C, 58.33%; H, 4.62% |
| UV (MeOH) | : $\lambda_{max}$ 270, 334 nm |
| IR (KBr) | : $\nu_{max}$ 3381. 1652, 1568 and 1501 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 7.11, Table 7.09 |

**3-*O*-Methyl-D-chiroinositol (TL-01, 7.03, 5 g)**

It was obtained as a white crystalline solid from methanol, m.p. 180-182 °C

| | |
|---|---|
| $[\alpha]_D^{25}$ | : +51.0° (*c*, 0.75 $H_2O$) |
| Found | : C, 43.40%; H, 7.33% |
| $C_7H_{14}O_6$ requires | : C, 43.29%; H, 7.21% |
| IR (KBr) | : $\nu_{max}$ 3403. 1072 $cm^{-1}$ |
| $^1H$ NMR | : Fig. 7.12, Table 7.10 |
| $^{13}C$ NMR | : Fig. 7.13, Table 7.11 |
| DEPT | : Fig. 7.14 |
| HMQC | : Fig. 7.15, Table 7.12 |
| HMBC | : Fig. 7.16, Table 7.13 |

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# Anti-inflammatory and antioxidant activity studies on *V. altissima* and *T. labialis*

The details of extraction, isolation and characterization of several chemical constituents from the ethyl acetate extractives of leaves of *V. altissima* and methanolic extractives of aerial parts of *T. labialis* were described in **chapters 2-7**.

The details of anti-inflammatory activity studies on crude extracts, 5-lipoxygenase inhibitory and antioxidant activities of some of the isolates obtained from the ethyl acetate extractives of *V. altissima* and methanolic extractives of *T. labialis* have been presented in **sections 1-3** in the following pages

## SECTION-1

### Anti-inflammatory activity studies on *V. altissima* and *T. labialis*

Inflammation can be defined as a defensive but exaggerated local tissue reaction in response to exogenous or endogenous insult.

Inflammation is a complex phenomenon comprises of biochemical as well as immunological factors. Many diseases as well as physical trauma including surgery induce inflammatory reactions. These reactions, although necessary to start the healing process, too often induces severe pain, which may even perpetuate the disease.

The drugs, which are used to alleviate or reduce inflammation and the pain, are known as anti-inflammatory agents.

Inflammation is characterized by accumulation of fluid, increased vascular permeability, migration of cells (macrophages, leukocytes, TNF etc.) and immunological reactions.[1]

Various animal and biochemical models corresponding to different stages of inflammation are used to evaluate an anti-inflammatory substance and the ability of the substance under study to reduce these inflammatory conditions is measured.

The screening models of anti-inflammatory agents are broadly divided into three categories based on different stages of inflammation.

i) Acute inflammation models
ii) Sub-acute inflammation models
iii) Chronic-inflammation models.

In the initial stages of inflammation, due to vasodilatation and increased vascular permeability there is accumulation of fluid, which leads to edema. In acute inflammation models edema is induced in animals by injecting various phlogistic agents (substances which causes inflammation) and the ability of inhibition of this edema by the substance under study is measured.

In the present study, *V. altissima* and *T. labialis* extracts were evaluated for their anti-inflammatory activity by an acute inflammation model, using carrageenan-induced edema in rats.

The use of carrageenan as a phlogistic agent was introduced by Winter, et. al.,[2] and is now a routine test and is commonly used in evaluating the activity of anti-inflammatory agents.

Carrageenan is a sulphated mucoplysaccharide derived from Irish Sea moss, *Chondrus crispus*. Many mediators appear to be involved in the carrageeenan edema including, histamine, 5-HT, kinins and prostaglandins.[3] Carrageenan acts through a proteolysis process with formation of kinin like mediators.[4,5] Carrageenan edema is suppressed by pre-treating the rats with cellulose sulphate, a kininogen depleting agent.[6] Vinger et al.[7] had reported that accumulation of edema in carrageenan induced models is a function of time and is biphasic. The first phase begins immediately after injection of the irritant and diminishes in an hour. The second period of accelerated edema formation begins at the end of the first hour and persists through the third hour. The initial phase involves the release of histamine and 5-HT and the second phase is mediated by kinins followed by prostaglandins.[3]

The volume of edema is determined by using a mercury plethysmometer.

**EXPERIMENTAL**

*Plant material* and *Preparation of extracts*

The details of extraction of the leaves of *V. altissima* and *T. labialis* were noted in **chapter-2**. The hexane, ethyl acetate, methanol and 70% aqueous methanol extracts of leaves of *V. altissima* and the hexane, ethyl acetate and methanol extracts of aerial parts of *T. labialis*, are used in the present study. Carrageenan is used as a phlogistic agent and diclofenac sodium is used as a positive control.

*Animals*

Wistar rats of either sex were obtained from the National Institute of Nutrition, Hyderabad. They were housed in groups of 4-6 animals in polypropylene cages and maintained on a standard diet. Animals were fasted overnight with free access to drinking water prior to the experiment.

*Anti-inflammatory activity*

Wistar rats of either sex weighing between 180-220 g were divided into six groups, with each group consisting of six animals. One group served as negative control (received 1% Tween-80, 10 mL/kg), and a second group, which served as positive control received 25 mg/kg, diclofenac sodium suspended in 1% Tween-80. The third, fourth, fifth, and sixth groups were treated with 250 mg/kg of the hexane, ethyl acetate, methanol, and 70% methanol extracts suspended in 1% Tween-80, respectively, by the oral route. Edema was produced after 1 hr of drug treatment, by injecting 0.1 mL (1% w/v in saline) carrageenan solution to the sub planter region of the left hind paw of rats of all groups. The paw volume was measured by a plethysmometer at zero and three hours after carrageenan injection. The difference between the initial and final paw volume was considered as the edema volume. The percent inhibition of paw edema was calculated by comparing the mean edema volume of treated group and control group.

comparing the mean edema volume of treated group and control group. The anti-inflammatory activity results of *V. altissima* and *T. labialis* have been presented in tables 8.01 and 8.02, respectively.

*Statistical analysis*

The edema volume was expressed in mL as mean±SEM ($n$=6). Student $t$-test was applied to evaluate the significance of the results.

**TABLE 8.01**

**Percent inhibition of carrageenan-induced rat paw edema by extracts of *V. altissima***

| S.No | Group | Dose | Mean Edema ±SEM | % inhibition |
|---|---|---|---|---|
| 1 | Control group | 10mL (vehicle) | 0.70±0.02 | |
| 2 | Diclofenac sodium | 25 mg/kg | 0.26±0.03 | 62.29** |
| 3 | *V. altissima* hexane extract | 250 mg/kg | 0.59±0.02 | 15.75 |
| 4 | *V. altissima* ethyl acetate extract | 250 mg/kg | 0.43±0.03 | 38.19** |
| 5 | *V. altissima* methanol extract | 250 mg/kg | 0.60±0.01 | 13.60** |
| 6 | *V. altissima* 70% methanol extract | 250 mg/kg | 0.56±0.02 | 20.05** |

n=6, **$P$<0.001

**TABLE 8.02**

**Percent inhibition of carrageenan-induced rat paw edema by extracts of *T. labialis***

| S.No | Group | Dose | Mean Edema ±SEM | % inhibition |
|---|---|---|---|---|
| 1 | Control group | 10mL (vehicle) | 0.63±0.01 | |
| 2 | Diclofenac sodium | 25 mg/kg | 0.15±0.01 | 76.19** |
| 3 | *T. labialis* hexane extract | 250 mg/kg | 0.61±0.02 | 3.17 |
| 4 | *T. labialis* ethyl acetate extract | 250 mg/kg | 0.59±0.03 | 6.34 |
| 5 | *T. labialis* methanol extract | 250 mg/kg | 0.45±0.03 | 28.57* |

Number of animals in each group 6. * $P<0.01$, ** $P<0.001$

## Results and Discussion

The anti-inflammatory activity of different extracts of the leaves of *V. altissima* was evaluated by carrageenan-induced rat paw edema model. The ethyl acetate extract caused significant inhibition (38.19%) of paw edema as compared to control group three hours after carrageenan injection, followed by 70% methanol extract (20.05%). The summary of the results is presented in table **8.01**.

The results incorporated in table **8.01** substantiate the folklore use of leaves of *V. altissima* to treat inflammatory conditions.

The extracts of *T. labialis* were screened for their anti-inflammatory activity by carrageenan-induced rat paw edema model. The methanolic extractives of *T. labialis* exhibited potent anti-inflammatory activity than hexane and ethyl acetate extracts. The summary of the results is presented in table **8.02**. The results obtained

in the present study substantiate the use of *T. labialis* in inflammatory conditions.

## SECTION-2

### 5-lipoxygenase inhibitory activity studies on *V. altissima* and *T. labialis*

Inflammation results from the complex series of actions and reactions triggered by the body's immunological response to tissue damage. Several inflammatory mediators, such as, histamine, eicosanoids, platelet activating factor, TNF etc. are involved in the inflammation process.[1]

Eicosanoids are the most significant inflammatory mediators. The main source of eicosanoids is arachidonic acid, a 20-carbon fatty acid containing four double bonds. Phospholipase-$A_2$ catalyses the conversion of phospholipids in to arachidonic acid. Many stimuli can liberate arachidonic acid. Arachidonic acid is metabolized to yield important hormone like substances which play a major role in the process of inflammation.[8] There are two primary enzymatic path ways, COX and LOX, in the metabolism of arachidonic acid. The cyclooxygenase mediated path way results in the formation of prostaglandins (PGs) and thromboxanes (TX), and the lipoxygenase path way yields leukotriens (LTs). These metabolites trigger various inflammatory processes.

Several anti-inflammatory drugs act by inhibiting the cocloxygenase[9] or lipoxygenase[10] enzymes and thus prevent the formation of various pro inflammatory mediators from arachidonic acid.

Several plant derived pentacyclic triterpenic acid derivatives, such as boswellic acids of *Boswellia serrata* oleo-gum-resin have been

reported to inhibit the 5-lipoxygenase enzymes and prevent the formation of inflammatory leukotriens.[11]

In view of the significant anti-inflammatory activity exhibited by the ethyl acetate extractives of *V. altissima* and the isolation of several triterpenic acid derivatives from this extract, the isolates of ethyl acetate extract of *V. altissima* have been screened for their 5-lipoxygenase inhibitory activity and the results are presented in this section.

Lipoxygenases are responsible for the dioxygenation of polyenoic fatty acids containing a 1,4-*cis,cis* system, especially arachidonic acid and produces lipid hydroperoxide. In the present study the 5-lipoxygenase enzyme inhibitory activity of the isolates of *V. altissima* has been determined by the modified ferric-xylenol orange peroxide assay (PCA FOX) of Gay, et al.[12] Polyenoic fatty acids are converted into leukotrienes by 5-lipoxygenase enzyme. These conjugated dienes form a colored complex with xylenol orange in presence of $Fe^{2+}$ ion. Optical density of the colored complex can be measured by a colorimeter at 560-600 nm.

## EXPERIMENTAL

### *Material and Methods*

#### *Test Compounds*

The details of extraction and isolation of different secondary metabolites from the ethyl acetate extract of *V. altissima* and methanolic extractives of *T. labialis* were noted in **chapter-2.** The triterpenoids, iridoids and flavonoids of *V. altissima* and the isolates of *T. labialis* are used in the present study. Nordihydroguaiaretic acid (NDGA) was used as a positive control.

*Chemicals and reagents*

Perchloric acid, acetic acid, potassium phosphate buffer, dimethyl sulfoxide, linoleic acid, xylenol orange.

*Enzyme*

5-lipoxygenase enzyme was obtained from the Department of Biotechnology, Central University, Hyderabad.

*5-Lipoxygenase Enzyme Inhibitory Activity*:

Test compounds were screened for 5-lipoxygenase enzyme inhibitory activity by the modified ferric-xylenol orange peroxide assay of Gay, et al.[12] The assay mixture contained 50 mM phosphate buffer (pH 6.3), 5-lipoxygenase, various concentrations of test substance in DMSO, and linoleic acid (80 mM), in a total volume of 0.5 mL. After 5 min of incubation, to the above reaction mixture, 0.5 mL ferric-xylenol orange reagent (in perchloric acid) was added and absorbance was measured after 2 min at 585 nm on a spectrophotometer. Percent inhibition was calculated by comparing absorbance of test substance with that of the control. All the tests were run in triplicate and averaged.

## TABLE 8.03

**5-Lipoxygenase inhibitory activity of isolates of *V. altissima* ethyl acetate extract**

| S.No | Test substance (structure number) | % inhibition at different concentrations | | | |
|---|---|---|---|---|---|
| | | 100 µM | 250 µM | 500 µM | 1000 µM |
| 1 | Corosolic acid (**5.02**) | 17.6 | 39.5 | 79.8 | - |
| 2 | Epicorosolic acid (**5.03**) | 16.3 | 36.0 | 78.6 | - |
| 3 | Ursolic acid (**5.01**) | 13.6 | 45.9 | 69.7 | - |
| 4 | Maslinic acid (**5.11**) | 10.9 | 36.1 | 71.9 | - |
| 5 | Euscaphic acid (**5.05**) | 8.3 | 22.2 | 54.7 | - |
| 6 | Euscaphic acid glucoside (**5.08**) | 10.1 | 16.1 | 21.5 | - |
| 7 | 6′-*O*-trans-caffeoylnegundoside (**3.03**) | NA | NA | NA | 11.98% |
| 8 | 6′-*O*-trans-feruloylnegundoside (**3.02**) | NA | NA | NA | 5.27% |
| 9 | Negundoside (**3.01**) | NA | NA | NA | 7.5% |
| 10 | Luteolin-7-*O*-glucoside (**6.03**) | NA | NA | NA | NA |
| 11 | **NDGA** | 67.4 | 91.5 | - | - |

NA-no activity.

**Results and Discussion**

The 5-lipoxygenase enzyme inhibitory activity of the compounds isolated from ethyl acetate extract of *V. altissima* was evaluated by Gay, et al., method. The summary of results is presented in table 8.03. The pentacyclic triterpenic acids with a free carboxylic acid group [corosolic acid (**5.02**), maslinic acid (**5.11**) and epicorosolic acid (**5.03**)] have exhibited potent inhibitory activity than the triterpenic acid esters (euscaphic acid ester glucoside, **5.08**). The iridoids [negundoside (**3.01**), caffeoyl negundoside (**3.03**) and feruloyl negundoside (**3.02**)] and flavonoids (luteolinglucoside, **6.03**) did not exhibit significant 5-lipoxygenase enzyme inhibitory activity.

The results incorporated in table **8.03** indicate that the anti-inflammatory activity of the ethyl acetate extractives of *V. altissima* could be attributed at least in part to its triterpenic acid components.

Among the isolates of methanolic extractives of *T. labialis* only vitexin exhibited moderate 5-lipoxygenase inhibitory activity (percent inhibition: dose in μM, 12: 500; 22: 1000; 36: 2000) daidzin, bergenin and 3-*O*-methyl-D-chiroinositol did not exhibit any activity even at 1000 μM concentration.

## SECTION-3

### Antioxidant activity studies on *V. altissima* and *T. labialis*

Free radicals and their metabolites which are formed in the body as a consequence of normal metabolic reactions, exposure to pollutants and UV radiation are increasingly recognized for their contribution to tissue injury and degenerative diseases, including arthritis, hepatic injury, ageing, ischemia, tumor promotion and carcinogenesis.[13]

Reactive oxygen species (ROS) are various forms of oxygen, which include free radicals such as superoxide ions ($O_2^{\cdot -}$) and hydroxyl ($OH^{\cdot}$), as well as non-free-radical species such as hydrogen peroxide ($H_2O_2$).[14]

In living organisms ROS are formed in different ways. Normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes are the main endogenous sources of most of the oxidants produced by the cells.[15,16] The exogenous sources of free radicals are UV radiation, organic solvents, tobacco smoke and pesticides.[17]

ROS are known to cause tissue injury and DNA damage that lead to mutations.[18] All aerobic organisms, including human beings, have antioxidant defenses that protect against oxidative damage.[19] However, the natural antioxidant mechanisms can be insufficient and hence dietary intake of antioxidant compounds has gained importance.

The role of oxygen free radicals in the inflammatory process is well established.[20] It has been reported that reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and peroxynitrite participate in the process of inflammation in various tissues.

In skin, ROS can be produced not only by chemical ionization or UV radiation but also enzymatically by polymorphonuclear leukocytes that infiltrate the sites of infection. In both cases, the excessively produced ROS can injure cellular biomolecules such as nucleic acids, proteins, carbohydrates and lipids, causing cellular and tissue damage, which in turn augments the state of inflammation. So, the use of antioxidants in inflammatory conditions has beneficial effects a fact supported by the anti-inflammatory and antioxidant potential of curcuminoids.[21] Several phenolic compounds are known to exhibit potent antioxidant activity.[22]

In view of the significant anti-inflammatory activity exhibited by the ethyl acetate extract of *V. altissima* and methanolic extractives of *T. labialis* and the isolation of several phenolic compounds (Feruloylnegundoside, caffeoylnegundoside, caffeoylgardoside, caffeoylloganic acid and flavonoids) from these extracts, these compounds have been screened for their antioxidant activity and the results are noted in the following paragraphs. Vitamin-C and butylated hydroxyanisole (BHA) were used as positive control. Details of isolation of chemical constituents were presented in **chapter 2**.

The antioxidant activity of the compounds was carried out by two different mechanisms, namely, superoxide scavenging and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities.

**1. Superoxide Free-radical scavenging activity:**

Superoxide radicals were generated in-vitro by non-enzymatic system and determined spectrophotometrically by following the Nitro Blue Tetrazolium (NBT) riboflavin photoreduction method of McCord and Fridovich.[23] The antioxidant activity of the compounds was expressed as the 50% inhibitory concentration ($IC_{50}$) that was measured from the plot drawn concentration (μg) verses percentage inhibition.

**2. DPPH-radical scavenging activity:**

DPPH-radical scavenging activity was determined by the method described by Lamaison, et al.,[24] based on the reduction of methanolic solution of the colored DPPH. The antioxidant activity of the compounds was expressed as the 50% inhibitory concentration ($IC_{50}$) that was measured from the plot drawn concentration (μg) verses percentage inhibition.

**Experimental**

*Material and methods*

*Chemicals and reagents*

Sodium dihydrogenorthophosphate, di-sodium hydrogenortho phosphate, ethylenediaminetetraacetic acid, riboflavin, nitro blue tetrazolium chloride, sodium cyanide, 1,1-diphenyl-2-picrylhydrazyl, methanol.

*Superoxide Free-radical Scavenging Activity*

Superoxide radical-scavenging activity of the test compounds isolated from *V. altissima* was determined by the method of McCord and Fridovich.[23] The assay mixture contained EDTA (6.0 μM), NaCN (3 μg), riboflavin (2 μM), NBT (50 μM) and various concentrations of test substances in methanol and phosphate buffer (58 mM, pH 7.8), in a final volume of 3 mL. The tubes were shaken well and the absorbance was measured before and after illumination at 560 nm. The percent inhibition of superoxide radical was measured by comparing the mean absorbance values of control and those of the test substances. $IC_{50}$ values were obtained from the plot drawn concentration in μg verses percentage inhibition.

*DPPH Radical-scavenging Activity*

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging activity was measured by the method of Lamaison, et al.[24] The reaction mixture contained $1.0 \times 10^{-4}$ mM methanolic solution of DPPH and various concentrations of test substances and kept in dark area for 50 min. The absorbance of the samples was measured on a spectrometer at 517 nm against a blank. All the tests were run in triplicate and averaged.

**TABLE 8.04**

**Superoxide radical scavenging activity of iridoids**

| Test substance (structure number) | Dose (in μg) | Mean OD | % inhibition | $IC_{50}$ (in μg) |
|---|---|---|---|---|
| 6'-*O*-*trans*-caffeoyl negundoside (**3.03**) | Control | 0.6639 | | 16 |
| | 10 | 0.4019 | 39.46 | |
| | 25 | 0.2296 | 65.42 | |
| | 50 | 0.0902 | 86.41 | |
| | | | | |
| 6'-*O*-*trans*- feruloyl negundoside (**3.02**) | Control | 0.5956 | | 137 |
| | 50 | 0.4391 | 26.28 | |
| | 100 | 0.3782 | 36.50 | |
| | 200 | 0.1819 | 69.46 | |
| | | | | |
| Negundoside (**3.01**) | Control | 0.6185 | | >200 |
| | 50 | 0.5917 | 4.33 | |
| | 100 | 0.5684 | 8.10 | |
| | 200 | 0.4881 | 21.08 | |
| | | | | |
| 2'-*O*-*p*-hydroxybenzoyl-6'-*O*-*trans*-caffeoylgardoside (**3.05**) | Control | 0.6119 | | 21 |
| | 10 | 0.4556 | 25.54 | |
| | 25 | 0.2689 | 56.05 | |
| | 50 | 0.0875 | 85.70 | |
| | | | | |
| 2'-*O*-*p*-hydroxybenzoyl-6'-*O*-*trans*-caffeoyl-8-epiloganic acid (**3.07**) | Control | 0.6468 | | 21 |
| | 10 | 0.448 | 30.74 | |
| | 25 | 0.2871 | 55.61 | |
| | 50 | 0.136 | 78.97 | |
| | | | | |
| Agnuside (**3.08**) | Control | 0.6537 | | >100 |
| | 25 | 0.6447 | 1:38 | |
| | 50 | 0.6019 | 7.92 | |
| | 100 | 0.4631 | 29.16 | |
| | | | | |
| Vitamin-C | Control | 0.583 | | 150 |
| | 100 | 0.480 | 17.66 | |
| | 200 | 0.189 | 67.50 | |
| | 400 | 0.014 | 97.52 | |
| | | | | |
| BHA | Control | 0.834 | | 174 |
| | 50 | 0.598 | 28.30 | |
| | 100 | 0.4696 | 43.69 | |
| | 200 | 0.3988 | 52.18 | |

**TABLE 8.05**

**DPPH-radical scavenging activity of iridoids**

| Test substance | Dose (in µg) | Mean OD | % inhibition | $IC_{50}$ (in µg) |
|---|---|---|---|---|
| 6′-*O*-*trans*-caffeoyl negundoside (**3.03**) | Control | 0.973 | | 10 |
| | 5 | 0.688 | 29.29 | |
| | 10 | 0.47 | 51.70 | |
| | 25 | 0.064 | 93.42 | |
| | | | | |
| 6′-*O*-*trans*- feruloyl negundoside (**3.02**) | Control | 0.902 | | 40 |
| | 25 | 0.603 | 33.15 | |
| | 50 | 0.317 | 64.86 | |
| | 100 | 0.152 | 83.15 | |
| | | | | |
| Negundoside (**3.01**) | Control | 0.981 | | >200 |
| | 50 | 0.963 | 1.83 | |
| | 100 | 0.931 | 5.10 | |
| | 200 | 0.845 | 13.86 | |
| | | | | |
| 2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoylgardoside (**3.05**) | Control | 1.0859 | | 5 |
| | 2.5 | 0.8344 | 23.16 | |
| | 5 | 0.548 | 49.53 | |
| | 10 | 0.118 | 89.13 | |
| | | | | |
| 2′-*O*-*p*-hydroxybenzoyl-6′-*O*-*trans*-caffeoyl-8-epiloganic acid (**3.07**) | Control | 1.0859 | | 6.8 |
| | 2.5 | 0.8619 | 20.63 | |
| | 5 | 0.6198 | 42.92 | |
| | 10 | 0.2647 | 75.62 | |
| | | | | |
| Agnuside (**3.08**) | Control | 1.0399 | | >100 |
| | 25 | 0.9831 | 5.46 | |
| | 50 | 0.8512 | 18.15 | |
| | 100 | 0.7233 | 30.45 | |
| | | | | |
| Vitamin-C | Control | 1.4813 | | 6.1 |
| | 2.5 | 1.1657 | 21.3 | |
| | 5 | 0.875 | 40.9 | |
| | 10 | 0.3366 | 77.3 | |
| | | | | |
| BHA | Control | 0.8982 | | 3.2 |
| | 2.5 | 0.5189 | 42.23 | |
| | 5 | 0.2855 | 68.21 | |
| | 10 | 0.107 | 88.09 | |

**TABLE 8.06**

**Superoxide radical scavenging activity of flavonoids**

| Test substance | Dose (in μg) | Mean OD | % inhibition | $IC_{50}$ (in μg) |
|---|---|---|---|---|
| Vitexin (**6.01**) | Control | 0.6083 | | 62 |
| | 25 | 0.5249 | 13.71 | |
| | 50 | 0.3238 | 46.77 | |
| | 100 | 0.1181 | 80.59 | |
| | | | | |
| Luteolin-7-*O*-glucoside (**6.03**) | Control | 0.6774 | | 8 |
| | 5 | 0.4044 | 40.30 | |
| | 10 | 0.2789 | 58.83 | |
| | 25 | 0.1069 | 84.22 | |

**TABLE 8.07**

**DPPH-radical scavenging activity of flavonoids**

| Test substance | Dose (in μg) | Mean OD | % inhibition | $IC_{50}$ (in μg) |
|---|---|---|---|---|
| Vitexin (**6.01**) | Control | 1.0539 | | 43 |
| | 10 | 0.9215 | 12.5 | |
| | 25 | 0.7162 | 32.5 | |
| | 50 | 0.4595 | 56.4 | |
| | | | | |
| Luteolin-7-*O*-glucoside (**6.03**) | Control | 1.0857 | | 7.4 |
| | 2.5 | 0.9253 | 14.77 | |
| | 5.0 | 0.7113 | 34.48 | |
| | 10 | 0.344 | 68.32 | |

**TABLE 8.08**

**Superoxide radical scavenging activity of isolates of *T. labialis***

| Test substance | Dose (in µg) | Mean OD | % inhibition | $IC_{50}$ (in µg) |
|---|---|---|---|---|
| vitexin (**6.01**) | Control | 0.6083 | | 62 |
| | 25 | 0.5249 | 13.71 | |
| | 50 | 0.3238 | 46.77 | |
| | 100 | 0.1181 | 80.59 | |
| | | | | |
| daidzin (**7.02**) | Control | 0.6403 | | >100 |
| | 25 | 0.6357 | 0.72 | |
| | 50 | 0.6023 | 5.93 | |
| | 100 | 0.5637 | 11.96 | |
| | | | | |
| bergenin (**7.01**) | Control | 0.7291 | | >100 |
| | 25 | 0.7207 | 1.15 | |
| | 50 | 0.6326 | 13.24 | |
| | 100 | 0.5941 | 18.52 | |

**TABLE 8.09**

**DPPH-radical scavenging activity of isolates of *T. labialis***

| Test substance | Dose (in µg) | Mean OD | % inhibition | $IC_{50}$ (in µg) |
|---|---|---|---|---|
| vitexin (**6.01**) | Control | 1.0539 | | 43 |
| | 10 | 0.9215 | 12.56 | |
| | 25 | 0.7262 | 31.09 | |
| | 50 | 0.4595 | 56.40 | |
| | | | | |
| daidzin (**7.02**) | Control | 1.0484 | | >100 |
| | 25 | 1.0392 | 0.88 | |
| | 50 | 1.0268 | 2.06 | |
| | 100 | 0.9433 | 10.02 | |
| | | | | |
| bergenin (**7.01**) | Control | 1.0425 | | >100 |
| | 25 | 0.952 | 8.68 | |
| | 50 | 0.8942 | 14.23 | |
| | 100 | 0.8218 | 21.17 | |
| | 2.5 | 1.1657 | 21.3 | |
| | 5 | 0.875 | 40.9 | |
| | 10 | 0.3366 | 77.3 | |

**Results and Discussion**

The antioxidant potential of the isolates of ethyl acetate extract of the leaves of *V. altissima* was evaluated by superoxide radical scavenging and DPPH-radical scavenging methods. The phenolic (iridoids and flavonoids) compounds exhibited potent antioxidant activity in comparison with known antioxidants, such as, vitamin-C and BHA. The results incorporated in tables **8.04-8.07** revealed that the phenolic compounds having orthodihydroxy substituents are more potent. As expected caffeoylnegundoside ($IC_{50}$; 16μg) and caffeoylgardoside ($IC_{50}$; 21 μg) have shown strong antioxidant activity in comparison with feruloylnegundoside ($IC_{50}$; 137 μg)

The antioxidant activity study results of isolates of methanolic extractives of *T. labialis* incorporated in tables **8.08** and **8.09** revealed that, only vitexin (**6.01**) exhibited moderate antioxidant activity. Bergenin and daidzin are weak antioxidants.

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# CONCLUSIONS

## CONCLUSIONS AND FUTURE SCOPE:

In view of the reported folklore use of ***Vitex altissima*** and ***Teramnus labialis***, in inflammatory conditions, the author has investigated these medicinal plants for their anti-inflammatory activities and isolated and characterized the active chemical constituents responsible for the activity.

The ethyl acetate extract of the leaves of *V. altissima* and the methanolic extractives of aerial parts of *T. labialis* exhibited significant anti-inflammatory activity in carrageenan-induced rat paw edema method.

Phytochemical investigations on ethyl acetate extractives of the leaves of *V. altissima*, led to the isolation and characterization of 23 chemical constituents belonging to different classes of secondary metabolites, such as iridoids, triterpenoids, flavonoids and lignan.

Six new iridoids (**3.02**-**3.07**), belonging to mussaenosidic acid, gardoside, 8-epiloganic acid and aucubin series have been isolated and characterized based on interpretation of high resolution NMR spectral data, in addition to two known iridoids, negundoside (**3.01**) and agnuside (**3.08**). The new iridoids having caffeoyl substituents, have exhibited significant anti-oxidant activity both in superoxide radical scavenging and DPPH-radical scavenging methods, in comparison with known antioxidants, vitamin-C and BHA.

Nine pentacyclic triterpenic acid derivatives (**5.01**-**5.03**, **5.05**, **5.07**, **5.09**-**5.11** and **5.13**) of ursane and oleanane skeleton having mono, -di and -tri hydroxyl substituents have been isolated and characterized. The triterpenic acid derivatives demonstrated moderate 5-lipoxygenase inhibitory activities.

A new acylated flavonoid, 2″-*O*-*p*-hydroxybenzoylorientin (**6.02**) and a new lignan, altissinone (**4.01**) have also been isolated and characterized from the ethyl acetate extractives of *V. altissima*. In addition, known flavonoids, namely, vitexin (**6.01**) and luteolin 7-*O*-glucoside (**6.03**) were also isolated. The flavonoids (**6.01** and **6.03**) exhibited moderate antioxidant activity.

Based on the present investigations, the anti-inflammatory activity of ethyl acetate extractives of *V. altissima* may be attributed to the 5-lipoxygenase inhibitory activity of triterpenic acid derivatives and the anti-oxidant activity of the phenolic constituents (iridoids and flavonoids).

Phytochemical investigations on the methanolic extractives of *T. labialis* resulted in the isolation and characterization of bergenin (**7.01**), daidzin (**7.02**), vitexin (**6.01**) and 3-*O*-methyl-D-chiroinositol (**7.03**). Vitexin exhibited moderate 5-lipoxygenase inhibitory and anti-oxidant activities.

The isolation and characterization of known anti-inflammatory compounds, bergenin, vitexin and daidzin from *T. labialis* substantiate the traditional use of *T. labialis* in inflammatory conditions.

In conclusion, the present study and the results obtained provide the corroborative scientific evidence for the folklore use of ***Vitex altissima*** and ***Teramuns labialis*** in inflammatory conditions.

However, further pharmacological investigations in both in-vivo and in-vitro models of sub-acute and chronic inflammatory conditions are necessary to explore the anti-inflammatory potential of the plants studied.

## FUTURE SCOPE

Novel iridoids isolated in the present study exhibited strong antioxidant activity in in-vitro studies. Because of the broad bioactivity profile recorded for iridoids, the new compounds have to be evaluated further by in-vivo experiments and screening for other activities also.

Further, the 5-lipoxygenase activity exhibited by the triterpenoids has to be studied further. We have found corosolic acid and its isomers to display potential 5-LOX inhibition. It would be beneficial to explore structure-activity studies on this group of compounds.

In the present study, only the leaves and that too only ethyl acetate extractives have been investigated. Other extracts have also shown mild to moderate anti-inflammatory activity. Detailed bioactivity guided isolation of chemical constituents from the other extracts provide complete details on the potential of leaves of *V. altissima.*

# APPENDICIES

# Published/Presented Papers

1 New Acylated Iridoid Glucosides from *Vitex altissima*, **Chenchugari Sridhar**, Gottumukkala V. Subbaraju, Yenamandra Venkateswarlu and Raju. T. Venugopal, ***Journal of Natural Products* 2004**, *67*, 2012-2016.

2 Flavonoids, Triterpenoids and a lignan from *Vitex altissima*, **Chenchugari Sridhar**, Karumanchi V. Rao, Gottumukkala V. Subbaraju, **Phytochemistry 2005** (in press).

3 Antiinflammatory Constituents of *Teramnus labialis*, **Chenchugari Sridhar**, Alluri V. Krishna Raju and Gottumukkala V. Subbararaju, ***Indian Journal of Pharmaceutical Sciences* 2005** (in press).

4 New Acylated Iridoid Glucosides from *Vitex Altissima*, Gottumukkala V. Subbaraju, **Chenchugari Sridhar** and Yenamandra Venkateswarlu, **2004 International Congress On Natural Products Research**, July 31-August 4, 2004, Arizona, USA.

## Biography of the Student

**Mr. C. Sridhar** did his undergraduate program in pharmacy in 1988 from Gulbarga University, Karnataka, India. Later he received his postgraduate degree in pharmacy from Birla Institute of Technology and Science, Pilani in 1990. His research interests include isolation, characterization and biological activity studies on secondary metabolites of medicinal plants and synthesis of Heterocyclic compounds. He began his academic career as a Lecturer in Pharmaceutical Chemistry at P. E. S. College of Pharmacy, Bangalore in 1990. He became Assistant Professor in 1995. Later he moved to Sri Padmavathi School of Pharmacy, Tirupati, as Associate Professor in Pharmaceutical Chemistry. As an academician he is actively involved in teaching, research and curriculum development programs of the institute. He is a member of several pharmacy professional bodies.

## Biography of the Research Supervisor

**Dr. Gottumukkala V. Subbaraju** was born in Pedakapavaram, Andhra Pradesh, India, in 1956. He received his postgraduate degree in organic chemistry with distinction in 1978 from Andhra University, Vishakapatnam, India and he was awarded Ph.D. degree in organic chemistry, in 1982 by the same University. He began his academic career in 1982 as a Lecturer in Chemistry at Andhra University P. G. Centre, Nuzvid, Andhar Pradesh, India. Later in 1985 he moved to Sri Venkateswara University, Tirupati, India. He became Assistant Professor in 1985 and then promoted to Associate Professor in chemistry in 1995. His research interests include chemistry of natural products, nutraceuticals and bioactivity evaluations. He was a visiting Scientist during 1990 to 1992 at Stevens Institute of Technology, New Jersey, USA and Postdoctoral researcher at University of Hawaii, USA during 1995-97. He has 10 patents to his credit and another 15 are in pipeline. He has published over a 100 research articles and presented his research work at several national and international conferences. He has guided 7 doctoral and 6 M. Phil. dissertations in the area of natural products. Currently, he is Vice-President, Research and Development at Laila Impex, Vijayawada, India and is leading a team of scientists involved in the process development, standardization and biological screening of herbal products including isolation and characterization of chemical constituents responsible for the activity.