CHEMICAL EXAMINATION OF SOME MEDICINAL PLANTS

Thesis

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DOCTOR OF PHILOSOPHY

(CHEMISTRY)

By

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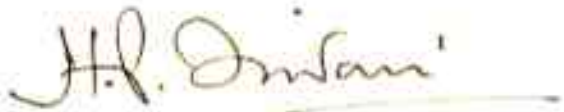
SUPERVISOR'S CERTIFICATE

Certified that the research work described in this thesis entitled "CHEMICAL EXAMINATION OF SOME MEDICINAL PLANTS" is original and was carried out by Shri R.S. Gupta, under my supervision during the period August 1969 to August 1974.



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A B S T R A C T

The thesis "Chemical examination of some medicinal plants" deals with the isolation and study of the constituents of the roots and stems of Cistanche tubulosa, roots of Aristolochia bracteata and roots and stems of Convolvulus pluricaulis.

The subject matter of the thesis is divided in four chapters. Chapter one contains a brief review of the literature on medicinal plants in general and on Cistanche, Aristolochia and Convolvulus genus in particular, followed by a detailed review of the literature of the chemical work done on Cistanche tubulosa, Aristolochia bracteata and Convolvulus pluricaulis.

Chapter two describes Cistanche tubulosa from whose roots and stems a mixture of hydrocarbons, Hentriacontan-16-one, β-Sitosterol, β-Sitosterol-D(+)-glucoside, another unidentified glucoside have been isolated and studied.

Chapter three deals with Aristolochia bracteata from whose roots Hentriacontane, Hentriacontan-16-one, Hentriacontol, β-Sitosterol, Aristolochic acid, free sugars and an alkaloid have been isolated and studied.

Chapter fourth describes Convolvulus pluricaulis from which Hentriacontane, Nonacosan-10-one, Hentriacontol, β-Sitosterol and Betaine have been isolated and studied.

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Ram Sewak Gupta

(RAM SEWAK GUPTA)

C O N T E N T S

CHAPTER I

I N T R O D U C T I O N

The study of natural products has been very fascinating since very beginning. In this process some very useful compounds have been discovered. Most natural products have usually been isolated from plants, since the practical difficulties in extracting them from animals are much greater in most cases.

In India quite a large number of plants are used for curing diseases. In all times people everywhere have attempted to utilize flora and fauna of their respective regions for the relief of human sufferings.

The importance of medicinally useful organic compounds derived from plants is enormous. In this connection such useful compounds as morphine, atropine, quinine, papaverine, reserpine, cocaine, caffeine, colchicine, penicillin and many others have been discovered. These compounds present a broad and representative range of pharmacological activities. In addition to this many crude drugs such as Digitalis purpurea leaves are still preferred by many physicians in their practice.

The study of plants particularly as a source of food is one of the oldest of human activities.

A knowledge of chemical constituents of plants is essential not only for the discovery of new therapeutic

agents, but also it is valuable in disclosing new sources of such economic materials as tannins, industrial oils, gums, and precursors for the synthesis of complex chemical substances.

The knowledge of the chemical structure of the active principles from plants has been of great value in the field of drug research. It has helped in many cases in establishing the relationship between the chemical constitution of these principles and their physiological activity. This knowledge has been utilized by chemists in obtaining a series of modified semi-synthetic compounds such as homoatropine, N-allylnormorphine which would either enhance therapeutic action of the drug or make it more specific with less toxic side effects.

It is evident that the investigations of indigenous medicinal plants has provided many effective remedies. Of over twenty two hundred Indian plants which have been reported to be of medicinal importance, only a few have been systematically examined.

A systematic investigation of the more important of these plants by using modern techniques is likely to be fruitful.

Many poisonous plants like Digitalis and Strophanthus yield glycosides of medicinal importance. Cardiac glycosides occur in small amounts in the seeds, leaves, stems, roots or bark of plants, belonging to the families of Liliaceae, Moraceae and Ranunculaceae. The sea onion (Scilla maritina) a bulbous herb (containing squillglycosides) is used in medicine by ancient Romans.

Compounds isolated from plants are also used as starting materials for the preparation of several compounds of medicinal importance. For example Diosgenin isolated from Dioscorea is used as starting material for the preparation of all the steroidal hormones.

In India due to difference of climatic conditions, it is possible to grow different kinds of plants.[1-5] About 2200 of such plants are listed in the Ayurvedic and Unani-tibb systems. These plants are still the main source of medicinal relief of the majority of the people of this sub-continent. Some of the other countries represented by books or review of publicationson medicinal botany are Mexico,[6] Poland,[7] New Guinea,[8] Nigeria,[9] U.S.S.R.[10-11] China,[12-13] Burma,[14] Malaya,[15] Africa,[16] Greece,[17] Australia,[18] New Zealand,[19-21] Taiwan[22-23] as well as many others.[24-26]

The publications describe the use of preparations derived from animal, mineral and vegetable sources. Some

of the drugs mentioned in them are serving mankind even today. These include Opium, Castoroil, Acasia, Calamus, Saffron, Oliveoil, Peppermint, Herbane, Aconite, Cannabis and Garlic.

The knowledge about the chemical nature of these preparations was very little in early times. This was due to the fact that the methods of extractions and ellucidation of structures were not much developed.

It was in nineteenth century that pure physiologically active substances were isolated from plant material and studied. The secrets of the pain relieving drug, opium, were unravelled step by step.

During the year 1820 morphine - a name deduced from the Graceo-Roman diety of sleep was first isolated by Serturner from opium. In 1833 Atropine was discovered by Mein, Gieger and Hosse. In the year 1823 Pelletier and Dumas discovered quinine. The great demand of this led to the synthesis of a number of compounds. Skraup's synthesis of quinoline in 1880 was followed by Fischer's synthesis of Kairin. Knorr synthesised Antipyrine in 1883, followed by the synthesis of Aspirin in 1899 by Drosser and Duisberg.

A knowledge of the chemical constituents of plants

is also useful to those who are interested in the expanding area of chemotaxonomy (biochemical systematics), in biosynthesis etc.

The medicinal importance of the plants Cistanche tubulosa, Convolvulus pluricaulis and Aristolochia bracteata has been recognised from very early days and their uses in medicine are well known even today. However, their systematic studies have not been carried so far. The present work is an effort in this direction.

CISTANCHE TUBULOSA

Description of the Plant

The plant belongs to the family of Orobanchaceae, a genus of about 28 species of herbs is widely distributed in Asia, Africa and the Madetarrian region.

Cistanche tubulosa is found extensively in Rajasthan, Punjab, Sind and Baluchistan.

It is an unbranched herb. Roots are parasite on Calotropis[3]. The general colour of the plant is yellow with an occasional tinge of purple colour. Stem is 1 meter to 1.5 meter high. It is 5 cm in diameter, furrowed, flesy, densely covered with triangular acute scales with numerous flowers.

Medicinal Uses

According to Ayurveda the plant is useful in heart diseases, anaemia, tumors and abdominal pains. The plant is also effective in curing of sores and is given in curd to stop diarrhoea[4]. The roots of the plant are known for its diuretic properties. It is also reported to be useful in cancer.[27]

The review of the Literature on Cistanche Tubulosa

In fact no work of much chemical importance has been reported on Cistanche tubulosa.

Alarjarov K.L.[28] and others have determined the alkaloids in different parts of different species. It was found that the maximum number of alkaloids are present in Cistanche ambiqua.

The only work which has been reported on Cistanche tubulosa is by T.S. Ranjan[29] of University of Delhi. His work is related to the morphogenesis of embryo which is of little chemical importance.

Convolvulus Pluricaulis (Chois)

Description of the plant

The plant belongs to the family Convolvulaceae.

This family consists of 55 genera and 1,650 species.[30] The genus convolvulus (200 Spp) contain purgative resins, some of which have long been used medicinally. Most plants of this family are annual or perennial herbs, often with twining stems, a few shrubs and trees. The Convolvulus pluricaulis is a perennial herb. The flowers are white occasionally pinkish. It is known by different names in different parts of India. In Punjab it is known as "Parprang dodak." In Sanskrit the plant is called "Shankhpuspi". The plant is distributed throughout the plains.

Medicinal Uses

The plant is used in curing number of diseases. In rural areas roots are used as purgative. The crude extracts of the plant are used for curing pain in the bladder, snake bite and as a domestic medicine for indigestion & swellings. It is also used as a brain tonic in the treatment of some cases of insanity.

Review of Literature

N.K. Basu[31] and P.C. Dandiya analysed the drug and isolated an alkaloid which they named 'Shankhpuspine' melting point 162-164°C, $C_{17}H_{23}NO_3$(decomposition).

They also prepared several derivatives.

Derivatives of the alkaloid Sankhpuspine

Derivative	Melting Point
Platinichloride	180°C
Picrate	76°C
Aurichloride	208°C

However they did not propose any structure for the base.

Presence of an essential oil has also been reported by the above workers, who determined some of its physical constants.

Basu and Bhan[32] reported the isolation of two water soluble bases. The first base has the molecular formula $C_3H_{14}NO_6$, melting point 84°C. They also prepared its hydrochloride melting point 214°C(decomp) and picrate, melting point 176°C. However, they could not isolate the other base in crystalline form. They prepared some derivatives of the second base and determined the melting points.

Hydrochloride, melting point 272°C (decomposition)
Oxalate, melting point 154°C.

S. Rakhit and Basu[33] have reported the isolation of

sterol I and sterol II. The sterol I has molecular formula $C_{28}H_{50}O_2$ and melting point 124-25°C. The sterol II has the molecular formula $C_{40}H_{60}O_5$, melting point 64-65°C.

Derivatives of Sterol I and Sterol II

Sterol I (A) Acetyl derivative, melting point, 117°C
(B) Digitonide, melting point 224-25°C

Sterol II (A) Acetyl derivative, melting point 88-89°C
(B) Digitonide, melting point 84-85°C

The same authors[34] in their second paper on this plant have reported the isolation of two bases A and B. The base A possesses the molecular formula $C_5H_{11}NO_2$, melting point 287-88°C and the base B, melting point 256°C (decomposition), molecular formula, $C_5H_9NO_2$. However, these authors could not identify either of Sterols I and II or bases A and B. They have also not assigned any structures to all above mentioned compounds. These authors have reported some pharmacological activity of this plant on rats and frogs.

Deshpande and Srivastava[35] have carried out further study of the plant C.pluricaulis. They have reported the isolation of 6-methoxy-7-hydroxy coumarin in pure

form, melting point 204°C. The molecular formula is $C_{10}H_8O_4$. These authors have also reported the presence of D-glucose, maltose, β-sitosterol and ceryl alcohol.

No flavonoids have been reported from this plant, but from its sister speceis C. arvensis[36] some flavonoids have been studied.

The isolation of clavine and lysergic acid alkaloids[37] have been reported from seeds of various convolvulus speceis.

Aristolochia bracteata (Retz)

Description of the plant

The plant belongs to the family Aristolochiaceae. This family comprises of about six genera and two hundred species of which about one hundred eighty belong to the genus Aristolochia. Most of the members are herbs or climbing plants with woody stems. Oil secreting cells are found throughout the family, sometimes forming transparent dots on the leaves. The oils of Asarum europeum (European snake wood) Asarum canadense (Canadian snake root) and Aristolochia serpentaria (Virginian snake root) have been examined.

The plant Aristolochia bracteata is very widely distributed throughout India and also grows in Ceylon, tropical and subtropical Asia, Africa and America.

The plant is known by different names in the different parts of India. According to Chopra and Nayer[4] some of the names are given below :

Sanskrit	Dhumrapatra
Hindi	Kiramar
Tamil and Malayanum	Aduthirapalai

Medicinal Uses

Aristolochia bracteata is of great pharmaceutical importance. It is extremely bitter plant and is used as a purgative. Juice of the leaves is given to cure neglected ulcers, and the roots are used for expelling round worms. The seeds are tonic and expectorant. They are also effective in muscular pain and are supposed to purify blood and hasten delivery.

In Rajasthan the roots are considered useful in expelling different kinds of worms from the stomach.

Ayurvedic medical practitioners consider this plant to possess laxative and diuretic properties. The powdered roots either alone or in a combined form act as a pain relieving for stomach. In over doses the drug produces violent gastro intestinal irritations. The powdered roots mixed with honey are given for curing leucoderma.

Literature Review

Knowledge of the constituents of Aristolochia species has long been in an unsatisfactory state. In recent years (1954-64) J.B. Stenlake and his colleagues have examined many species.

C.R. Mehta, Y.P. Dutta and N.G.Rana[38] have isolated and identified β-sitosterol, oleic acid, myristic acid, palmitic acid, stearic acid, and lauric acid from the seeds of Aristolochia bracteata by extraction with petroleum ether (60-80°C). From acetone extract they have separated aristolochic acid from the same seeds.

K.V. Jagannatha Rao, L.Rama Chandra Rao and Y. Suryanarayana Murthy[39] have separated aristolochic acid (melting point, 275-77°C with decomposition) and aristored from the petroleum ether extract of the plant.

M.S. Sastry[40] have isolated ceryl alcohol, β-sitosterol, aristolochic acid from the leaves and fruits, potassium chloride and potassium nitrate salts were isolated from the roots of the plant.

Some work has been reported from few other species of Aristolochia.

A.V. Subbaratnum and William B.Cooke[41] have reported the isolation of the following compounds from Aristolochia indica.

1. Allantoin, m.p. 236-37°C
2. A bitter yellow crystalline substance, m.p. 245-250°C
3. Aristolochic acid, m.p. 279-286°C (decomposition)

T.R. Govindachari[42-45] and his coworkers have reported the isolation of sesquiterpene hydrocarbons from Aristolochia indica namely ishwarane and aristolochin. The same authors have also reported the sesquiterpenoids, namely ishwarone and ishwarol from the same species of the plant.

Aristolochia rutunda has also been studied by Salvatore and Carbone[46] and have isolated aristolactum and aristolochic acid.

From Aristolochia sipho various flavonoids[47] and related compounds have been reported.

Object of the present work

A careful review of the literature available on Cistanche tubulosa clearly indicates that no systematic work has been done on chemical constituents of the plant.

It has been proposed by Spenser[48] that aristolochic acid is biosynthesised from norlaudanosoline alkaloid. The main aim was to try and isolate the above alkaloid which so far has not been isolated from any of the species.

Further two sterols and some bases have been isolated from Convolvulus pluricaulis, but the chemical constitution of these compounds have not been established.

Therefore the object of the present work is to isolate various groups of compounds from above mentioned plants and study them by chemical and physical (UV, IR, NMR and mass spectra) methods.

On the basis of these studies the compounds have been assigned structures and identified.

CHAPTER II

ISOLATION AND STUDY OF MIXTURE OF HYDROCARBONS, HENTRIACONTAN-16-ONE (PALMITONE), β-SITOSTEROL, β-SITOSTEROL-D(+)-GLUCOSIDE, ANOTHER UNIDENTIFIED GLUCOSIDE FROM THE ROOTS AND STEMS OF CISTANCHE TUBULOSA

The roots and stems of Cistanche tubulosa were extracted with petroleum ether and the neutral fraction was chromatographed on neutral alumina. Elution of column with petroleum ether, petroleum ether and benzene (10:1), benzene and chloroform (2:1) gave compounds, CA melting point 60-66°C, CB, melting point 81°C and CC, melting point 137°C successively.

Study of the compound CA

This compound moves along with the solvent front on neutral alumina tlc plate when developed with petroleum ether. The melting point range (60-66°C), indicates that it is the mixture of more than one compound.

The mixture is highly soluble in chloroform, ether, benzene, and hexane and sparingly soluble in acetone, methanol and ethanol.

Chemical tests

This mixture indicates the absence of nitrogen, sulphur, halogens and also other functional groups.

The mixture gave a negative tetranitromethane test showing this to be a mixture of saturated hydrocarbons.

Spectral studies

UV spectrum did not give any absorption from 200-700 nm.

The IR spectrum of this mixture in KBr shows bands at 725 cm^{-1} and 714 cm^{-1} indicating n-alkane chain[49-50]. Strong absorption bands at 2920 cm^{-1} and 2850 cm^{-1} indicate a large number of $-CH_2$ groups[51].

On the basis of above chemical and spectral studies the CA seems to be the mixture of long chain hydrocarbons.

Study of the compound CB

This compound is a white solid, melting point, 81°C. The elemental analysis of the compound corresponds to the molecular formula, $C_{31}H_{62}O$.

Chemical studies

With hydroxylamine it forms fine needles which were crystallised from ethyl alcohol. Melting point was found to be 59°C.

However the compound does not react with sodium bisulphite to give a crystalline derivative. It gave negative tests for steroids and triterpenes.

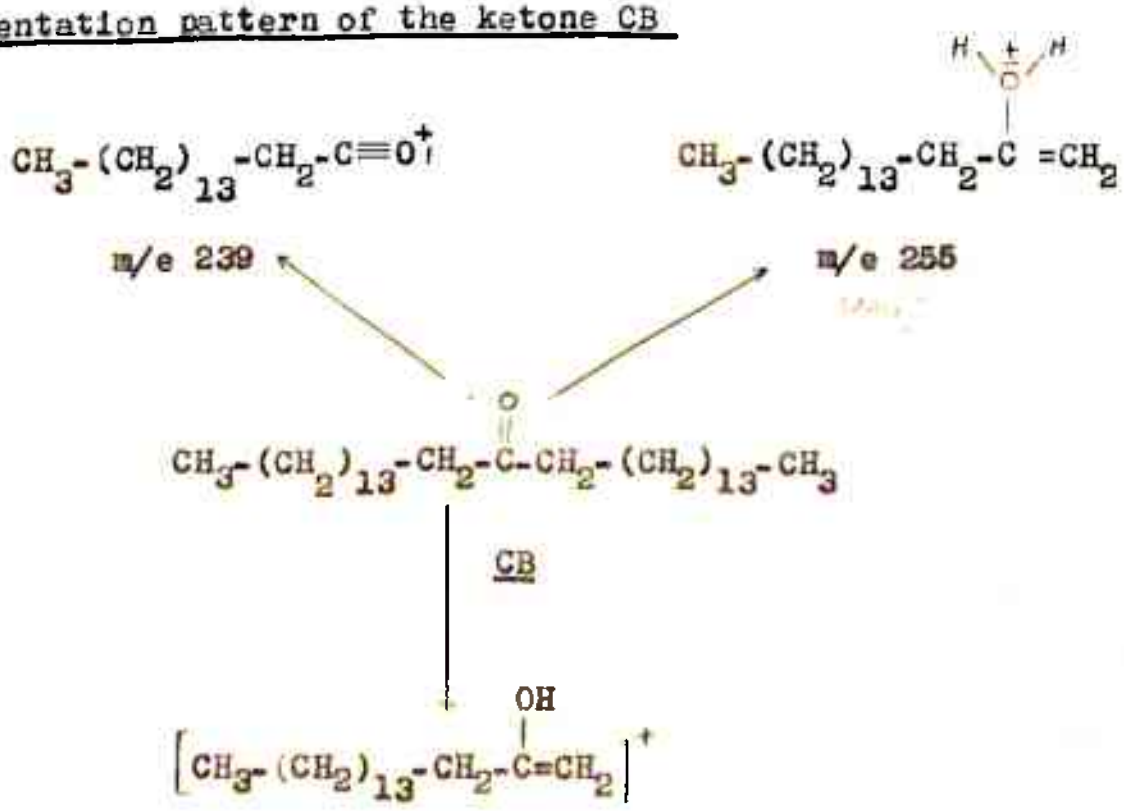
The IR spectrum of the compound in KBr shows a strong peak at 1733 cm^{-1}, indicating that the compound has a carbonyl function. The other important peaks are at 722 cm^{-1} and 715 cm^{-1} indicating a n-alkane chain.

The molecular formula of the ketone is supported by molecular ion peak m/e 450 in mass spectrum. Mass spectrometry of ketones give a characteristic fragmentation pattern which helps in the determination of the position of the ketone group.

In the mass spectrum of the ketone CB, no significant $(M-15)^+$ peak was observed. This indicates that the ketone has a straight chain.[69]

The most important peaks obtained in the spectrum of CB are at m/e 239, m/e 255 and m/e 254. These mass fragments can be explained on the basis of the fragmentation pattern given by V. Wollrab of nonacosan-7-one derived from the secondary alcohols occuring in wax of flower Rosa damascena.[70]

Fragmentation pattern of the ketone CB



The proposed structure for the ketone is further supported by nuclear magnetic resonance spectrum of the compound. This spectrum shows a signal at 9.12 τ assigned to six methyl protons, and a strong signal at 8.72 τ due to methylene protons. A multiplet centred at 7.60 τ is assigned to four methylenic protons next to carbonyl function.

On the basis of above chemical and spectral studies, the compound CB was identified as Hentriacontan-16-one.

Identification of Hentriacontan-16-one was further confirmed by mixed melting point, IR spectrum and by Co-tlc, with an authentic sample.

Study of the compound CC

The elemental analysis of the compound corresponds to the molecular formula, $C_{29}H_{50}O$.

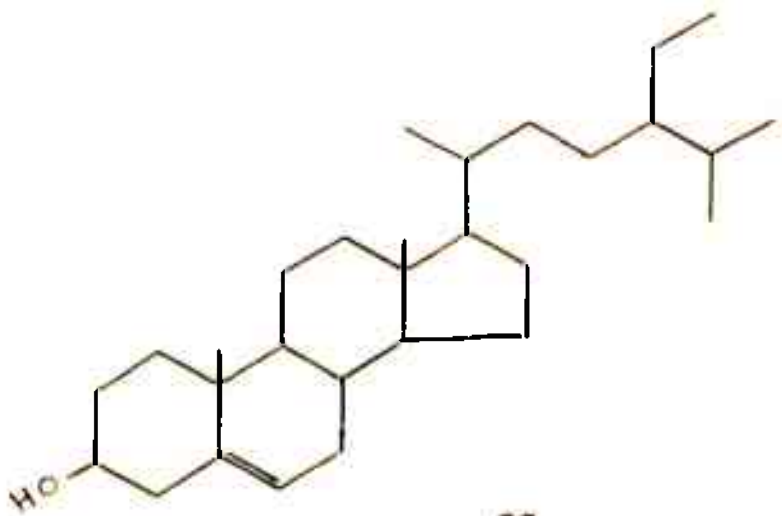
The compound gave a green colour in Liebermann-Burchard test[52], red colouration in Salkowski test[53], and a yellow colouration with tetranitromethane[54]. When alcoholic solution of digitonin was added to the alcoholic solution of the compound, a precipitate was formed (digitonide, m.p. 226°C)[55]. On the basis of the colour reactions and molecular formula, the compound under study

appears to be a steroid. IR spectrum of the compound showed peak at 3625 cm^{-1} indicating the presence of hydroxyl group.

The compound on acetylation with acetic anhydride and pyridine formed an acetate, m.p. 128°C, $[\alpha]_D$ -38° [56] (chloroform). IR spectrum of the acetate showed peaks at 1742 cm^{-1} (acetate -CO) and 1236 cm^{-1} (O-$COCH_3$) [57].

The elemental analysis of the acetate corresponds to the formula, $C_{31}H_{52}O_2$, indicating the presence of one hydroxyl function in the compound CC. This is also in agreement with the observations that most of the steroids are oxygenated at C_3, usually as alcohols.

On the basis of above observations, the compound under study appears to be β-sitosterol.



CC

The identity of the compound was further established by mixed m.p., Co-tlc and superimposable IR spectrum of the sterol and its acetate with authentic samples of β-sitosterol and its acetate.

Treatment of the alcoholic extract of Cistanche tubulosa

Alcoholic extract of the defatted roots of Cistanche tubulosa was further extracted with solvent ether, chloroform, ethylacetate and n-butanol in a liquid-liquid extractor.

The neutral fraction of the ethyl acetate extract, on usuel processing and chromatography on silica gel column gave a white crystalline compound CD, melting point 289°-290°C (decomposition), $[\alpha]_D$ -40° (pyridine).

Homogeneity of the compound was established by thin layer chromatography on silica gel plate.

Elemental analysis of the compound CD corresponds to the molecular formula, $C_{35}H_{60}O_6$. The compound gave yellow colouration with tetranitromethane. In Liebermann-Burchard test the compound first gave a pink colour which changed to bluish green. A red colour was observed in Salkowski test.

The alcoholic solution of the compound did not give

precipitate with digitonin. The compound gave positive Molisch's test, but did not give characteristic colour with aniline phthalate reagent (Hough).

On the basis of above observations, the compound under study seems to be a phytosterolin in which the reducing group of the sugar is involved in the glycosidic linkage.

IR spectrum of the compound showed strong absorption at 3400 cm^{-1}, indicating the presence of hydroxyl function in the compound. Absorption bands between 1150 cm^{-1} and 1010 cm^{-1} indicate various C-O-C groups.[58]

The phytosterolin on acetylation with mixture of acetic anhydride and pyridine gave an acetate, m.p. 164°C, $[\alpha]_D$ -35° (chloroform). IR spectrum of the acetate showed peaks at 1762 cm^{-1} (acetate -CO) and 1224 cm^{-1} ($O-COCH_3$).

Determination of acetyl percentage by Robinson and Bailey method as described by J.L. Norula,[59] showed the presence of four hydroxyl groups.

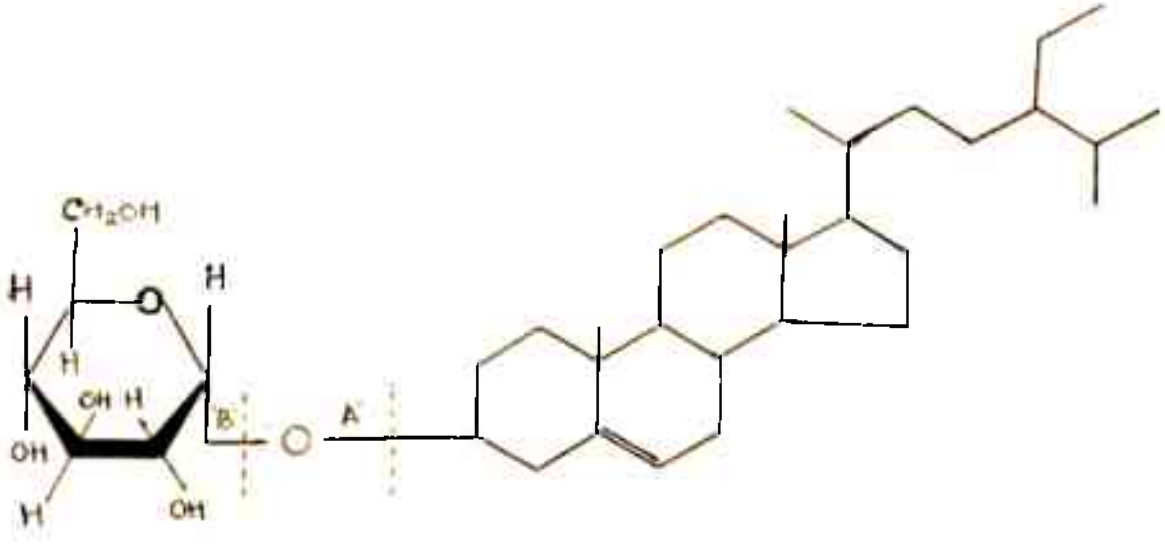
NMR spectrum of the acetate disclosed a triplet at 7.98 τ assigned to the protons of $CH_3-\overset{O}{\overset{\|}{C}}-$ (one signal superimposed with the other signal). Signal at 5.82 τ assigned to the protons of CH_2OAC and deformed envelop between 4.95 τ to 4.70 τ assigned to the protons of

CHOAC, signal at 9.26 τ assigned to methyl group attached to C-13. A triplet at 9.10 τ is due to the methyl group attached to C-28, a doublet at 9.19 τ is due to methyl group attached to C-25 and a singlet at 9.0 τ is due to methyl group attached at C-10. Multiplet envelop between 8.92 τ to 7.64 τ is due to methylenes of the steroid ring and $-CH_2$ of the side chain. A deformed triplet at 4.68 τ is due to the ethylenic protons.

The phytosterolin CD on acid hydrolysis yielded β-sitosterol and D(+)-glucose. The sugar was identified by Co-paper chromatography.

β-sitosterol was identified by mixed m.p., Co-tlc and by superimposable IR spectrum with an authentic sample of β-sitosterol.

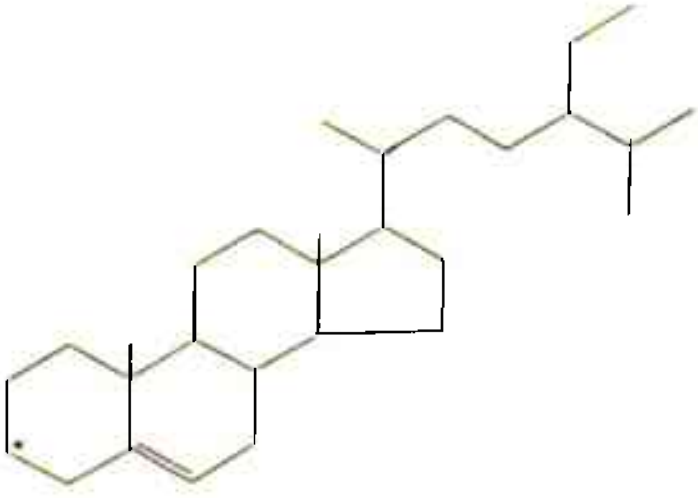
On the basis of the above observations the compound under study appears to be β-sitosterol-D(+)-glucoside and can be represented as.



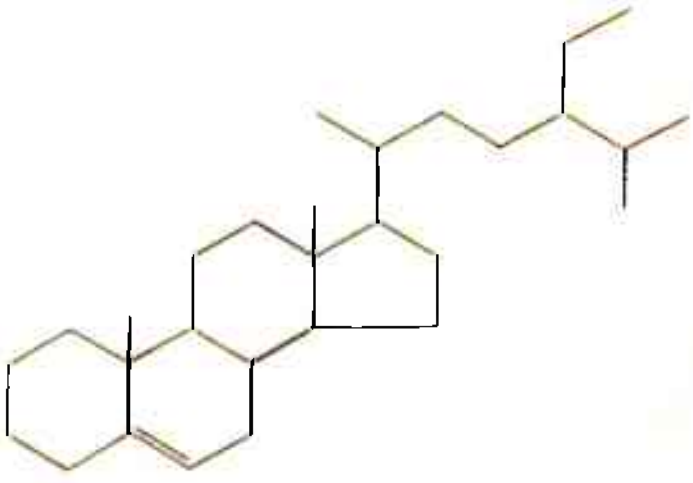
CD

Mass spectral studies of glycoside

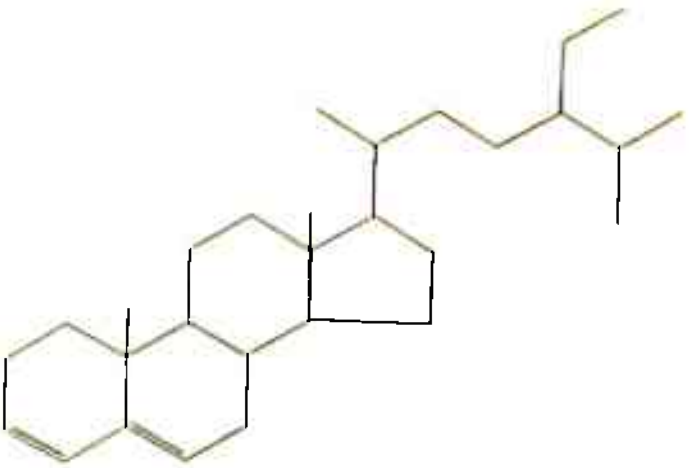
From its mass spectrum the compound appears to be unstable even using direct inlet probe. Towards the end of the spectrum, intense peaks at m/e 396, m/e 397 and peaks at m/e 398 and m/e 414 were obtained. Peak at m/e 397 can arise by the cleavage of the carbon oxygen bond at 'A' (CD). Peak at m/e 398 is being formed by cleavage of carbon oxygen bond at 'A' and a hydrogen rearrangement. Peak at m/e 396 corresponds to the fragment obtained after elimination of the sugar. Peak at m/e 414 which corresponds to the mass of β-sitosterol may arise by cleavage of carbon-oxygen bond at 'B' and a hydrogen rearrangement.



m/e 397



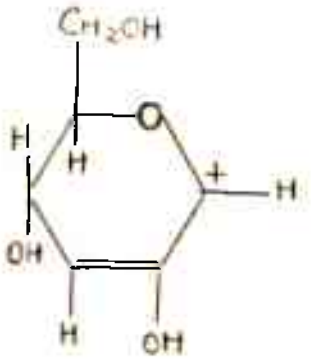
m/e 398



m/e 396

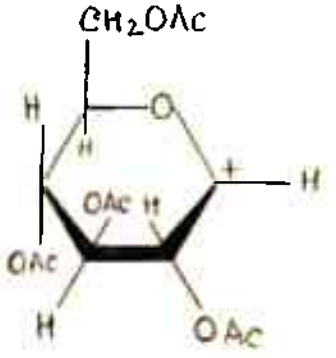
The other peaks in the mass spectrum are at m/e 381, 303,288,275,273,255,231,229,213.

The occurrence of peaks at m/e 145, 127, 109, 73, 61, and 60 in the lower region of the mass spectrum indicates that glycone part of the glycoside may be glucose.[60]



m/e 145

Mass spectrum of the acetate of CD showed peaks at m/e 396, 397, 398, 414, 381, 255, 329, 303, 275, 288, 273, 229, 231 and 213. Beside these peaks, spectrum also showed a strong peak at m/e 331 which may arise by the fragment obtained from cleavage of the carbon-oxygen bond at 'B'.



m/e 331

Other signals are at m/e 289, 271, 229, 211, 157, 115, 109 and 57. These fragments are characteristic of glucose tetra-acetate.

Thus by the study of mass spectrum of glucoside and its tetra acetate its identity as β-sitosterol-D(+)-glucoside was completely established.

Treatment of the n-butanol extract of Cistanche tubulosa

The n-butanol extract was separated into acidic, basic, and neutral fractions. The acidic and basic fractions gave negligible residues on evaporation. The

neutral fraction on chramatography on neutral alumina column gave a white crystalline compound CE, melting point 162°C.

Study of the compound CE

The homogeneity of the compound was established by thin layer chromatography on silica gel G plates, using chloroform: methanol (9:1) as an irrigating solvent and concentrated sulphuric acid for revealing the spots. R_f determined in chloroform and methanol (9:1) was found to be 0.32.

The alcoholic solution of the compound CE did not give precipitate with digitonin. The compound gave positive Molisch's test, but did not give characteristic colour with aniline phthalate reagent (Hough). In Liebermann-Burchard test the compound first gave a pink colour which changed to bluish green.

IR spectrum of the compound showed strong absorption at 3450 cm^{-1}, indicating the presence of hydroxyl function in the compound. Absorption bands between 1150 cm^{-1} and 1010 cm^{-1} indicate various C-O-C groups.[58]

On the basis of the above observations the compound under study seems to be a steroidal glycoside in which the reducing group of the sugar is involved in the glycosidic linkage.

The compound was hydrolysed in presence of hydrochloric acid. This process yielded aglycone and D(+)-glucose, which was identified by Co-paper chromatography.

R_f value of the aglycone was determined in pure chloroform (0.38).

Furtner study of the glycoside and its aglycone could not be carried out because the glycoside was obtained in very small amount.

E X P E R I M E N T A L

Melting points were taken on THOMAS-HOOVER Unimelt melting point apparatus and are uncorrected. Optical rotations were measured on HILGER STANDARD POLARIMETER model MK-III. Ultra-violet absorption spectra were recorded on manual HITACHI PERKIN-ELMER 139 UV-VIS spectrophotometer and Infra-red spectra were taken on HITACHI-PERKIN-ELMER 237B. Nuclear magnetic resonance spectra were taken on VARIAN A-60D instrument at Central Drug Research Institute, Lucknow where Mass spectra and Elemental analysis were also carried out. The NMR spectra refer to deuterochloroform ($CDCl_3$), unless otherwise stated.

WOLEM neutral alumina, STAHL silica gel G were used for thin layer chromatography. REIDAL neutral alumina and BDH silica gel were used for column chromatography.

Extraction and isolation

Air-dried and powdered roots of Cistanche tubulosa (5 Kg), collected locally, were extracted in a Soxhlet apparatus with petroleum ether (40^{o}-60^{o}C) for 50 hours.

The solvent was distilled under reduced pressure using water suction pump and the yellow viscous residue (48 g) so obtained, was taken in solvent ether. The ether solution was washed with 10% sodium bicarbonate solution. On acidification of this alkaline extract, no worthwhile residue was obtained. The ether solution was then washed with 2N-sulphuric acid. Basification of this extract with dilute ammonia gave a negligible residue.

Ether soluble neutral material was thoroughly washed with distilled water and dried over anhydrous sodium sulphate. Evaporation of the ether gave a viscous solid (30.4 g). Chromatography of the viscous solid on tlc plates using different solvent systems and spraying the plate with concentrated sulphuric acid and heating for 10 minutes at 120°C showed three main spots. 5% aqueous phosphoric acid was also used as spraying reagent. One spot gave colour on heating at 100°C for 10 minutes, indicating the presence of a steroid. Crude solid also gave positive Liebermann-Burchard test for steroids.

Treatment of neutral ether soluble material

12.0 g of the solid was dissolved in minimum quantity of solvent ether and was adsorbed on 35 g of neutral alumina. It was then placed on the top of chromatography column (40"x3").

packed previously with 400 g of neutral alumina.

Development of the column was carried out utilising a discontinuous gradient technique, beginning with petroleum ether (40°-60°C) and finally with methanol. Elutes were collected in fractions of 50 ml each and evaporated to dryness under vacuum. Fractions from the column were divided into three portions on the basis of tlc pattern. Each portion was rechromatographed on neutral alumina column of Brockmann activity - 1.

Chromatography of portion I

Portion I (2.0 g) was chromatographed on alumina using petroleum ether (40°-60°C) for elution. 25 ml fractions were collected. First fraction was discarded. Fractions 2 to 6 were combined on the basis of tlc pattern. The petroleum ether was evaporated under reduced pressure. A white solid (1.0 gm), m.p. 60°C - 66°C was obtained.

Remaining fractions were mixed together, evaporated to dryness under reduced pressure and were combined with portion II.

Chromatography of portion II

Portion II (0.85 g) was dissolved in minimum quantity of hexane and placed on top of an alumina column (24"x$\frac{1}{2}$").

The column was washed with petroleum ether (50 ml) and the washings were discarded. It was then eluted with petroleum ether: Benzene (10:1). 25 ml fractions were collected. Fractions 4 to 10 were combined according to tlc pattern, evaporated to dryness and crystallised several times with hexane to give white shining crystals CB (0.25 g), m.p. 81°C. Elutes from benzene were combined with portion III.

Chromatography of portion III

Portion III (7.5 g) was chromatographed on neutral alumina (120 g) column (30"x1½"). Elution was carried out successively with benzene, mixture of benzene:chloroform and pure chloroform. Finally the column was washed with ethanol. 25 ml fractions were collected.

Benzene fractions (150 ml) were discarded. Elutes from benzene: chloroform (2:1) were combined on tlc pattern. The compound was crystallised with acetone to give a white crystalline compound CC (3.5 g), m.p. 137°C.

Thin layer chromatography of CA

Activated silica gel G plate spotted with hexane solution of the compound was developed in a tank saturated with petroleum ether (40°-60°C). The developed plate was air dried and sprayed with concentrated sulphuric acid. The chromatogram on heating at 120°C for 15 minutes developed a single black spot at solvent front.

Solubility of CA

This is highly soluble in chloroform, ether, benzene and hexane and is sparingly soluble in acetone, methanol and ethanol.

IR spectrum of CA in KBr

The observed peaks and their assignments are as follows :

Peaks cm^{-1}	Assignment
2920	C-H stretching
2850	C-H stretching
1460	C-H bending
1375	C-H bending
725	n-Alkane chain
714	n-Alkane chain

Thin layer chromatography of the compound CB

Activated silica gel G plate was spotted with chloroform solution of the compound and developed in a tank saturated with petroleum ether: benzene (4:1). The developed plate was air dried and sprayed with concentrated sulphuric acid. The chromatogram on heating at 120°C for 20 minutes developed a single black spot (R_f 0.75).

Elemental analysis of the compound CB

Found	Calculated for $C_{31}H_{62}O$
C = 82.44 %	C = 82.66 %
H = 14.12 %	H = 13.77 %

Solubility of the compound CB

The compound is soluble in chloroform, ether, and sparingly soluble in ethanol and methanol. It is insoluble in water.

IR spectrum of the compound CB

The observed peaks and their assignments are as follows :

Peaks cm^{-1}	Assignments
2915	C-H stretching
2855	C-H stretching
1733	C=O stretching
1460	C-H bending of $-CH_3$ and $-CH_2$
1408	$-CH_2-CO-$
1375	C-H bending of $-CH_3$
1182	C-O stretching
1170	C-O stretching
722 715	n-alkane chain

Preparation of oxime of compound CB

A mixture of 50 mg of the compound and 50 mg of hydroxylamine hydrochloride was taken in 50 ml round bottom flask. To this 5 ml of alcohol and few drops of pyridine were added. The contents of the flask were refluxed on the water bath for 60 minutes. Alcohol was distilled off and 5 ml water was added. The residue was cooled in ice bath and filtered. The crude oxime was crystallised from alcohol, melting point was found to be 59°C.

Thin layer chromatography of the compound CC

Activated plates of silica Gel G were spotted with a chloroform solution of the compound and were developed in a tank saturated with

(a) chloroform: Benzene (1:1)

and

(b) pure chloroform

The developed plates were air dried and sprayed with concentrated sulphuric acid. The chromatograms were heated at 120°C for 15 minutes. A single black spot appeared in each case

R_f (a) 0.25

(b) 0.43

Elemental analysis of the compound CC

Found	Calculated for $C_{29}H_{50}O$
C = 83.88%	C = 84.05%
H = 12.2%	H = 12.07%

Reactions of the compound CC

(i) Liebermann-Burchard reaction - The compound was taken in few drops of acetic acid and 2 ml of acetic anhydride. When a drop of concentrated sulphuric acid was added, a green colour developed.

(ii) Salkowski reaction - The compound was dissolved in chloroform and a few drops of concentrated sulphuric acid were added. A deep red colour was observed.

(iii) Ruzicka's reaction - When a few drops of chloroform solution of tetranitromethane were added to a chloroform solution of the compound, a yellow colour was observed.

(iv) When the compound in ethanol was treated with alcoholic solution of digitonin a precipitate was formed.

IR spectrum of the compound CC in CCl_4

The observed peaks and their assignments are as follows:

Peaks cm^{-1}	Assignments
3625	O-H stretching
2950	C-H stretching
2870	C-H stretching
1465	C-H bending
1385) 1362)	gem dimethyl
1048	C-O stretching
1020	C-O stretching

Acetylation of the compound CC

The compound (50 mg) was acetylated with a mixture of acetic anhydride (5 ml) and pyridine (1 ml) by usual method. The acetylated product was crystallised from ethanol to give white flakes (48 mg), m.p. 128°C, $[\alpha]_D$ -38° (chloroform).

Elemental analysis of the acetyl derivative of the compound CC

Found	Calculated for $C_{31}H_{52}O_2$
C = 81.40%	C = 81.57%
H = 11.54%	H = 11.40%

IR spectrum of the acetyl derivative of the compound CC in CCl_4

The observed peaks and their assignments are as follows:

Peaks cm^{-1}	Assignments
2950	C-H stretching
2870	C-H stretching
1742	Acetate CO
1465	C-H bending
1380) 1360)	Gem-dimethyl
1236	O-CO-CH_3

Digitonoid derivative of the compound CC

A saturated solution of sterol in absolute alcohol was treated with an equal volume of a saturated solution of digitonin in absolute alcohol. The mixture was refluxed on water bath for two hours and cooled, when the digitonide separates out. It was filtered and crystallised with absolute alcohol, m.p. 227^{o}-228^{o}C.

Benzoylation of the compound CC

The compound (50 mg) was treated with benzoyl-chloride (10 ml) and two drops of pyridine. The mixture was heated on water bath and allowed to stand overnight

at room temperature. The mixture was then poured in ice cold water. The solid was filtered off, washed with 2% aquous KOH and then thoroughly with water. It was crystallised from a mixture of methanol: Acetone (1:1), m.p. 144°C.

Treatment of alcoholic extract of Cistanche tubulosa

The defatted roots of Cistanche tubulosa (5 Kg) were extracted with ethanol in a soxhlet extractor. Ethanol was distilled under reduced pressure. 100 ml distilled water was added and the mixture was subjected to liquid - liquid extraction successively with solvent ether, chloroform, ethyl acetate and n-butanol.

The ethyl acetate solution was washed with little water, dried over anhydrous (Na_2SO_4) and extracted with 10% sodium bicarbonate solution. The ethyl acetate solution was then washed with 2N sulphuric acid (4x30 ml). The ethyl acetate solution left after this was washed with water, dried over Na_2SO_4 and evaporated under reduced pressure to give a brown solid (2.5 gms).

Chromatography of the brown solid

The brown solid 2.5 gm was chromatographed on neutral alumina (60.0 g) column. Elution was carried out successively with chloroform, mixture of chloroform and methanol.

Finally the column was washed with methanol and 25 ml fractions were collected.

Chloroform fractions (200 cc) were discarded. Elutes from chloroform and methanol (9:1) were combined on tlc pattern. The compound was crystallised with absolute alcohol to give a white crystalline compound CD (1.25 g), melting point 289°C-290°C (decomposition), $[\alpha]_D$-40° (pyridine).

Thin layer chromatography of the compound CD

Activated plate of silica gel G was spotted with ethanol solution of the compound and developed in a tank saturated with chloroform: methanol (90:10). The air dried plate was sprayed with concentrated sulphuric acid. A single pink spot developed. It was then heated at 120°C for 15 minutes, the pink spot turned black and no other spot appeared.

Elemental analysis of the compound CD

Found	Calculated for $C_{35}H_{60}O_6$
C = 73.22%	C = 72.91%
H = 10.55%	H = 10.41%

Reactions of the compound CD

(i) Liebermann-Burchard reaction - The compound was taken in a few drops of acetic acid and 2 ml of acetic anhydride. On adding a few drops of concentrated sulphuric acid a pink colour developed which immediately changed to bluish green.

(ii) Salkowski reaction - When a few drops of concentrated sulphuric acid were added to the solution of the compound in chloroform, a red colour developed.

(iii) Ruzicka's reaction - When a few drops of the chloroform solution of tetranitromethane were added to a chloroform solution of the compound, a yellow colour was observed.

IR spectrum of the compound CD in KBr

Peaks cm^{-1}	Assignments
3400	O-H stretching
2955	C-H stretching
2925	C-H stretching
2850	C-H stretching
1650	C=C
1455	C-H bending
1380 / 1365	gem-dimethyl
1150 - 1015	various C-O-C
800 - 825	-

Acetylation of the compound CD

The compound (0.12 g) was taken in acetic anhydride (8 ml) and pyridine (2 ml). The solution was stirred mechanically with slight warming for 8 hours. The reaction mixture was then left over night and was poured over crushed ice. The white solid, which separated out, was filtered and washed thoroughly with water. The acetyl derivative thus obtained was recrystallised with absolute alcohol and dried at 100°C in vacuum, m.p. 164°C, $[\alpha]_D$ -35° ($CHCl_3$)

Thin layer chromatography of the acetate

Activated silica gel G plate was spotted with chloroform solution of the compound and was developed in a tank saturated with chloroform. The developed plate was air dried, sprayed with concentrated sulphuric acid, and heated at 120°C for 15 minutes. Single black coloured spot appeared (R_f 0.65).

Elemental analysis of the acetate:

Found	Calculated for $C_{43}H_{68}O_{10}$
C = 69.55%	C = 69.35%
H = 9.10%	H = 9.27%

Determination of acetyl percentage

The acetyle percentage in the acetyl derivative of the compound was determined as follows :

Procedure:

A 10 mg of the sample was weighed into a small porcelain boat and introduced into 25 ml round bottom pyrex flask.

10 ml of N/25 aquous NaOH were added to the flask and a micro reflux condenser attached. The solution was boiled gently for about 12 hours. After hydrolysis was complete, any alkali adhering to the condenser and rubber stopper was washed into the flask and the excess of alkali titrated with N/100 HCl using phenolphthalein as the indicator. A blank correction was run simultaneously and heated under identical conditions.

Found	Calculated for $C_{35}H_{56}O_6(COCH_3)_4$
Acetyl percentage = 23.98	Acetyl percentage = 23.10

IR spectrum of the acetyl derivative of CD in CCl_4

The observed peaks and their assignments are as follows :

Peaks cm^{-1}	Assignments
2930	C-H stretching
2854	C-H stretching
1762	Acetate - CO

Peaks cm^{-1}	Assignments
1464	C-H bending
1365) 1375)	gem-dimethyl
1248	O-$COCH_3$
1215	C-O-C stretching
1025	-

NMR spectrum of the compound CD

Signals τ	Assignment
9.26	Methyl protons
9.19	Methyl protons
9.10	Methyl protons
9.00	Methyl protons
8.92 to 7.64	Methylene protons
7.98	Protons of $CH_3-\overset{O}{\overset{\|}{C}}-$
5.82	Protons of CH_2OAC
4.95 to 4.70	Protons of CHOAC
4.68	Ethylenic protons

Mass spectrum of the compound CD

Main Peaks (Mass numbers)	Relative Intensity
414	15
398	40
397	80

Main Peaks (Mass numbers)	Relative Intensity
396	100
381	60
351	18
303	10
288	10
275	15
255	55
229	15
213	20
145	32
127	15
73	22

Hydrolysis of the compound CD and identification of sugars

The compound (0.1 g) and 5% aquous sulphuric acid (25 ml) were taken in a round bottom flask and refluxed for 4 hours. The reaction mixture was allowed to cool at room temperature and then extracted with solvent ether (5 x 25 ml). The ether extract was washed with water and dried over Na_2SO_4. The solvent was distilled off. The residue thus obtained was crystallised with absolute alcohol, m.p. 136°- $137^{\circ}C$, $[\alpha]_D$ - $35^{\circ}(CHCl_3)$

The compound was readily soluble in chloroform, benzene and solvent ether but sparingly soluble in hexane and ethanol. The aqueous solution left after extraction with solvent ether was neutralised with barium carbonate and filtered. The filterate was concentrated under reduced pressure. The concentrate gave positive Molisch's test and reduced Fehling's solution. It also gave colour with aniline-hydrogen - phthalate reagent, indicating the presence of a reducing sugar. On descending paper chromatography in ethyl acetate: acetic acid: water (10:3:3) a single spot appeared when the chromatostrip was sprayed with p - anisidine hydrochloride and heated at 110°C for 10 minutes (R_f 0.17). This indicated the presence of only one sugar which from its R_f value appeared to be glucose. This was confirmed by Co-paper chromatography with an authentic sample of D(+)-glucose.

Thin layer chromatography of aglycone

Activated silica gel G plate was spotted with chloroform solution of the compound and developed in a tank saturated with chloroform. The developed plate was air dried and sprayed with concentrated sulphuric acid and heated at 120°C for 15 minutes, single black spot developed (R_f 0.44).

Elemental analysis of aglycone

Found	Calculated for $C_{29}H_{50}O$
C = 84.50%	C = 84.05%
H = 11.7%	H = 12.07%

Reactions of the aglycone

(i) Ruzicka's reaction - When a few drops of tetranitromethane in chloroform were added to the chloroform solution of the compound a yellow colour was observed.

(ii) Liebermann-Burchard reaction - The compound was taken in acetic anhydride and a few drops of acetic acid. Addition of a few drops of concentrated sulphuric acid produced a green colour.

(iii) Salkowski reaction - When a drop of concentrated sulphuric acid was added to the solution of the compound in chloroform a yellow colour developed which changed to red after sometime.

Acetylation of aglycone

Compound was acetylated with a mixture of acetic anhydride and pyridine. Acetyl derivative was crystallised with chloroform methanol mixture, m.p. 127°C, $[\alpha]_D$ -37°($CHCl_3$).

Elemental analysis of the acetyl derivative

Found	Calculated for $C_{31}H_{52}O_2$
C = 81.11%	C = 81.57%
H = 11.71%	H = 11.40%

IR spectrum of the aglycone

The observed peaks and their assignments are as follows :

Peaks cm^{-1}	Assignment
3623	O-H stretching
2950	C-H stretching
2868	C-H stretching
1660	C=C
1464	C-H bending
1380 }	gem-dimethyl
1365 }	
1050	C-O stretching

Treatment of n-butanol extract

The n-butanol extract was washed with water and dried over anhydrous Na_2SO_4. The solution was then separated into acidic, basic and neutral fractions as follows.

The n-butanol solution was treated with 10% sodium bicarbonate solution (5 x 25 ml). The n-butanol solution

was then extracted with 2N sulphuric acid (4 x 30 ml). The neutral n-butanol extract so left, was washed with water, dried over Na_2SO_4 and evaporated under reduced pressure to give a "reddish solid" (50 mg).

The sodium bicarbonate extract was neutralyzed by hydrochloric acid and extracted with chloroform. The chloroform solution after evaporation under reduced pressure did not yield any residue.

The sulphuric acid extract was neutralyzed by sodium hydroxide, extracted with chloroform and dried over Na_2SO_4. On evaporating chloroform under reduced pressure no residue was left.

Chromatography of the"reddish solid"

The above solid was chromatographed on neutral alumina (20 g) column. The column was eluted with chloroform and with a mixture of chloroform and methanol (9:1) successively. Finally the column was washed with methanol (250 ml) and 25 ml fractions were collected.

Chloroform fractions showed no spot on tlc and hence were discarded. Elutes from chloroform and methanol (9:1) were combined according to tlc pattern. The solvent mixture was evaporated under reduced pressure to yield a white solid CE.

Thin layer chromatography of the compound CE

Activated plate of silica gel G was spotted with ethanol solution of the compound and developed in a tank saturated with chloroform: methanol (10:1.5). The air dried plate was sprayed with concentrated sulphuric acid. A single reddish spot developed. It was heated at 120°C for 15 minutes, the spot turned black and no other spot appeared.

Reactions of the compound CE

(i) Liebermann-Burchard reaction - The compound was taken in a few drops of acetic acid and 2 ml of acetic anhydride. On adding a few drops of concentrated sulphuric acid a pink colour developed which immediately changed to bluish green.

(ii) Ruzicka's reaction - When a few drops of the chloroform solution of tetranitromethane were added to a chloroform solution of the compound, a yellow cclour was observed.

IR spectrum of the compound CE in KBr

Peaks cm^{-1}	Assignment
3400	O-H stretching
2955	C-H stretching
2925	C-H stretching

Peaks cm^{-1}	Assignment
2850	C-H stretching
1650	C=C
1380) 1365)	gem-dimethyl
1150-1015	Various C-O-C

Hydrolysis of the compound CE and identification of sugar

The compound (10 mg) and 7% aquous sulphuric acid (5.0 ml) were taken in a round bottom flask, and refluxed for 5 hours. The reaction mixture was allowed to cool at room temperature and then extracted with chloroform (4 x 10 ml). The chloroform extract was washed with water and dried over anhydrous Na_2SO_4. The solvent was distilled off. A very small amount of residue was obtained.

The compound was readily soluble in chloroform, benzene and solvent ether, but sparingly soluble in hexane and ethanol. The aqueous solution left after extraction with chloroform was neutralised with barium carbonate and filtered. The filterate was concentrated under reduced pressure. The concentrate gave positive Molisch's test and reduced Fehling's solution. It also gave colour with aniline hydrogen-phthalate reagent, indicating the presence of a reducing sugar. On descending paper chromatography

in ethylacetate: acetic acid: water (10:3:3) a single spot appeared when the chromatostrip was sprayed with p-anisidine hydrochloride andheated at 110°C for 10 minutes (R_f 0.17). This indicated the presence of only one sugar which from its R_f value appeared to be glucose. This was confirmed by Co-paper chromatography with an authentic sample of D(+)-glucose.

Thin layer chromatography of the aglycone

Activated silica gel G plate was spotted with chloroform solution of the compound and developed in a tank saturated with chloroform. The developed plate was air dried, sprayed with concentrated sulphuric acid and heated at 120°C for 10 minutes, a single black spot developed (R_f 0.38).

Reactions of the aglycone

(i) Salkowski reaction - When a drop of concentrated sulphuric acid was added to the solution of the compound in chloroform a yellow colour developed which changed to red after sometime.

(ii) Ruzicka's reaction - When a few drops of tetra-nitromethane in chloroform were added to the chloroform solution of the compound, a yellow colour was observed.

CHAPTER III

ISOLATION AND STUDY OF HENTRIACONTANE, HENTRIACONTAN-16-ONE HENTRIACONTOL, β-SITOSTEROL, ARISTOLOCHIC ACID, FREE SUGARS AND ALKALOID FROM THE ROOTS OF ARISTOLOCHIA BRACTEATA (Retz)

The roots of A. bracteata were extracted with petroleum-ether and the neutral fraction was chromatographed on neutral alumina column. Elution of column with petroleum ether, petroleum ether and benzene (10:1), pure benzene, benzene and chloroform (1:1) gave compounds AA, m.p. 66°C, AB, m.p. 81°C, AC, m.p. 85°C and AD, m.p. 137°C successively. Examination of AA, AB, AC and AD on thin layer chromatography showed them to be homogeneous.

Study of the compound AA

The elemental analysis of the compound corresponds to the molecular formula $C_{31}H_{64}$. This formula is supported by the molecular ion peak m/e 436.

The IR spectrum of the compound in KBr shows bands at 725 cm^{-1} and 714 cm^{-1}, indicating a long n-alkane chain. Strong absorption bands at 2920 cm^{-1} and 2850 cm^{-1} indicate a large number of $-CH_2$ groups.[51] NMR spectrum of the compound gives only two signals, one at 9.12 τ assigned to $-CH_3$ protons and another strong signal at 8.74 τ assigned to $-CH_2$ protons. This also indicates it to be a straight chain aliphatic saturated hydrocarbon.[61]

Mass spectrum of the compound AA shows no $(M-15)^+$ peak, but an intense molecular ion peak and fragments of 14 mass units were observed. This further shows that the compound

under study is a straight chain aliphatic hydrocarbon.[62] The hydrocarbon has been identified as Hentriacontane (AA).

$$CH_3-CH_2-(CH_2)_{27}-CH_2-CH_3$$

AA

Identification of Hentriacontane was further confirmed by mixed melting point and superimposable IR spectrum with an authentic sample.

Study of the compound AB

The compound AB is a white solid, melting point 82°C. The elemental analysis of the compound corresponds to the molecular formula, $C_{31}H_{62}O$.

Chemical studies

It forms fine needle shaped crystals with hydroxylamine. The melting point of the oxime derivative was determined to be 59°C.

It gave negative tests for steroids and triterpenes. It also does not react with sodium bisulphite to form a crystalline derivative.

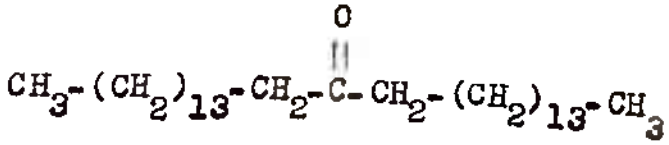
Spectral studies

IR spectrum of the compound in carbon tetrachloride showed a strong peak at 1729 cm^{-1} indicating that the

compound has a carbonyl function. The other important peaks are at 722 cm^{-1} and 715 cm^{-1} indicating a n-alkane chain. Thus the compound CB appears to be a saturated aliphatic ketone.

The molecular formula is supported by the molecular ion peak m/e 450 in mass spectrum. The other important peaks are at m/e 239 and m/e 255 (100 %).

On the basis of these studies the ketone was identified as Hentriacontan-16-one (AB)



AB

The above structure for the ketone has been further confirmed by its nuclear magnetic resonance spectrum. The NMR spectrum shows the signal at 9.12 τ assigned to six methyl protons, and a strong signal at 8.72 τ due to methylene protons. A multiplet centred at 7.60 τ is assigned to four methylenic protons next to carbonyl function.

Identification of Hentriacontan-16-one was further confirmed by mixed melting point, superimposable IR spectrum and by Co-tlc with an authentic sample.

Study of the compound AC

The compound is a white solid, m.p. 85°C. The elemental analysis of the compound corresponds to molecular formula, $C_{31}H_{64}O$. The compound gave a negative tetranitromethane test, showing this to be a saturated compound.[54]

IR spectrum of the compound in carbon tetrachloride showed a peak at 3630 cm^{-1} indicating the presence of a hydroxyl group in the compound. Presence of the hydroxyl group was further supported by a triplet at 6.32 τ in the NMR spectrum, which also had a singlet at 9.10 τ and a strong signal at 8.68 τ assigned to methyl and methylene protons respectively. The IR spectrum of the compound in KBr showed peaks at 715 cm^{-1} and 725 cm^{-1}, indicating the presence of normal alkane chain. The compound with acetic anhydride and pyridine formed an acetyl derivative, m.p. 75°C. The IR spectrum of the acetate in CCl_4 showed peaks at 1740 cm^{-1} (acetate CO) and 1235 cm^{-1} (-O-CO). Elemental analysis of the acetate corresponds to the molecular formula $C_{33}H_{66}O_2$. This compound was identified as Hentriacontol.

The identity of the compound was finally established by mixed m.p., Co-tlc and a superimposable IR spectrum of the compound with an authentic sample of Hentriacontol (AC).

$$CH_3-(CH_2)_{29}-CH_2OH$$

AC

Study of the compound AD

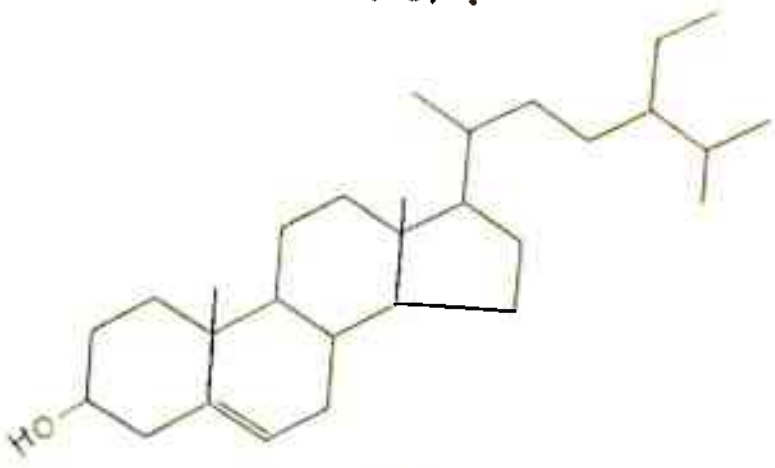
The elemental analysis of the compound corresponds to the molecular formula $C_{29}H_{50}O$.

The compound gave a green colour in Liebermann-Burchard test[52], red colouration in Salkowski test[53] and a yellow colouration with tetranitromethane[54]. When alcoholic solution of digitonin was added to the alcoholic solution of the compound, a precipitate was formed (digitonide, m.p. 226°C).

On the basis of the colour reactions and molecular formula, the compound under study appears to be a steroid. IR spectrum of the compound in KBr showed peak at 3625 cm^{-1}, indicating the presence of hydroxyl group.

The compound on acetylation with acetic anhydride and pyridine formed an acetate, m.p. 128°C, $[\alpha]_D$ - 38° (chloroform). IR spectrum of the acetate showed peaks at 1748 cm^{-1} (acetate-CO) and 1236 cm^{-1} (O-CO-CH_3).

The elemental analysis of the acetate corresponds to the formula $C_{31}H_{52}O_2$, indicating the presence of one hydroxyl function in the compound AD. On the basis of above observations the compound under study appears to be β-sitosterol.



AD

The identity of the compound was further established by mixed m.p., Co-tlc and superimposable IR spectra of the sterol and its acetate with authentic samples of β-sitosterol and its acetate.

Treatment of the alcoholic extract of Aristolochia - bracteata

Alcoholic extract of the defatted roots of A. bracteata was further extracted with solvent ether, chloroform and ethyl acetate successively in a liquid-liquid extractor. The solvent ether extract was then separated into acidic, basic and neutral fractions. The acidic fraction upon usual processing gave a yellow crystalline compound AE melting point, 278°- 280°C (decomposition).

Study of the compound AE

The homogeneity of the compound was established by thin layer-chromatography over cellulose plates using butanol, acetic acid and water in the ratio (25:0.1:25) as an irrigating solvent. Concentrated sulphuric acid and chlorosulphonic acid (Stahl) were used for revealing the spots.

The R_f value in the above system was determined to be 0.92

The elemental analysis of the compound AE corresponds to the molecular formula, $C_{17}H_{11}O_7N$.

The compound AE gave a positive Lassaigne test for nitrogen and evolved carbon dioxide on treatment with sodium bicarbonate solution.

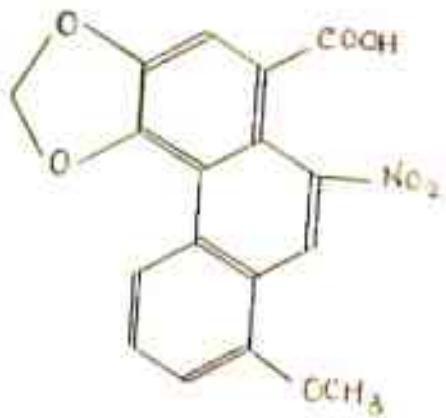
The infra-red and ultraviolet spectra of this product resembled with those reported[64,65] for Aristolochic acid.

The acid AE on methylation with an etherial solution of diazomethane gave a methyl ester $C_{18}H_{13}O_7N$, which was crystallized as yellow needles from hot methanol. Melting point, 282°C (reported m.p. 281°C).

The acid after reduction with zinc dust and 2N hydrochloric acid was refluxed in glacial acetic acid for 20 minutes. This procedure yielded a lactam as a yellow precipitate.

The yellow impure product was purified by sublimation under vacuum (0.1 mm/ at 70°C), melting point 317°C (reported,[64] m.p. 319°C).

On the basis of above observations AE was identified as Aristolochic acid which can be represented as



Aristolchic Acid

AE

The identify of the compound AE as aristolochic acid was further confirmed by mixed melting point, Co-tlc and superimposable IR spectrum with an authentic sample.

Study of the basic fraction

A crude base was obtained from solvent ether extract and it was named as AF

Study of the compound AF

The homogeneity of the base AF was established by thin layer chromatography on neutral alumina plates using chloroform and methanol (95:5) as a developing solvent

and Dragendorff's reagent[68] and concentrated sulphuric acid for visualizing the spots. Dragendorff's reagent gave a single orange colour spot.

All attempts to crystallize the alkaloid AF failed. The alkaloid developed a brown colour, when exposed to air.

Compound AF gave a positive Lassaigne test for nitrogen. UV absorption spectrum of the compound in methanol showed absorption maxima at 215 nm and 273 nm. IR absorption spectrum showed the characteristic band at 3640 cm^{-1} assigned to -OH group. Peaks at 3525 cm^{-1} and 3450 cm^{-1} were assigned to -NH stretching vibrations and at 1640 cm^{-1} due to the bending vibrations[61] of -NH. On the basis of above observations the compound under study appears to be an alkaloid.

As the alkaloid AF was obtained in a very small amount, it was not possible to continue further work on it.

Study of the aqueous extract of A. bracteata

In the aqueous extract, glucose fructose have been identified by Co-paper chromatography. Quantitative estimation of sugars was carried out by using phenol-sulphuric acid method[66]. The following results were obtained:

Glucose = 2.26%

Fructose = 1.84%

EXPERIMENTAL

The plant *A. bracteata* was collected locally and also from nearby villages. It was air dried for about two weeks. The roots were separated from rest of the plant and were made into the powdered form.

About 3.0 Kg roots were extracted in soxlet apparatus with petroleum ether (b.p. 60° - 80°C) for 70 hours. Solvent was distilled under reduced pressure, and the dark reddish residue so obtained was dissolved in hot methanol-acetone (1:1) mixture and kept in a refrigerator. A reddish waxy solid which separated out, was filtered. The filterate was concentrated and again kept in a refrigerator, when some more solid separated. This solid was mixed with previously obtained solid. The process was repeated thrice. The solid material so obtained was collected and the filterate which contains mainly colouring matter was discarded.

The solid waxy mass (14.2 g) was dissolved in solvent ether and separated into acidic, basic and neutral fractions. The neutral, ether soluble fraction was washed thoroughly with water and dried over anhydrons sodium sulphate. The solvent was evaporated to dryness to give a solid (12.40 g).

Thin layer chromatography of this crude solid showed four main spots in different solvent systems. The plates were sprayed with concentrated sulphuric acid and heated for 15 minutes at 120°C.

Treatment of neutral material

The solid (11.5 g) was dissolved in minimum quantity of chloroform and was adsorbed on 40g of neutral alumina. It was then placed on the top of a chromatographic column (36"x3"), packed previously with 400g of neutral alumina. Development of the column was carried out with solvents of increasing polarity, starting with petroleum ether and ending with chloroform. Elutes were collected in fractions of 50 ml each and evaporated to dryness under vacuum. Fractions from the column were divided into four portions on the basis of tlc pattern and were designated as A,B,C and D. Each portion was rechromatographed on neutral alumina of Brockmann activity.

Chromatography of Portion A

Portion A (2.25 g) was chromatographed on alumina using petroleum ether for elution. 25 ml fractions were collected. First fraction was discarded. Fractions 2 to 7 were combined on the basis of tlc pattern. The

residue on repeated crystallization with hexane gave white shining flakes AA (1.2 g), melting point 67°C.

Remaining fractions were mixed together, evaporated to dryness under reduced pressure and were combined with portion B.

Chromatography of portion B

Portion B (1.5 g) was adsorbed on 4.0 g of neutral alumina and placed on the top of a column (30"x1") packed previously with neutral alumina (80g). The column was washed with petroleum ether (60 ml) and the washings were discarded. It was then eluted with petroleum ether: benzene (10:1) mixture 25 ml fractions were collected. Fractions 4 to 10 were combined on the basis of tlc pattern. The combined elute was evaporated to dryness under vacuum. The white amorphous solid was crystallised twice with hexane to give a white crystalline compound AB (0.70 g), melting point 82°C. Later fractions of petroleum ether: benzene (10:1) and elutes from benzene were found to be mixtures of at least two compounds. The fractions showing these mixtures were mixed with portion C.

Chromatography of portion C

Portion C (0.95 g) was adsorbed on alumina (4g) and was placed on top of alumina (60g) column (25"x3/4").

The column was washed with 75 ml of petroleum ether:benzene (1:1) and the washings were discarded. The column was then eluted with benzene and finally with chloroform. 25 ml fractions were collected. The first four fractions of benzene elutes were discarded. The fractions 5 to 12 were combined on the basis of tlc pattern. Later elutes of benzene and elutes from chloroform were combined and rechromatographed. The compound was crystallised twice with chloroform at room temperature to give a white crystalline compound AC (0.35 g), melting point 85°C.

Chromatography of portion D

Portion D (4.0 g) was chromatographed on neutral alumina (80.0 g) column (25"x1"). Elution was carried out successively with benzene, mixture of benzene: chloroform and pure chloroform. Finally the column was washed with ethanol. 25 ml fractions were collected.

Benzene fractions (150 ml) were discarded. Elutes from benzene: chloroform (1:1) were combined on the basis of tlc pattern. The compound was crystallised with acetone to give a white crystalline compound AD (1.5 g), melting point 137°C.

Thin layer chromatography of the compound AA

Hexane solution of the compound was spotted on activated silica gel G plate. The plate was developed

in a tank saturated with petroleum ether (40^{o} - 60^{o}C). The developed plate was air dried and sprayed with concentrated sulphuric acid. The chromatogram on heating at 120^{o}C for 15 minutes developed a single black spot at solvent front.

Elemental analysis of the compound AA

Found	Calculated for $C_{31}H_{64}$
C = 84.52%	C = 84.32%
H = 14.82%	H = 14.67%

Solubility of the compound AA

The compound is highly soluble in chloroform, ether, benzene and hexane. It is sparingly soluble in acetone, methanol and ethanol.

IR spectrum of the compound AA in KBr

The observedpeaks and their assignments are as follows :

Peaks cm^{-1}	Assignment
2920	C-H stretching
2850	C-H stretching
1460	C-H bending
1375	C-H bending
725	n-alkane chain
714	n-alkane chain

NMR spectrum of the compound AA in CCl_4

Signal τ	Assignment
9.12	methyl protons
8.75	methylene protons

Thin layer chromatography of the compound AB

A little amount of the compound was dissolved in chloroform. Activated silica gel G plate was spotted with this solution and developed in a tank saturated with petroleum ether: benzene (4:1). The developed plate was air dried and sprayed with concentrated sulphuric acid. The chromatogram on heating at 120°C for 30 minutes developed a single black spot (R_f 0.75).

Elemental analysis of the compound AB

Found	Calculated for $C_{31}H_{62}O$
C = 82.36%	C = 82.66%
H = 14.2%	H = 13.77%

Solubility of the compound AB

It is soluble in ether, chloroform and sparingly soluble in ethanol and methanol. It is insoluble in water.

IR spectrum of the compound AB

The observed peaks and their assignments are as follows :

Peaks cm^{-1}	Assignments
2915	C-H stretching
2855	C-H stretching
1720	C=O stretching
1460	C-H bending of $-CH_3$ and $-CH_2$
1408	$-CH_2-CO-$
1375	C-H bending of $-CH_3$
1182	C-O stretching
1170	C-O stretching
722) 715)	n-alkane chain

Preparation of oxime of compound AB

A mixture of 40 mg of the compound and 40 mg of hydroxylamine hydrochloride was taken in 50 ml round bottom flask. To this 5 ml of alcohol and few drops of pyridine were added. The contents of the flask were refluxed on water bath for 60 minutes. Alcohol was distilled off and 5 ml water was added. The residue was cooled in ice bath and filtered. The crude oxime was crystallised from ethanol melting point was found to be $58^{o}C$.

Thin layer chromatography of the compound AC

Activated silica gel G plate was spotted with chloroform solution of the compound and developed in a tank of benzene: chloroform (1:1).

The developed plate was air dried and sprayed with concentrated sulphuric acid. The chromatogram on heating at 120°C for 15 minutes developed a single black spot (R_f 0.46).

Elemental analysis of the compound AC

Found	Calculated for $C_{31}H_{64}O$
C = 82.70%	C = 82.30%
H = 14.32%	H = 14.15%

Solubility of the compound AC

The compound is soluble in benzene, chloroform, ether and is sparingly soluble in acetone, ethanol and methanol.

IR spectrum of the compound AC in CCl_4

The observed peaks and their assignments are as follows:

Peaks cm^{-1}	Assignments
3630	O-H stretching
2925	C-H stretching
2852	C-H stretching
1462	C-H bending
1040	C-O stretching

NMR spectrum of the compound AC

Signals τ	Assignments
9.10 singlet	Methyl protons
8.68 singlet	methylene protons
6.32 triplet	proton of -OH group attached to a methylene group

Acetylation of the compound AC

The compound (50 mg) was taken in a mixture of acetic anhydride (5 ml) and pyridine (10 ml) and was stirred mechanically with slight warming for 8 hours. The mixture was kept overnight. It was then poured over crushed ice. A white solid separated out. This was extracted with solvent ether. The etherial solution was thoroughly washed with water, dried over Na_2SO_4 and evaporated to dryness. The residue was crystallised with ethanol, m.p. 75°C.

Thin layer chromatography of the acetyl derivative of AC

Activated silica gel G plate was spotted with chloroform solution of the compound and was developed in a tank of petroleum ether: benzene (1:1). The developed plate was air dried and sprayed with concentrated sulphuric acid. The chromatogram on heating at 120°C for 15 minutes gave a single black spot (R_f 0.65).

Elemental analysis of the acetyl derivative of AC

Found	Calculated for $C_{33}H_{66}O_2$
C = 80.32%	C = 80.16%
H = 13.16%	H = 13.36%

IR spectrum of acetyl derivative of AC in CCl_4

The observed peaks and their assignments are as follows :

Peaks cm^{-1}	Assignments
2925	C-H stretching
2852	C-H stretching
1740	Acetate-CO
1465	C-H bending
1362	C-H bending
1235	O-$COCH_3$

Thin layer chromatography of the compound AD

A small quantity of the compound was dissolved in chloroform. Activated silica gel G plates were spotted with this solution and were developed in a tank saturated with

(a) chloroform: benzene (1:1)

(b) pure chloroform

The developed plates were air dried and sprayed with concentrated sulphuric acid. The chromatograms

were heated at 120°C for 20 minutes. A single red spot turning black appeared in each case.

R_f (a) 0.23

(b) 0.45

Elemental analysis of the compound AD

Found	Calculated for $C_{29}H_{50}O$
C = 83.96%	C = 84.05%
H = 12.15%	H = 12.07%

Reactions of the compound AD

(i) Liebermann-Burchard reaction - The compound was taken in a few drops of acetic acid and 2 ml of acetic anhydride. When a drop of concentrated sulphuric acid was added a green colour developed.

(ii) Salkowski reaction - The compound was dissolved in chloroform and a few drops of concentrated sulphuric acid were added. A deep red colour was observed.

(iii) Ruzicka's reaction - When a few drops of chloroform solution of tetranitromethane were added to a chloroform solution of the compound, a yellow colour was observed.

(iv) When the compound in C_2H_5OH was treated with alcoholic solution of digitonin a precipitate was formed.

IR spectrum of the compound AD in CCl_4

The observed peaks and their assignments are as follows :

Peaks cm^{-1}	Assignment
3625	O-H stretching
2950	C-H stretching
2870	C-H stretching
1465	C-H bending
1385) 1362)	gem dimethyl
1048	C-O stretching
1020	C-O stretching

Acetylation of the compound AD

The compound (60 mg) was acetylated with a mixture of acetic anhydride (5 ml) and pyridine (1 ml) by usual method. The acetylated product was crystallised from ethanol to give white flakes (50 mg), m.p. 128°$C_{[\alpha]_D}$ - 38° (chloroform).

Elemental analysis of the acetyl derivative of the compound AD

Found	Calculated for $C_{31}H_{52}O_2$
C = 81.42%	C = 81.57%
H = 11.52%	H = 11.40%

Treatment of the alcoholic extract of A.bracteata

The defatted roots of A.bracteata (3 Kg) were extracted with ethanol in a soxhlet extractor. Ethanol was distilled under reduced pressure. 75 ml distilled water was added and the mixture was subjected to liquid-liquid extraction successively with solvent ether and ethylacetate.

Etherial solution was washed with little water, dried over Na_2SO_4 and extracted with 10% sodium bicarbonate solution. Sodium bicarbonate extract on acidification gave a brown coloured precipitate. The brown precipitate was extracted from the aqueous solution with chloroform (5 x 50 ml). The chloroform extract was washed with water(3 x 25 ml) dried over Na_2SO_4 and the chloroform was evaporated. A yellow coloured solid (0.82 g) was obtained.

Treatment of acidic fraction

The layer chromatography of the sample of the yellow coloured product on cellulose plate revealed two spots of which only one was prominent.

Purification of AE by preparative tlc

The compound AE was purified by preparative tlc[67] using cellulose plate which had been sprayed with a

buffer solution (pH9.2), consisting of sodium carbonate (0.05 M) and sodium bicarbonate (0.05M) and had then been dried at room temperature. The compound AE was applied in ethanol solution and plates were developed in butanol: acetic acid and water in the ratio (25:0.1:25) as an irrigating solvent. The compound AE was extracted from the cellulose with ethanol. The solvent was evaporated, the residue was suspended in chloroform, and the compound was regenerated by addition of dilute hydrochloric acid. The product was recrystallized from ethanol, m.p. 280(d) (reported m.p. 281° - 286°C).

Solubility of the compound AE

The compound is soluble in methanol, ethanol, sparingly soluble in acetone, chloroform and insoluble in water.

Reactions of the compound AE

The compound gave a positive test for nitrogen and for carboxylic group.

Esterification of the compound AE

The compound (20 mg) was dissolved in warm dioxane (2 ml). After cooling, diazomethane in ether was added

and the mixture was allowed to stand for about four hours. The solvent was evaporated and the residue was dissolved in chloroform (30 ml). The chloroform layer was washed with water (20 ml) dried over Na_2SO_4 and concentrated. The ester was crystallized from hot methanol as yellow needles (15 mg), m.p. 282°C.

The ester was sparingly soluble in most of the solvents.

Preparation of the lactam of the compound AE

50 mg of the compound was mixed with 200 mg of zinc dust (activated by brief treatment with 2N hydrochloric acid, washed with water and ether, dried at 70°C for two hours) in glacial acetic acid (10 ml) was refluxed gently for 25 minutes. The solution was filtered and diluted with water to give a yellow precipitate which was purified by sublimation under vacuum (0.1 mm / at 70°C), melting point 319°C.

Treatment of the basic fraction

The aqueous extract, obtained by treating alcoholic extract with 2N sulphuric acid, was neutralized by sodium hydroxide. The solution was extracted with chloroform in a separating funnel. The chloroform extract was dried over anhydrous Na_2SO_4 for overnight. On evaporating chloroform under reduced pressure, a brownish substance was left (10 mg).

Thin layer chromatography of AF

The plate was prepared with a slurry of alumina (wolem) in water. The air dried plate was activated by heating at 120°C for 15 minutes. The plate was spotted with chloroform solution of the basic fraction and developed in a tank saturated with chloroform: methanol (95:5). The developed plate was air dried and sprayed with Dragendorff's reagent. One orange coloured spot developed (R_f 0.95).

The compound gave a positive test for nitrogen.

Solubility of the compound AF

It is soluble in ethyl alcohol, chloroform, solvent ether but insoluble in water.

UV spectrum of the compound AF in methanol

The compound gave the following bands

λ_{max} 215 nm

λ_{max} 272 nm

IR spectrum of the compound AF in chloroform

The IR spectrum of the compound showed absorption peaks at the following wave numbers :

3640 cm^{-1}, 3525 cm^{-1}, 3450 cm^{-1}, 2940 cm^{-1}
2850 cm^{-1}, 1735 cm^{-1}, 1640 cm^{-1}, 1470 cm^{-1}
1375 cm^{-1}, 1265 cm^{-1}, 1185 cm^{-1}, 1040 cm^{-1},
970 cm^{-1} and 865 cm^{-1}.

Detection of sugars in A. bracteata

The alcoholic extract of A. bracteata was tested for sugars. It gave positive Molisch's test, reduced Fehling's solution and produced colour with aniline hydrogen phthalate reagent, indicating the presence of reducing sugars. Paper chromatography of the sugar mixture showed the presence of two sugars. These sugars were identified by Co-paper chromatography (descending) on Whatmannpaper No.1 in ethyl acetate: acetic acid: water (10:3:3). p-Anisidine hydrochloride was used as a spraying reagent. The sugars identified were glucose (R_f 0.17) and fructose (R_f 0.25).

Quantitative estimation of sugars

Air dried and powdered roots (100 g) of A. bracteata were extracted with 75% ethanol in a soxhlet apparatus for 50 hours and was distilled to concentrate the solution.

Animal charcoal was added to the solution and the solution was filtered through sintered funnel. It was then treated with neutral lead acetate. Excess of Pb^{++} were removed by addition of sodium hydrogen phosphate and the precipitate was filtered off. The solvent was then removed under reduced pressure. The residue was dissolved in water and transferred to 100 ml measuring flask. The volume was made up to the mark.

Paper chromatography (Quantitative)

Chromatography (descending) was done on Whatmann No. 1 chromatographic paper. Two sheets (8" x 18") were taken. One sheet was used as a blank. A base line was drawn. The paper was spotted along the base line at five places each with 10 sugar solutions. Chromatograms were developed in a chamber saturated with ethylacetate: acetic acid: water (10:3:3). The chromatogram was allowed to overflow for 8 hours, so that sugars are clearly separated. The paper chromatogram was dried at room temperature. One marginal strip was cut and sprayed with p-anisidine hydrochloride. On heating the chromatostrip at 110°C for 15 minutes the appearance of the spots marked the distance the sugars had travelled in the strip and the unsprayed section. After reassembling the chromatostrips, the best-line of demarcation was drawn between the two spots, and the sectionswere cut out. Corresponding blanks were cut from the blank paper. The strips containing glucose, fructose and their corresponding blanks were transferred to wide mouth test tubes, and 20 ml water was added to each test tube. The tubes were then allowed to stand for 30 minutes with occasional shaking and warming. The elutes were filtered through glass wool and the concentration of sugar was determined by phenol sulphuric acid method.

2 ml of sugar solution was taken with 1 ml of 5% phenol (in water) in a pyrex test tube, to this solution 5 ml of sulphuric acid (A.R.) were added rapidly. A pink colour developed in each case. The test tube was allowed to stand for 30 minutes and the absorbance was measured at 490 nm in a UV spectrophotometer. The same procedure was followed with blank.

A standard absorption curve between micrograms of sugar and corresponding absorbance was plotted for glucose and fructose. A straight line was obtained in each case. The amount of sugar was then determined by refering to the standard curve and thus the percentage of glucose and fructose was calculated in the roots of A. brecteata. The procedure was repeated thrice under identical conditions. The percentage of glucose and fructose obtained in each case was within ± 1%. The average of the percentage of sugars was calculated on the basis of the weight of dry roots.

Glucose = 1.42%

Fructose = 2.20%

CHAPTER - IV

ISOLATION AND STUDY OF HENTRIACONTANE, NONACOSAN-10-ONE, HENTRIACONTOL, β-SITOSTEROL AND BETAINE FROM CONVOLVULUS PLURICAULIS.

Freshly dried plants of Convolvulus pluricaulis (3.5 Kg) were powdered and extracted with petroleum ether. The neutral fraction of the extract was chromatographed on neutral alumina column. The column was eluted with petroleum ether, petroleum ether: benzene (95:5), pure benzene, benzene: chloroform (1:1). This procedure gave four compounds CA, m.p. 67°C, CB, m.p. 74°C, CC, m.p. 86°C and CD, m.p. 137°C.

Examination of these compounds on thin layer chromatography showed them to be homogeneous.

Study of the compound CA

The elemental analysis of the compound corresponds to the molecular formula $C_{31}H_{64}$. This formula is supported by molecular ion peak m/e 436 in mass spectrum. Mass spectrum does not show $(M-15)^+$ peak, but an intense molecular ion peak and fragements of 14 mass units were observed. This indicates that the compound is a straight chain aliphatic hydrocarbon.[62]

IR spectrum of the compound in KBr shows characteristic bands at 725 cm^{-1} and 714 cm^{-1} indicating a long n-alkane chain.[49,50] Strong absorption bands at 2920 cm^{-1} indicate the presence of large number of $-CH_2$ groups.[51]

NMR spectrum of the compound gives only two signals, one at 9.12 τ which is due to methyl protons. The

second strong signal at 8.75 τ is due to methylene protons. Therefore NMR spectrum furrther indicates that the compound is a straight chain saturated aliphatic hydrocarbon.

On the basis of above studies the compound CA has been identified as Hentriacontane.

$$CH_3-CH_2-(CH_2)_{27}-CH_2-CH_3$$

CA

Identification of Hentriacontane was further confirmed by mixed m.p. and superimposable IR spectrum with an authentic sample.

Study of the compound CB

The compound CB is a white solid, melting point 74°C. The elemental analysis of the compound corresponds to the molecular formula $C_{29}H_{58}O$.

Chemical studies

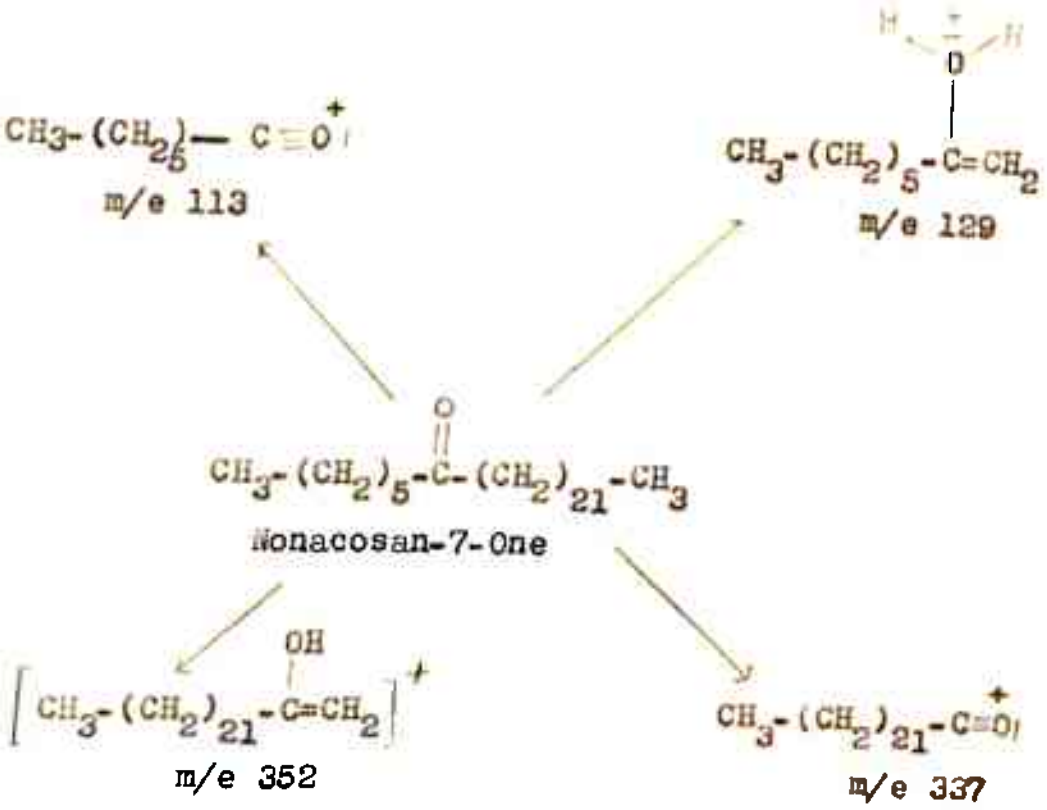
The compound did not give yellow colour with tetranitromethane, indicating its saturated character.[54] It gave negative tests for steroids and triterpenes. With hydroxylamine it formed an oxime derivative which crystallised from ethyl alcohol into fine needle shaped crystals, m.p. 49°C.

Spectral studies

The IR spectrum of the compound in KBr shows a strong peak at 1733 cm^{-1} indicating the presence of carbonyl function. The peaks at 722 cm^{-1} and 715 cm^{-1} indicate a n-alkane chain. Therefore the compound appears to be a saturated aliphatic long chain ketone. The NMR spectrum of the compound shows a signal at 9.12 τ assigned to six methyl protons, and a strong signal at 8.72 τ due to methylene protons. A multiplet centred at 7.60 τ is assigned to four methylenic protons next to carbonyl function.

Mass spectral study of the ketone CB

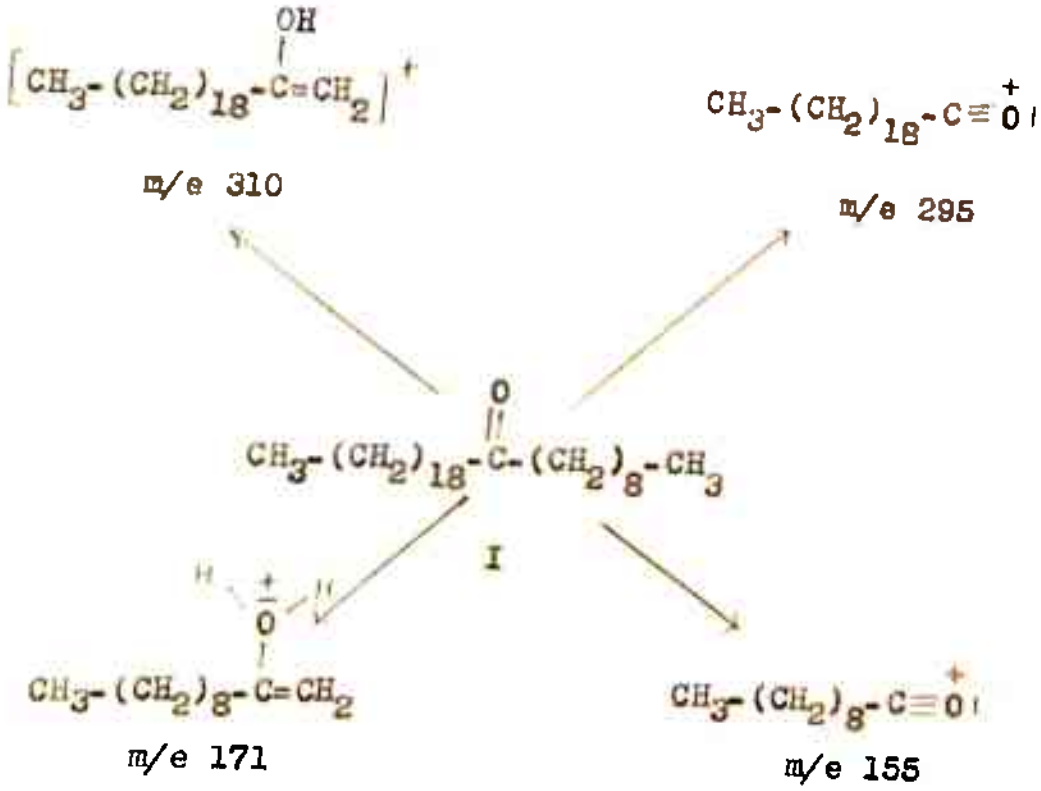
Mass spectrometry of ketones give a characteristic fragmentation pattern which helps in the determination of the position of the ketone group. Ketones are known to undergo frequent rearrangements in mass spectrum through a six membered ring intermediate, followed by fragmentation with the retention of the positive charge on the oxygen containing fragments. Fragmentation pattern of Nonacosan-7-one as observed by V. Wollrab[70] in mass spectrum is given below.



In the mass spectrum of the ketone CB no significant $(M-15)^+$ peak was observed. This indicates that ketone CB has a straight chain.[71] Mass spectrum shows $(M+1)^+$ peak characteristic of asymmetrical ketones.[72,73] The ratio of M^+ to $(M+1)^+$ is two.

The molecular formula of the compound CB is supported by molecular ion peak m/e 422. The other important peaks obtained in the spectrum of CB are m/e 310, m/e 295, m/e 171 and m/e 155. These mass fragments can be explained

on the basis of the fragmentation pattern given by V. Wollrab, by assigning structure (I) to the ketone CB



Study of the compound CC

The compound is a white solid, m.p. 84°C. The elemental analysis corresponds to the molecular formula, $C_{31}H_{64}O$. The compound gave negative tetranitromethane test showing this to be a saturated compound.

IR spectrum of the compound CC in KBr shows a peak at 3425 cm^{-1} indicating the presence of a hydroxyl group in the compound. The absorption band at 1050 cm^{-1} is assigned to C-O stretching. The presence of the hydroxyl group is further supported by the triplet at 6.34 τ in NMR spectrum, which also has a singlet at 9.22 τ and a strong signal at 8.68 τ assigned to methyl and methylene protons respectively. It is clear that the single oxygen present in the compound is in the form of a hydroxyl function.

With acetic anhydride and pyridine the compound formed an acetyl derivative, m.p. 76°C. IR spectrum of the acetate in CCl_4 shows peaks at 1742 cm^{-1} (acetate CO) and 1235 cm^{-1} ($O-COCH_3$). The elemental analysis of the acetate corresponds to the molecular formula $C_{33}H_{66}O_2$. The compound CC has been identified as Hentriacontol.

$$CH_3-(CH_2)_{29}-CH_2OH$$
CC

The identity of the compound was finally established by mixed m.p., Co-tlc and a superimposable IR spectrum of the compound and of the acetate with the authentic samples.

Here it may be mentioned that Deshpande and Srivastava[35] have carried out the study of this plant and have reported

the presence of ceryl alcohol. However in our studies no ceryl alcohol was obtained.

Study of the compound CD

This compound is a white crystalline solid, m.p. 137°C. The elemental analysis of the compound corresponds to the molecular formula $C_{29}H_{50}O$.

The compound gave a green colour in Liebermann-Burchard test[52], red colouration in Salkowski test[53] and a yellow colouration with tetranitromethane[54]. When alcoholic solution of digitonin was added to the alcoholic solution of the compound, a precipitate was formed (digitonide, m.p. 226°C).

IR spectrum of the compound in KBr shows peak at 3625 cm^{-1} indicating the presence of hydroxyl group.

On the basis of colour reactions and molecular formula, the compound appears to be a steroid.

The compound on acetylation with acetic anhydride and pyridine formed an acetate, m.p. 128°C, $[\alpha]_D$ -38° (chloroform). IR spectrum of the acetate shows peaks at 1748 cm^{-1} (acetate CO) and 1236 cm^{-1} (O-CO-CH_3).

On the basis of above observations the compound under study was identified as β-sitosterol.

The identity of the compound was further confirmed by mixed m.p., Co-tlc and superimposable IR spectra of the sterol and its acetate with authentic samples of β-sitosterol and its acetate.

Treatment of the alcoholic extract of Convolvulus pluricaulis

The alcoholic extract of the defatted roots and stems of Convolvulus pluricaulis was concentrated under reduced pressure to a semi-solid mass. This semi-solid mass was then extracted with ethanol. The ethanol extract was concentrated under reduced pressure and was kept in the refrigerator for 48 hours. A white crystalline compound was separated which was named as CE.

Study of the compound CE

The compound CE is a white solid, which on recrystallisation from alcohol-acetone mixture melts at 232-233°C (decom).

The homogeneity of the compound was established by think-layer chromatography on silica gel G plates using methanol: acetone: hydrochloric acid in the ratio (90:10:10) as an irrigating solvent and Dragendroff's reagent for revealing the spots.

The compound CE gave a positive Lassaigne test for nitrogen and a positive test for chloride ion with silver nitrate. It also gave positive test for alkaloids with Dragendorff's reagent and ammonium reineckate reagent.[76]

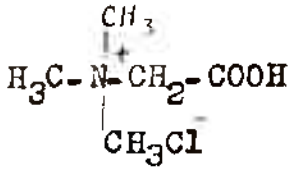
The elemental analysis of the compound corresponds to the molecular formula $C_5H_{12}O_2NCl$.

The R_f value determined in methanol: acetone: hydrochloric acid (90:10:10) was found to be 0.64.

IR spectrum of the compound in KBr shows strong absorption at 2810 cm^{-1} indicating N-methyl[74] groups in the compound. A peak at 2565 cm^{-1} is typical for substituted amines.[74] A very strong absorption at 1735 cm^{-1} is assigned to C=O stretching vibrations. Another strong peak at 3400 cm^{-1} indicates hydroxyl group in the compound. A peak at 2500 cm^{-1} is attributed to the overtone and combination band typical for salts.[74] Peak at 1400 cm^{-1} is assigned for N-Me deformation vibrations.[75]

On the basis of above observations the compound appears to be a hydrochloride of the basic compound. The free base was liberated by treating the compound with moist silver carbonate. The redish brown residue on repeated crystallization with absolute alcohol yielded hygroscopic crystals, m.p. 292-294°C (decomposition).

On the basis of above chemical and spectral studies, the compound under study appears to be Betaine hydrochloride.



The following derivatives were prepared

Derivatives	Melting point
hydroiodide	188-190°C
picrate	183-184°C
reineckate	152-154°C

E X P E R I M E N T A L

Air dried and powdered roots and stems of Convolvulus pluricaulis (3.5 Kg), collected locally, were extracted in a soxhlet apparatus with petroleum ether (60°-80°C) for about 50 hours. The solvent was distilled under reduced pressure and the yellow viscous residue (35 g) soobtained, was taken in solvent ether. The ether solution was washed with 10% $NaHCO_3$ solution. On acidification of this alkaline extract, no worthwhile residue was obtained. The ether solution was then washed with 2N-sulphuric acid. Basification of this extract with dilute ammonia gave a negligible residue.

Ether soluble neutral material was thoroughly washed with distilled water and dried over anhydrous sodium sulphate. Evaporation of the ether gave a viscous solid (20.5 g). Chromatography of the viscous solid on tlc plates using different solvent systems and spraying the plates with concentrated sulphuric acid and heating for 10 minutes at 120°C showed four main spots. 5% aqueous phosphoric acid was also used as spraying reagent.

Treatment of neutral ether soluble material

12.0 g of the solid was dissolved in minimum quantity of the solvent ether and was adsorbed on 30 g of neutral alumina. It was then placed on the top of chromatography

column (40"x3") packed previously with 350 g of neutral alumina.

Development of the column was carried out with solvents of increasing polarity, starting with petroleum ether and ending with chloroform. Elutes were collected in fractions of 50 ml each and evaporated to dryness under vacuum. Fractions from the column were divided into four portions on the basis of tlc pattern and were designated as A,B,C and D. Each portion was rechromatographed on neutral alumina of Brockmann activity - 1.

Chromatography of portion A

Portion A (1.5 g) was chromatographed on alumina using petroleum ether for elution. 25 ml fractions were collected. First fraction was discarded. Fractions 2 to 6 were combined on the basis of tlc pattern. The residue on repeated crystallization with hexane gave white shining flakes CA(1.0 g), m.p. 67°C.

Remaining fractions were mixed together, evaporated to dryness under reduced pressure and were combined with portion B.

Chromatography of portion B

Portion B (1.0 g) was adsorbed on 3.0 g of neutral alumina and placed on the top of a column (30"x1")packed

previously with neutral alumina (60 g). The column was washed with petroleum ether (50 ml) and washings were discarded. It was then eluted with petroleum ether: benzene (10:0.5) mixture and 25 ml fractions were collected. Fractions 3 to 7 were combined on the basis of tlc pattern. The combined elute was evaporated to dryness under reduced pressure. The amorphous solid was crystallised twice with acetone to give a white crystalline compound CB (0.50 g), m.p. 74°C. Later fractions of petroleum ether: benzene and elutes from benzene ~~and elutes from benzene~~ were mixed with portion C.

Chromatography of portion C

Portion C (2.04 g) was adsorbed on alumina (8 g) and was placed on top of prepacked alumina (120 g) column (40"x3"). The column was washed with 50 ml of petroleum ether: benzene (1:1) and the washings were discarded. The column was then eluted with benzene and finally with chloroform. 25 ml fractions were collected. The first three fractions of benzene elutes were discarded. The fractions 4 to 11 were combined on the basis of tlc pattern. The solvent mixture was evaporated. The compound was crystallised twice with chloroform at room temperature to give a white crystalline compound CC (1.25 g), melting point 84°C. The chloroform fractions were combined with portion D.

Chromatography of portion D

Portion D (2.5 g) was chromatographed on neutral alumina (60.0 g) column (25"x1"). Elution was carried out with benzene, mixture of benzene: chloroform (1:1) and pure chloroform. Finally the column was washed with ethanol. 25 ml fractions were collected. Benzene fractions (100 ml) were discarded. Elutes from benzene: chloroform (1:1) were combined on the basis of tlc pattern. The solvent mixture was evaporated. The compound was crystallised with acetone to give a white crystalline solid CD(1.0 g), m.p. 137°C.

Thin layer chromatography of the compound CA

Small amount of the compound was dissolved in hexane. This solution was spotted on activated silica gel G plate. The plate was developed in a tank saturated with petroleum ether (60°-80°C). The developed plate was air dried and sprayed with concentrated sulphuric acid. The chromatogram on heating at 120°C for 15 minutes developed a single black spot at solvent front.

Elemental analysis of the compound CA

Found	Calculated for $C_{31}H_{64}$
C = 84.60%	C = 84.32%
H = 14.84%	H = 14.67%

IR spectrum of the compound CA in KBr

The observed peaks and their assignments are as follows:

Peaks cm^{-1}	Assignments
2920	C-H stretching
2850	C-H stretching
1460	C-H bending
1375	C-H bending
714	n-alkane chain

NMR spectrum of the compound CA in CCl_4

Signal τ	Assignments
9.12	methyl protons
8.75	methylene protons

Thin layer chromatography of the compound CB

A small amount of the compound was dissolved in chloroform. Activated silica gel G plate was spotted with the solution and developed in a tank saturated with petroleum ether: benzene (5:1). The developed plate was air dried and sprayed with concentrated sulphuric acid. The chromatogram on heating at 120°C for 20 minutes developed a single black spot (R_f 0.72).

Elemental analysis of the compound CB

Found	Calculated for $C_{29}H_{58}O$
C = 81.92%	C = 82.12%
H = 13.6%	H = 14.0%

Solubility of the compound CB

It is soluble in ether, chloroform and sparingly soluble in ethanol. It is insoluble in water.

IR spectrum of the compound CB

The observed peaks and their assignments are as follows :

Peaks cm^{-1}	Assignments
2915	C-H stretching
2855	C-H stretching
1720	C=O stretching
1182	C-O stretching
1170	C-O stretching
722) 715)	n-alkane chain

Preparation of oxime of the compound CB

A mixture of 30 mg of the compound and 30 mg of hydroxylamine hydrochloride was taken in 50 ml round bottom flask. To this 5 ml of alcohol and few drops of pyridine were added. The contents of the flask were refluxed on water bath for an hour. Alcohol was

distilled off and 5 ml water was added. The residue was cooled in ice bath and filtered. The crude oxime was crystallised from ethanol, m.p. 49°C.

Thin layer chromatography of the compound CC

Activated silica gel G plate was spotted with chloroform solution of the compound and developed in a tank of benzene: chloroform (1:1).

The developed plate was air dried and sprayed with concentrated sulphuric acid. The chromatogram on heating at 120°C for 15 minutes developed a single black spot (R_f 0.48).

Elemental analysis of the compound CC

Found	Calculated for $C_{31}H_{64}O$
C = 82.60%	C = 82.30%
H = 14.02%	H = 14.15%

Solubility of the compound CC

The compound is soluble in benzene, chloroform, ether and is sparingly soluble in acetone, ethanol and methanol.

IR spectrum of the compound CC in CCl_4

The observed peaks and their assignments are as follows :

Peaks cm^{-1}	Assignments
3640	O-H stretching
2925	C-H stretching
2850	C-H stretching
1462	C-H bending
1040	C-O stretching

Acetylation of the compound CC

The compound (40 mg) was taken in a mixture of acetic anhydride (5 ml) and pyridine (1.0 ml) and was stirred mechanically with slight warming for 5 hours. The mixture was kept overnight. It was poured over crushed ice. A white solid separated out. This was extracted with solvent ether. The etherial solution was thoroughly washed with water, dried over Na_2SO_4 and evaporated to dryness. The residue was crystallised with ethanol, m.p. 76°C.

Thin layer chromatography of the acetyl derivative of CC

Activated silica gel G plate was spotted with chloroform solution of the compound and was developed in a tank of petroleum ether: benzene (1:1). The developed plate was air dried and sprayed with concentrated sul-

phuric acid. The chromatogram on heating at 120°C for 15 minutes gave a single black spot (R_f 0.64).

IR spectrum of acetyl derivative of CC in CCl_4

The observed peaks and their assignments are as follows :

Peaks cm^{-1}	Assignments
2925	C-H stretching
2850	C-H stretching
1745	acetate CO
1460	C-H bending
1235	O-$COCH_3$
1020	C-O stretching

Thin layer chromatography of the compound CD

A small quantity of the compound was dissolved in chloroform. Activated silica gel G plates were spotted with this solution and were developed in a tank saturated with

(a) chloroform: benzene (1:1)

(b) pure chloroform

The developed plates were air dried and sprayed with concentrated sulphuric acid. The chromatograms were heated at 120°C for 15 minutes. A single red spot turning black appeared in each case.

R_f (a) 0.22

(b) 0.45

Reactions of the compound CD

(i) Liebermann-Burchard reaction - The compound was taken in a few drops of acetic acid and 2 ml of acetic anhydride. When a drop of concentrated sulphuric acid was added a green colour developed.

(ii) When the compound in C_2H_5OH was treated with ethanol solution of digitonin a precipitate was formed.

IR spectrum of the compound CD in CCl_4

The observed peaks and their assignments are as follows

Peaks cm^{-1}	Assignments
3620	O-H stretching
2960	C-H stretching
2875	C-H stretching
1460	C-H bending
1385) 1360)	gem dimethyl
1045	C-O stretching
1020	C-O stretching

Acetylation of the compound CD

The compound (50 mg) was acetylated with a mixture of acetic anhydride (5 ml) and pyridine (1 ml) by usual method. The acetylated product was crystallised from ethyl alcohol to give white crystalline compound, m.p. 128°C, $[\alpha]_D$ -38° (chloroform).

Treatment of the alcoholic extract of Convolvulus pluricaulis

The powder left after petroleum ether extraction was further extracted with ethanol in a soxhlet apparatus for 60 hours. Ethanol was distilled under reduced pressure to a semi-solid mass. This was then triturated with 250 ml absolute alcohol. The supernatent liquid was decanted. It was then concentrated to 100 ml under reduced pressure and kept in a refrigerator for 48 hours. A white crystalline solid (CE) settled down. This was crystallised from absolute alcohol.

Thin layer chromatography of the compound CE

Activated silica gel G plate was spotted with alcoholic solution of the compound. The plate was developed in a tank saturated with methanol: acetone and hydrochloric acid in the ratio (90:10:10). The plate was air dried and sprayed with Dragendorff's reagent. A single pink coloured spot was observed (R_f 0.64).

Elemental analysis of the compound CE

Found	Calculated for $C_5H_{12}O_2NCl$
C = 38.7%	C = 39.08%
H = 7.45%	H = 7.81%
N = 8.68%	N = 9.12%
Cl=24.04%	Cl=23.12%

Reactions of the compound CE

(i) To the aqueous solution of the compound, silver nitrate solution was added. A white precipitate was formed.

(ii) A few drops of alcoholic solution of ammonium reineckate was added to the aqueous solution of the compound. A dark violet precipitate was formed.

IR spectrum of the compound in KBr

Peaks cm^{-1}	Assignments
3400	O-H stretching
2958	C-H stretching
2810	N-CH_3 groups
2623) 2563)	Overtone and combination band typical for salts
1735	C-O stretching
1400) 1420)	N-Me deformation vibrations
1125) 980)	C-N stretching

Picrate of the compound CE

The compound (20 mg) was dissolved in distilled water and added to an alcoholic solution of picric acid. A reddish yellow coloured compound separated out. The picrate was filtered and crystallised from ethyl alcohol m.p., 183°C-184°C.

Reineckate of the compound CE

The compound (20 mg) was dissolved in distilled water. To this solution, alcoholic solution of ammonium reineckate was added, a dark violet precipitate was formed which was crystallised from ethyl alcohol m.p., 152-154°C.

Hydroiodide of the compound CE

10.0 g of KI and 15.3 g of iodine were dissolved in 25 ml of distilled water. A few drops of this reagent were added to the alcoholic solution of the compound prepared by adding 20 mg in 10 ml of water. A precipitate was formed. It was filtered, washed with water and crystallised from alcohol m.p., 186-190°C (decomposition).

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