CHEMICAL EXAMINATION OF SOME INDIAN MEDICINAL PLANTS

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SUPERVISOR'S CERTIFICATE

Certified that the research work described in this thesis entitled, "Chemical Examination of some Indian Medicinal Plants" is original and was carried out by Shri A.N. Misra under my supervision during the period June 1968 to November 1971.

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A B S T R A C T

The thesis "Chemical examination of some Indian medicinal plants" deals with the isolation and study of the constituents of the roots of Boerhaavia diffusa (Linn) and roots and stems of Trianthema pentandra (syn Zaleya Govindia) NC. Nair.

The subject matter of the thesis is divided in three Chapters. Chapter one contains a brief review of the literature on medicinal plants in general and on Boerhaavia and Trianthema genus in particular followed by a more detailed review of the literature of the chemical work done on Boerhaavia diffusa and Trianthema pentandra. Chapter two deals with Boerhaavia diffusa from whose roots Hentriacontane, Hentriancontol, β-sitosterol, ursolic acid, β-sitosterol-D(+)-glucoside, Glucose, Fructose, Sucrose and alkaloids have been isolated and studied. Chapter three deals with Trianthema pentandra from whose roots and stems Hentriancontane, nonacos-1-ene-4-one, Hentriacontol, and mixture of β-sitosterol-D(+)-glucoside and stigmasterol-D(+)-glucoside have been isolated and studied.

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CHAPTER I

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

The use of plants for the alleviation of human sufferings is as ancient as civilization itself. In all ages people everywhere have attempted to utilize the flora and fauna of their respective regions for the relief of human ailments. The modern system of medicine owes much to past knowledge for remedies developed by different civilizations in different periods of history.

The importance of plant derived medicinals in modern medicine is often underestimated. Such useful compounds as digitoxin, rutin, papain, morphine, codeine papaverine, atropine, scopolamine, quinine, quinidine, reserpine, cocaine, ephedrine, colchicine and caffeine, to mention a few, present a broad and representative range of pharmacological activities. In addition, crude drugs as Digitalis purpurea leaves and Rauwolfia serpentina roots are still preferred by many physicians in their practice; whereas extract from Podophyllum peltatum (podophyllin), Rhamnus purshiana (anthraquinones), Cassia species (anthraquinones), and Plantago species (mucilage) are widely utilized for their medicinal activity. In fact, a survey has pointed that 47% of some 300 million new prescriptions written by physicians in 1961 contained as one or more active ingredients, a drug of natural

origin. Further, between 1950 and 1960 prescriptions containing drugs of natural origin increased by 7.7%[1]. Uptil only about a hundred years ago, all medicinal preparations were derived from natural sources.

One could spend a life time surveying the published books and periodicals describing the native flora of various regions and the medicinal uses ascribed for each plant. De Laszlo[2] has compiled list of some 1500 references on books, journals, and periodicals concerning phytotherapy and Dragendorff's Die Heilpflanzen der Verschienden Volker and Zeilen[3] should not be overlooked as source of new leads.

In India, the wide range of climatic conditions, from temperate (Kashmir) to tropical (Kerala), makes it possible to grow many different kinds of plants[4-9] and about 2200 of such plants are listed in the Ayurvedic and Unani-tibb systems, which are still the main source of medicinal relief of the majority of the people of this subcontinent. Some of the other countries represented by books or review publications on medicinal botany are Mexico[10-11], Poland[12], New Guinea[13], the Philippines[14], Nigeria[15], the U.S.S.R.[16-17], China[18-20], Burma[21], Puerto Rico[22], Malaya[23], Africa[24], Greece[25], Australia[26], New Zealand[27-30], Taiwan[31-32] and Haiti[33],

as well as others[34-38].

These texts describe the use of preparations derived from animal, mineral and vegetable sources with almost universal emphasis on the use of plant materials. Some of the drugs mentioned in them are serving mankind even today. These include opium, castor oil, squill, acacia, calamus,coriender,saffron, hyoscyamus, colchicum, gentian, olive oil,peppermint,herbane, aconite, cannabis, ephedra, and garlic.

The knowledge accumulated in early ages was distorted and mutilated as nations passed through political upheavels or when great civilizations decayed. This distortion reached its peak in the middle ages when medicine become associated for a considerable period with witchcraft and supernaturalism. Efforts at salvaging the ancient materia medica started around the sixteenth century and it was in the nineteenth century that men of science began to rationalise the treatment of disease. It was in the nineteenth century that pure physiologically active substances were isolated from the plant material with which man had treated himself through the ages. The secrets of the pain relieving drug, opium, were unravelled step by step. Derosne discovered narcotine. Hesse extracted a dozen alkaloids

and Berturner isolated the major alkaloid morphine. The isolation and characterisations of active principles from other plant material followed rapidly. Nicotine from tobacco, caffeine from tea dust, hyoscyamine from hyoscyamus, strychnine from nuxvomica, emitin from ipecae, and the most important of all quinine from cinchona. A large number of these pure active principles notably quinine for malaria and emitin for amoebic dysentery, soon replaced the corresponding crude drugs in clinical practice. Such remedies were almost wholly derived from plant materials.

A knowledge of the biological activities and/or chemical constituents of plant is desirable not only for the discovery of new therapeutic agents, but because such information may be of value in disclosing new sources of such economic materials as tannins[39], industrial oils[40-46], gums[47], precursors for the synthesis of complex chemical substances[48], etc.

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 constitutions of these principles and their physiological activity. This knowledge has been utilized by chemists in obtaining a series of modified semisynthetic

compounds, such as, atropine, homoatropine, reserpine, syrosingopine, morphine and N-allylnormorphine, which would either enhance the therapeutic action of a drug or make it more specific with less toxic effects.

The most important glycosides used in therapy are a closely related group, which because they increase the tone, excitability, and contractibility of cardiac and arterial muscles, are called cardiac glycosides. They occur in many poisonous plants, like Digitalis and Strophanthus.

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 progesterone, cortisone, testosterone, and ○rosterone and several other sex hormones.

A knowledge of the chemical constituents of plants would also be valuable to those interested in the expanding area of chemotaxonomy (biochemical systematics), in biosynthesis, and in deciphering the actual value of folkloric remedies.

The medicinal importance of the plants Boerhaavia diffusa and Trianthema pentandra has been recognised all over the country from time immemorial and their use

in medicine is even today very widespread. Inspite of the importance of these plants, their study has not received much attention. The present work is an effort in this direction.

Boerhaavia diffusa (Linn)

The plant belongs to the family Nytagenaceae. Members of this family are reported to yield alkaloids[49]. One of its member, Mirabilis jalpa, yields eight yellow pigments[50]. Plants of this family show antitumour activity against sarcoma 180 in mice[50]. A proteinous antitumour substance has been reported from Mirabilis multiflora. This material has shown activity against Lewis lung carcinoma, walker carcinosarcoma 256 (intramuscular) and lymphosarcoma[51].

B. diffusa is a perennial creeping weed with pinkish flowers. The roots are large and fusiform. The plant is distributed throughout India and also grows in Baluchistan, Ceylon, Tropical and subtropical Asia, Africa and America.

The plant is known by different names in the different parts of India. According to Kirtikar and Basu[52], some of the names are given below:

Arabic	Hadakuki, Sabaka
Bengali	Gadhapurna, Punarnaba
Hindi	Sant, Thikri
Persian	Devasapat
Sanskrit	Bhauma, Punarbhava Punarnava, etc.
Urdu	Bashkhira
Marathi	Kharaparya, Raktavasu
Gujarati	Dholisaturdi

Medicinal uses of Boerhaavia diffusa

According to Ayurveda the plant is useful in heart diseases, anaemia, inflammations, asthma, "vata" and "kapha". The leaves are useful in dyspepsia, tumours[53], enlargement of the spleen and abdominal pains. The plant in combination with other drugs is prescribed for snake-bite (Charaka, Sushruta, Vagbhata) and scorpion-sting (Sushruta). The root ground in rice water is given internally for snake-bite (Rasaratnakara, Yogaratnakara), alone or in combination with the root of either Gossypium herbaceum or Glycyrrhiza glabra it is administered internally and externally for scorpion-sting (Yogaratnakara, Nighantaratnakara, Brihannighantaratnakara).

According to Yunani-tibb the leaves act as appetiser, alexiteric; used in aphthalmia and for eye wounds;

also useful in pain of joints. The seeds are tonic, expectorant, and carminative. They are also effective in muscular pain, lumbago, scabies, scorpion-sting. They are supposed to purify the blood and hasten delivery.

The root is well known for its diuretic properties. It is also a very good expectorant. Taken in large doses it acts as an emetic. The plant is used in jaundice, ascites, anasarca, scanty urine, and internal inflammations. Mixed with dried ginger it is given in urticaria. It is also useful in cancer[54].

In Punjab, the drug is considered useful for eyes. In Western India, it is used for dropsical swellings. In the West Indies and in Goa the herb is a popular remedy for gonorrhoea.

Vaidyans consider the root of this plant to possess laxative, diuretic and stomachic properties. The powdered root either alone or combined with oxide of iron acts as a diuretic in anaemia. This drug is considered to be a sovereign remedy for dropsy. The intravenous injections of the alkaloid of Boerhaavia diffusa, in cats produce a distinct and persistent rise of blood pressure and a marked diuresis. The diuresis is mainly due to the action of alkaloid on the renal epithelium, although the rise

in blood pressure may contribute towards it. Clinically 1 to 4 drachms of the liquid extract from either the dry or fresh plant produce diuresis in case of oedema and ascites, especially due to early liver, peritoneal and kidney conditions. When the liquid extract is used the presence of a large amount of potassium salts no doubt reinforces the action of the alkaloid. The drug appears to exert a much more powerful effect on certain types of cases of ascities, i.e., those due to early cirrhosis of the liver and chronic peritonitis (Hale White) than some of the other diuretics known.

Literature Review

Ghoshal[55] analysed the drug and found an alkaloid body, a waxy amorphous mass and sulphates, chlorides, nitrates in the ash. Chopra, Ghosh, Ghosh and De[56] found an unusually large quantity of potassium nitrate (6.4%). They found also an alkaloid in very small quantity (0.01%) which they named Punarnavine. Agarwal and Dutt[57] failed to detect any alkaloid, but isolated an acid (m.p. 108°-109°C) which they named as boerhaavic acid, and determined its molecular formula $C_{10}H_{18}O_3$. The same authors[58], a year later isolated an alkaloid which melted with decomposition at 235°C with previous shrinking at 187°C. Those authors did not carry out any elemental analysis,

but prepared the hydrochloride, m.p. 135°C. Chopra, Chatterji and Ghosh[59] made a comparative study of Trianthema monogyna and B. diffusa. The melting point of the crude base isolated by them was 175°C. However, it is not very clear from their paper, whether this base is from T. monogyna or B. diffusa. The melting points of the picrate and platinichloride, prepared by them were 117°C and 120°C respectively. Inspite of the difference in the melting points of the derivatives prepared from the crude bases obtained from the two drugs. Chopra et al on the basis of pharmacological actions said that punarnavine occurs in B. diffusa and T.pentandra.

Basu and Sharma[60] have made a detailed study of the alkaloids of the plant B. diffusa. They have reported the isolation of punarnavine in pure form (m.p. 236° - 237°C with decomposition) and determined its molecular formula $C_{17}H_{22}N_2O$. They prepared several derivatives platinichloride, m.p. 118°C (decomposition) picrate, m.p. 114°C-115°C (decomposition) sulphates, m.p. 204°C - 205°C.

S. Sankara Subramanium and S. Ramakrishna[61] have studied chemical difference between two species of Boerhaavia. They have studied the aerial part of B. diffusa and B. punarnava collected in Pondichery. They have determined the ash contents of B. diffusa (11.8%)

and B. punarnava (9.6%). The calcium content of the ash (determined by micromethod) of B. diffusa and B. punarnava were (1.2%) and (0.94%) respectively. They have estimated the potassium in the ash of B. diffusa (2.3%) and B. punarnava (1.1%). They have reported the presence of quercetin and iso-quercetin in B. punarnava. No anthoxanthine pigments from B. diffusa has been reported, but a greenish blue fluorescent spot under U.V., Rf. 0.14, in n-butanol:acetic acid: water (4:1:5) has been reported. They have also reported the presence of an alkaloid. They have also identified free and combined amino acids present in B. diffusa.

Recently pharmacological activity of the plant B. diffusa has been studied at Central Drug Research Institute, Lucknow by Bhakuni et al[62]. The plant has been found pharmacologically active.

Trianthema Pentandra (Syn Zalia Govindia) N.C. Nair

The plant belongs to family Aizoaceae. It is a perennial weed. The stems are much branched. The roots are fusiform.

The plant is known by different names in different parts of India, in Punjab it is known as Biskhapra.

In north India and Rajasthan the plant is called as santhi or sata. In Las Bela the plant is known as Lular wahu.

The plant is distributed in tropical countries especially in India and tropical Africa.

Medicinal uses

The plant is used as an astringent in abdominal diseases, and is stated to produce abortion. In Las Bela the plant is used as a cure both for pain in the bladder and for snake bite (Kirtikar & Basu). Locally the stems and leaves are used as cattle food and also as vegetables by inhabitants of the area. It is widely used in Pilani and the surrounding area as domestic medicine for diarrhoea, indigestion, liver swelling, jaundice and also said to improve the eyesight. The roots are powdered and used in gum swelling and gum bleeding[63].

Review of Literature

No work has been reported on chemical constituents of T. pentandra. A little work has been done on T. monogyna. Chopra et al[59] carried out some chemical and pharmacological work on its constituents. They are of the opinion

that the drug contains an alkaloid identical with Punarnavine isolated by Agrawal and Dutt[64] from B. diffusa. These conclusions are mainly based on the similarity of the pharmacological action of the two drugs. The chemical data submitted by them in support of their conclusions is meagre. They have mentioned the melting point of the base as 175°C, but it is not clear whether this base is from B. diffusa or from T. monogyna. The melting points of the two derivatives, platinichlorides as well as picrates, prepared by them from the crude alkaloid of the respective plants were not the same. They have not given any analytical data on the alkaloid. Basu and Sharma[65] have reported the isolation of a new alkaloid trianthemene (0.07%) in pure crystalline form; m.p. 127°C, molecular formula $C_{32}H_{46}O_6N_2$. They have also prepared several derivatives and determined their melting points. However they did not propose any structure for the base.

Derivatives of alkaloid trianthemene and their m.pts

Derivatives	Melting points
Platinichloride	121° - 122°C
Oxalate	138°C
Aurichloride	150° - 153°C
Sulphate	110° - 111°C

Recently pharmacological activity of Trianthema monogyna has been studied at the Central Drug Research Institute, Lucknow by Dhar and coworkers[66]. The plant has been found pharmacologically very active.

OBJECT OF THE PRESENT WORK

It is clear from the review of literature available on B. diffusa and T. pentandra that no systematic work on chemical constituents of these plants has been done. Basu and Sharma[60,65] have reported the isolation of alkaloid punarnavine and trianthemine from the plant B. diffusa and T. pentandra respectively, but they have only determined their molecular formulae and have prepared some derivatives.

The object of present work on these plants is to study the various groups of compounds present in detail, and assign structures to them on the basis of their physico-chemical data .

CHAPTER II

ISOLATION AND STUDY OF HENTRIACONTANE, HENTRIACONTOL, β-SITOSTEROL, URSOLIC AND, β-SITOSTEROL-D(+)-GLUCOSIDE, GLUCOSE, FRUCTOSE, SUCROSE AND ALKALOIDS FROM THE ROOTS OF BOERHAAVIA DIFFUSA LINN

The roots of B. diffusa were extracted with petroleum ether and the neutral fraction was chromatographed on neutral alumina column. Elution of column with petroleum ether, petroleum ether:benzene (4:1), pure benzene and benzene:chloroform (1:1), gave compounds BA, m.p.66°C, BB, m.p. 86°C, BC, m.p. 85°C and BD, m.p. 137°C successively. Examination of BA, BB, BC and BD on thin layer chromatography showed them to be homogeneous.

Study of the compound BA

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.

Mass spectrum of the compound BA showed no $(M-15)^+$ peak, but an intense molecular ion peak and fragments of 14 mass units were observed. This shows that the compound under study is a straight chain aliphatic hydrocarbon[67].

The IR spectrum of the compound (in KBr) showed bands at 725 cm^{-1} and 714 cm^{-1}, indicating a long n-alkane chain[68,69]. Strong absorption bands at 2920 cm^{-1} and 2850 cm^{-1} indicate a large number of $-CH_2$ groups[70]. NMR spectrum of the compound gave 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[71]. The hydrocarbon

has been identified as Hentriacontane and can be represented as

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

BA

Identification of Hentriacontane was further confirmed by mixed mp. and superimposable IR spectrum with an authentic sample[72].

Study of the compound BB

IR spectrum of the compound in CCl_4 showed a peak at 1735 cm^{-1} indicating the presence of a carbonyl function. When the compound in alcohol was treated with an alcoholic solution of 2:4 dinitrophenyl hydrazine, no hydrazone derivative was formed as indicated by tlc of the reaction mixture. The compound was not reduced with lithium aluminium hydride. UV spectrum showed an absorption maxima at 222 nm. As the compound BB isolated from the plant was in small amount, further work was not possible.

Study of compound BC

The elemental analysis of the compound corresponds to molecular formula $C_{31}H_{62}O$. The compound gave a negative tetranitromethane test showing this to be saturated compound[73].

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} ($-O-COCH_3$). 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. Hentriacontol can be written as

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

BC

Study of the compound BD

The elemental analysis of the compound corresponds to the molecular formula $C_{29}H_{58}O$. This molecular formula is supported by the molecular ion peak at m/e 414. Other

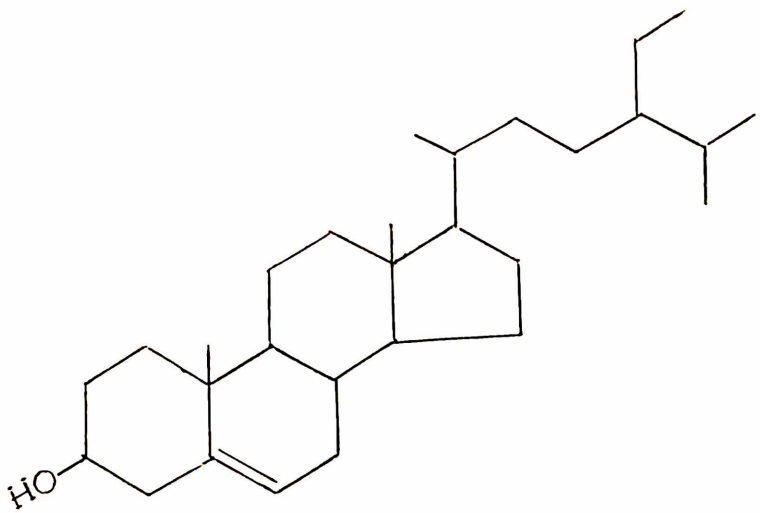
important fragments in mass spectrum are at m/e 399 (M^+-CH_3); 396 (M^+-HOH), 381 (M^+-CH_3+HOH), 273 (M^+-side chain), 255 (M^+-side chain + HOH), 329, 303, 275, 288, 273, 229, 231, and m/e 213[75].

The compound gave a green colour in Libermann-Burchard test[76], red colouration in Salkowski[77] test, and a yellow colouration with tetranitromethane[73]. When alcoholic solution of digitonin was added to the alcoholic solution of the compound, a precipitate was formed (digitonide, m.p. 226°C)[78]. On the basis of the colour reactions, molecular formula, and its fragmentation pattern, 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 $\left[\alpha\right]_D$ - 38°[79] (chloroform). IR spectrum of the acetate showed peaks at 1742 cm^{-1} (acetate - CO) and 1236 cm^{-1} (O-$COCH_3$)[80].

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 BD. This is further supported by a multiplet at 6.33 τ in NMR spectrum. This is also in conformity 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



BD

The NMR spectrum of the compound disclosed signals at 9.27 τ assigned to the methyl group attached to C_{13}, a triplet at 9.1 τ due to methyl attached to C_{28}, a doublet at 9.18 τ due to two methyl groups attached to C_{25}, and a singlet at 9.02 τ due to methyl attached at C_{10}. However while six methyl groups are present in β-sitosterol but only four signals were observed in NMR spectrum. This may be due to the overlapping of some of the signals. Multiplet envelop at 8.90 τ to 7.62 τ is due to methylenes of the steroid ring system and methylenes of the side chain. The deformed triplet at 4.66 τ is due to olefinic protons and multiplet at 6.33 τ is due to the proton attached to the carbon having hydroxyl group[81].

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 Boerhaavia diffusa

Alcoholic extract of the defatted roots of B. diffusa were further extracted with solvent ether in a liquid-liquid extractor. The acidic fraction of the ether extract upon usual processing and chromatography on silica gel column gave a white crystalline compound BE, m.p. 278°-279°C.

Study of the compound BE

The homogeneity of the compound was established by thin layer chromatography on silica gel G plates using chloroform:methanol (95:5) as an irrigating solvent and concentrated sulphuric acid and chlorosulphonic acid (Stahl)[82] for revealing the spots.

The elemental analysis of the compound BE corresponds to the molecular formula $C_{30}H_{48}O_3$. The compound has $[\alpha]_D$ + 68°C (pyridine).

The compound BE gave a yellow colour changing to red in Salkowski reaction and a pink colouration in

Liebermann-Burchard test. It produced a red colour with a greenish yellow flourescence when chloroform solution of the compound was boiled with an excess of acetyl chloride and a little of zinc chloride (Tschugagiw)[83]. A yellow colour which changed to red was observed when a small quantity of the compound was treated with Noller's reagent[84]. The compound developed a reddish violet colour in Brieskorn test[85] and a yellow colour with tetranitromethane. No precipitate was formed when an ethanolic solution of the compound was treated with an ethanolic solution of digitonin. The appearance of a reddish violet colour in Brieskorn test and the absence of a precipitate with digitonin ruled out the possibility of the compound being a steroid.

From the molecular formula and colour reactions it is evident that the compound BE is a triterpenoid. Yellow colouration with tetranitromethane indicates that compound is unsaturated.

IR spectrum of the compound BE showed strong absorption at 3535 cm^{-1} assigned to the O-H stretching vibration of -COOH group[86]. Absorption at 3624 cm^{-1}, which is assigned to -OH alcoholic[87], appears as a shoulder probably due to the overlapping of the hydroxyl absorption of the carboxyl function. Absorption band at 1715 cm^{-1} is due to the carboxyl carbonyl[86]. Absorption

peaks at 835 cm^{-1}, between 800-820 cm^{-1} and at 1650 cm^{-1} show the presence of C=C[88]. An intense peak at 990 cm^{-1} and medium intensity peaks at 1025 cm^{-1} and 962 cm^{-1} are characteristic of tetracyclic triterpenes having carboxyl and other oxygenated groups[89,90].

Bands at 1282 cm^{-1} and 1245 cm^{-1} are characteristic of all tetracyclic triterpenic acids[89,91,92].

On the basis of above observations, BE appears to be a tetracyclic triterpenic acid having an alcoholic group and a carbon-carbon double bond.

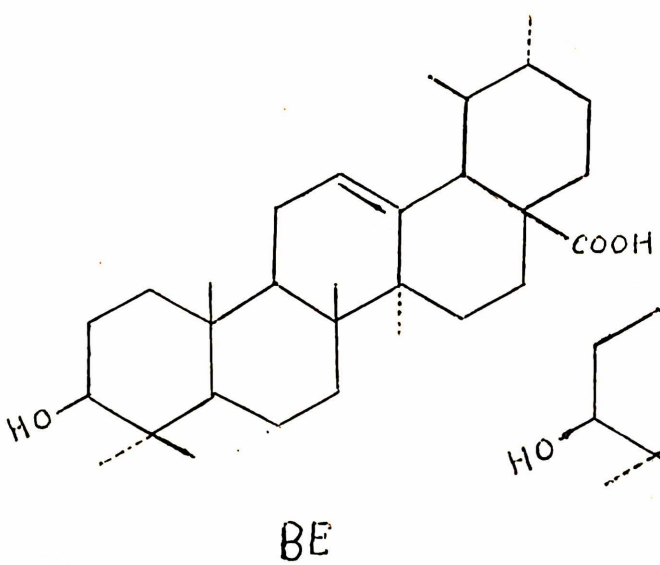
The acid BE on methylation with an etherial solution of diazomethane gave the methyl ester $C_{31}H_{50}O_3$, m.p. 168°-169°C $[\alpha]_D$ + 61° (pyridine). The monomethyl ester could not be saponified easily (8% alcoholic alkali) to the original acid, indicating the hindered nature of the carboxyl function[93].

The acid BE on acetylation with acetic anhydride and pyridine gave acetyl derivative $C_{32}H_{50}O_4$, m.p. 287°C, $[\alpha]_D$ + 64° (chloroform).

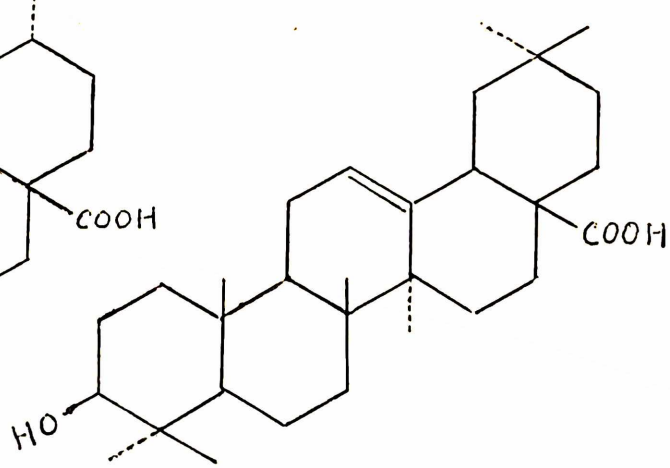
Methyl ester of the acid BE on acetylation with acetic anhydride and pyridine in the usual manner gave acetyl methyl ester $C_{33}H_{52}O_4$, m.p. 241°C, $[\alpha]_D$ + 79° (pyridine). The methyl ester failed to undergo selenium-

dioxide oxidation indicating that the acid does not possess an oleanane skeleton[94].

The acid BE on oxidation with Cr_2O_3/H_2SO_4 in acetone[95] gave a product which responded to positive Zimmerman colour test for 3-keto group[96]. This indicates that the OH group is secondary in nature and is at position 3. The compound under study therefore appears to be ursolic acid.



BE



Ursolic acid Oleanolicacid

Snatzke, Lampert and Tschesche[97] have studied the IR spectrum of the triterpenes in two spectral ranges (A, between 1392 cm^{-1} - 1355 cm^{-1} and B, between 1330 cm^{-1} - 1245 cm^{-1}) characteristic of oleanolic acid and ursolic acid and their esters. Tetracyclic triterpenic acids or

their esters show only one strong absorption in these ranges. Acids which do not belong to the type mentioned above possess their intensive bands outside the described limits.

The IR spectrum of the acid (in KBr) under study shows three absorption bands in region A at 1386 cm^{-1}, 1360 cm^{-1} and 1372 cm^{-1} characteristic of ursolic acid. These bands can be attributed to the methyl groups at C_{19} and C_{20}[98]. In region A oleanolic acid show only two bands. This is due to the overlapping of the gem-dimethyl with absorption of the angular methyl group[99].

In region B spectrum showed three peaks at 1315 cm^{-1}, 1280 cm^{-1} and 1245 cm^{-1} characteristic of ursolic acid[97].

With the study of IR spectrum of the compound, the identity of the compound BE as ursolic acid was established.

IR spectrum also showed bands at 1200 cm^{-1}, 1178 cm^{-1}, 1162 cm^{-1} and 1118 cm^{-1}, which are assigned to the oscillations of -COOH. This is in agreement with the observations of Snatzke and Tschesche in tetracyclic triterpenic acids[100,101].

Identity of the compound BE as ursolic acid was further confirmed by mixed m.p., Co-tlc on silica gel G plate and superimposable IR spectrum with an authentic sample of ursolic acid[102].

Study of the basic fraction

Crude bases obtained from solvent ether extract upon chromatography on neutral alumina gave two compounds BF and BG.

Study of compound BF

All attempts to crystallise the alkaloid BF failed. The alkaloid developed a dark brown colour when exposed to air even for a short time or when heated during attempts for crystallisation. It developed colour even when kept in an atmosphere of nitrogen in a refrigerator, though the rate of development of colour was slow. The alkaloid BF was purified by sublimation under vacuum (0.1 mm/at 70°), m.p. 279°-280°C (decomposition).

The homogeneity of the base BF, m.p. 279°-280°C (decomposition) was established by thin layer chromatography on neutral alumina plates using chloroform as a developing solvent and Dragendorff's reagent[103] and concentrated sulphuric acid for visualizing the spots. Dragendorff's reagent gave a single orange colour spot.

Compound BF 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[71] of -NH. On the basis of above observations the compound under study appears to be an alkaloid.

The alkaloid formed a methiodide, m.p. 211°-212°C, and a picrate m.p. 119°C.

As the alkaloid BF was unstable and the percentage yield was very low, it was not possible to continue further work on it.

Study of compound BG

Chloroform:methanol (98:2) elute of the column gave alkaloid BF, m.p. 292°-293°C(decomposition). As in the case of alkaloid BF, attempts to crystallise this alkaloid BG also failed. This alkaloid also developed brown colour on standing for short time. Purity of the compound was established by thin layer chromatography on activated neutral alumina plate.

The compound gave positive Lassaigne test for nitrogen. UV absorption spectrum of the compound in methanol showed absorption maxima at 225 nm, 290 nm and 375 nm. IR spectrum of the compound in chloroform showed peak at 3645 cm^{-1}, indicating the presence of

-OH group[71]. The presence of -NH-group is established by the absorption peaks at 3525 cm^{-1}, 3400 cm^{-1} characteristic stretching bands of -NH- and a band at 1625 cm^{-1} due to the bending vibration of -NH-. Peak at 1722 cm^{-1} indicates the presence of a carbonyl group in the compound. On the basis of colour with Dragendorff's reagent and IR spectrum, the compound BG appears to be an alkaloid.

The compound formed methiodide, m.p. 216°-217°C (decomposition) and a picrate, m.p. 213°-214°C.

Basu and Sharma[104] have reported the isolation of an alkaloid Punarnavine, m.p. 236°-237°C (decomposition) from B. diffusa in 0.041% yield. Inspite of very careful extraction and processing it was not possible to get more than 0.005% yield of the alkaloids. To avoid the possibility, that the climatic variations may be the reason for the poor yield of the alkaloids, a fresh attempt was made to isolate the alkaloids from B. diffusa collected from gangetic planes near Allahabad. The procedure described by Basu and Sharma[104] for isolation of Punarnavine was carefully repeated with a slight modification to avoid prolonged heating and exposure to atmosphere. There was practically no difference in the total yield of bases (0.006%).

It thus appears that the alkaloid punarnavine reported by Basu and Sharma[104], is a mixture of two alkaloids.

Treatment of ethylacetate extract of B. diffusa

The residue from the alcoholic extract of defatted roots of B. diffusa after extraction with ether was further extracted with ethyl acetate. Ethyl acetate extract on concentration and subsequent cooling in a refrigerator for four days gave a white crystalline compound BH, m.p. 289°-290°C (decomposition), $[\alpha]_D$ -40° (pyridine). Homogeneity of the compound was established by thin layer chromatography on silica gel G plate.

Elemental analysis of the compound BH 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 obtained 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 ofthe 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[105].

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 peak at 1762 cm^{-1} (acetate-CO) and 1224 cm^{-1} ($O-COCH_3$).

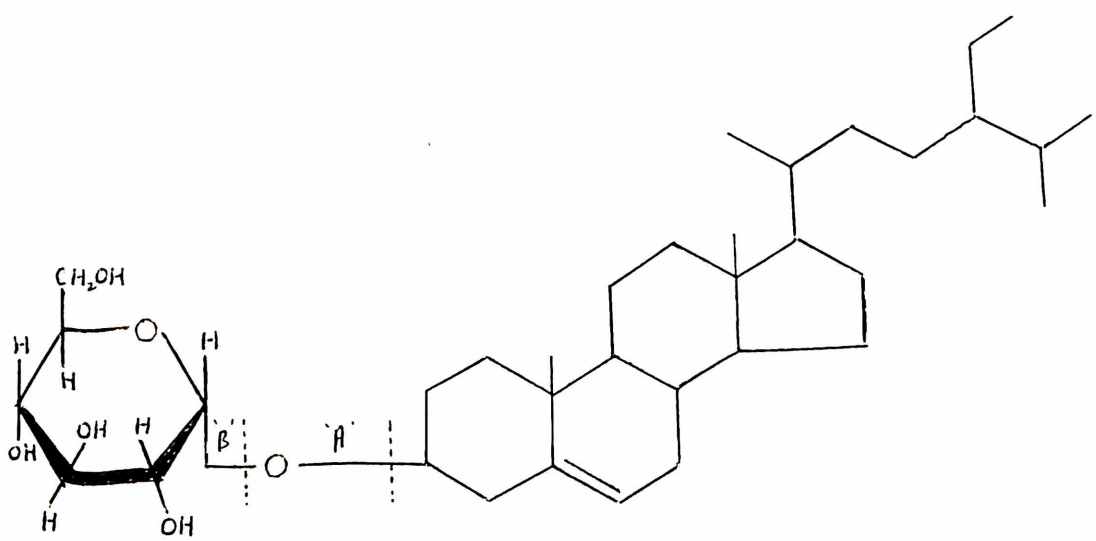
Determination of acetyl percentage by Wiesenberger's method as described by Belcher[106] showed the presence of four hydroxyl groups.

NMR spectrum of the acetate disclosed a triplet at 7.98 τ assigned to the protons of $CH_3\overset{\overset{O}{\|}}{C}-$ (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 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.

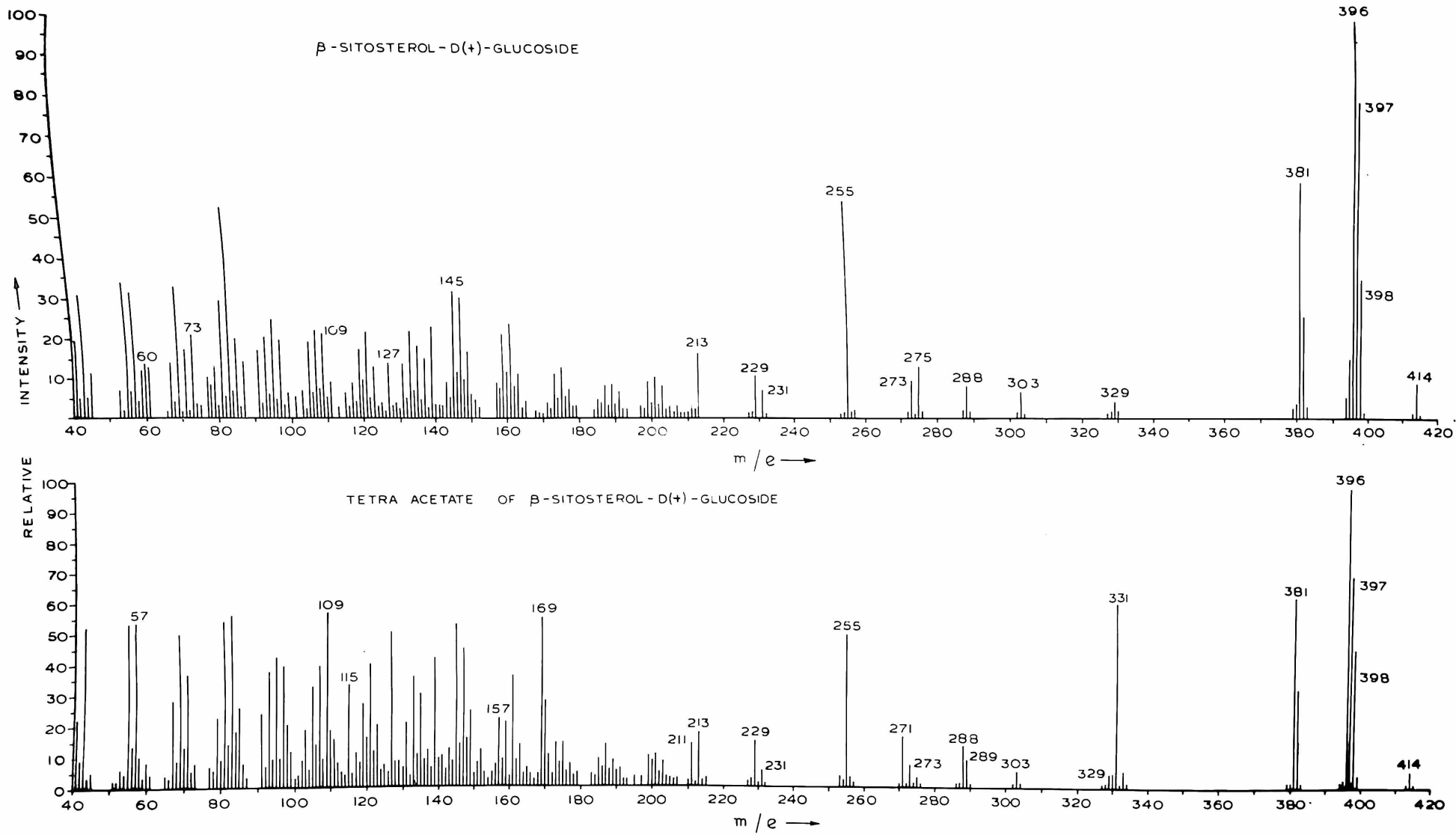
The phytosterolin BH on acid hydrolysis yielded β-sitosterol and D(+)-glucose, which was identified by Co-paper chromatography and from osazone, m.p. 203°C (reported 204°C).

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



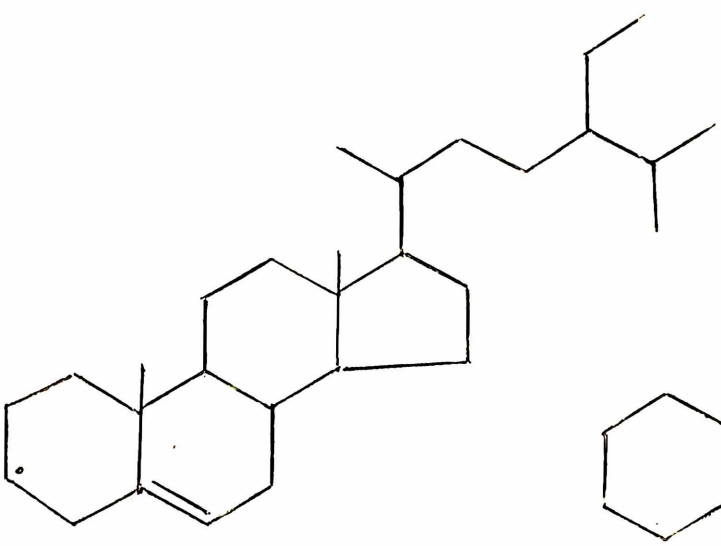
BH



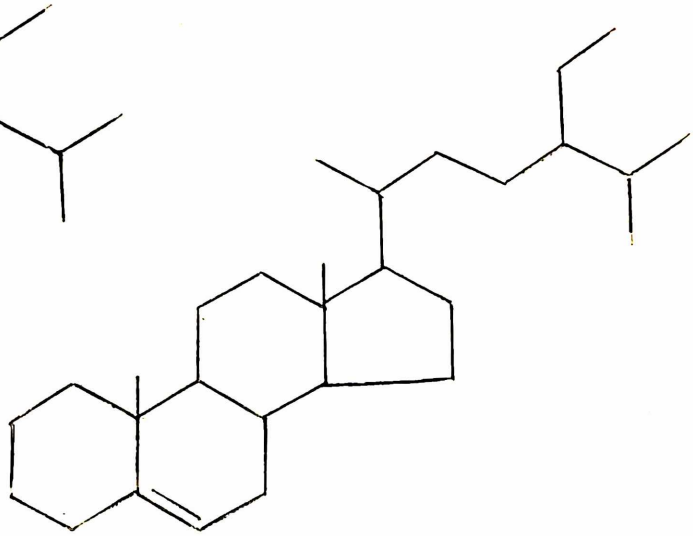
MASS SPECTRA OF β-SITOSTEROL-D(+)-GLUCOSIDE AND ITS TETRAACETATE

Mass spectral studies of glycoside

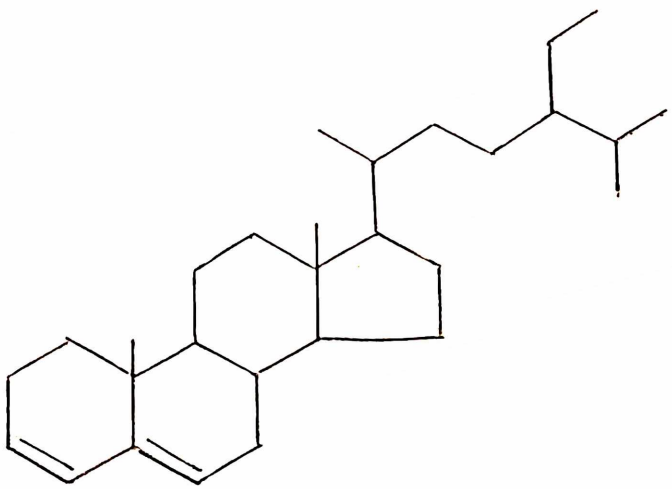
From its mass spectrum the compound appears to be unstable even using direct inlet probe. Towards the end of the spectrum intense peaks at m/e 396, m/e 397 and peaks at m/e 398 and m/e 414 were obtained. Peak at m/e 397 can arise by the cleavage of the carbon oxygen bond at 'A' (BH). Peak at m/e 398 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



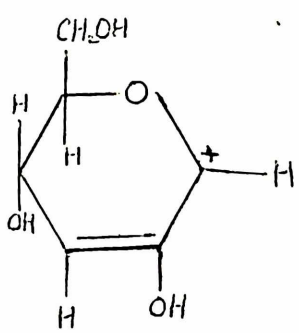
m/e 398



m/e396

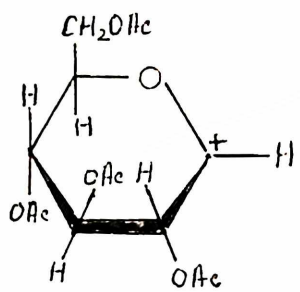
The other peaks in the mass spectrum are at m/e 381, 303, 288, 275, 273, 255, 231, 229, 213.

The occurrence of peaks at m/e 145, 127, 109, 73, 61, and 60 in the lower region of the mass spectrun indicates that the glycone part of the glycoside may be glucose[107].



m/e 145

Mass spectrum of the acetate of BH showed peaks at m/e 396, 397, 398, 414, 381, 255, 329, 303, 275, 288, 273 229, 231 and 213. Besides 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 tetraacetate.

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

Study of the aqueous extract of B.diffusa

In the aqueous extract, glucose, fructose and sucrose have been identified by co-paper chromatography. Quantitative estimation of sugars was carried out by using phenol

sulphuric acid method[108]. The following results were obtained

Glucose = 1.66%

Fructose = 2.87%

Sucrose = 1.29%

-x-

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 Boerhaavia diffusa (8 Kg), collected locally, were extracted in a Soxhlet apparatus with petroleum ether (b.p.60°-80°C) for 48 hours.

The solvent was distilled under reduced pressure using water suction pump and the yellow viscous residue (51.98 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 the acid 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 (40.4 g). Chromatography of the viscous solid on tlc plates using different solvent systems and spraying the plate with concentrated sulphuric acid and heating for 15 minutes at 120°C showed four 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 gave positive Libermann-Burchard test for steroids.

Treatment of neutral ether soluble material

15 g of the solid was dissolved in minimum quantity of solvent ether and was adsorbed on 50 g of neutral alumina. It was then placed on the top of chromatography

column (36" x 3"), packed previously with 450 g of neutral alumina.

Development of the column was carried out utilising a discontinuous gradient elution 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 four 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 (1.5 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 tlc pattern. The residue on repeated crystallisation with hexane gave white shining flakes BA (0.45 g), m.p. 66°C.

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

Chromatography of portion II

Portion II (0.25 g) was dissolved in minimum

quantity of hexane and placed on top of an alumina column (24" x 1/2"). The column was washed with petroleum ether (75 ml) and the washings were discarded. It was then eluted with petroleum ether:benzene (4:1). 25 ml fractions were collected. Fractions 4 to 10 were combined on tlc pattern, evaporated to dryness and crystallised several times with hexane, to give white shining crystals BB (0.09 g) m.p. 86°C. Elutes from benzene were combined with portion III.

Chromatography of portion III

Portion III (0.82 g) was adsorbed on alumina (4 g) and was placed on top of alumina (60 g) column (24" x 3/4"). The column was washed with 100 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 seven fractions of benzene elutes were discarded. Fractions 8 to 18 were combined on 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 BC (0.25 g), m.p. 85°C.

Chromatography of portion IV

Portion IV (8.45 g) was chromatographed on neutral alumina (140 g) column (30" x $1\frac{1}{2}$"). 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 (200 ml) were discarded. Elutes from benzene:chloroform (1:1) were combined on tlc pattern. The compound was crystallised with acetone to give a white crystalline compound BD (4.25 g), m.p. 137°C.

Thin layer chromatography of the compound BA

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.

Elemental analysis of the compound BA

Found	Calculated for $C_{31}H_{64}$
C = 84.5%	C = 84.32%
H = 14.62%	H = 14.67%

Solubility of the compound BA

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

IR spectrum of the compound BA (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

Nuclear magnetic resonance spectrum of the compound BA (in CCl_4)

Signals τ	Assignment
9.12	methyl protons
8.75	methylene protons

Thin layer chromatography of the compound BB

Activated silica gel G plate was spotted with

chloroform solution of the compound and developed in a tank saturated with petroleum ether:benzene (3:1). 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 (Rf 0.68).

Solubility of the compound BB - The compound is soluble in chloroform, carbontetrachloride, ether and hexane. It is sparingly soluble in ethanol, methanol and insoluble in water.

IR spectrum of the compound BB (in CCl_4)

Following peaks were observed in IR spectrum:

2930 cm^{-1}, 2850 cm^{-1}, 1735 cm^{-1}, 1462 cm^{-1}, 1375 cm^{-1}, 1252 cm^{-1}, 1170 cm^{-1}, 1000 cm^{-1}.

Thin layer chromatography of the compound BC

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 (Rf 0.46).

Elemental analysis of the compound BC

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

Solubility of the compound BC - The compound is soluble in benzene, chloroform, ether and is sparingly soluble in acetone, ethanol, and methanol.

IR spectrum of the compound BC (in CCl_4)

The observed peaks and their assignments are as follows:

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

Nuclear magnetic resonance spectrum of the compound BC

Signals τ	Assignment
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 BC:

The compound (50 mg) was taken in a mixture of acetic anhydride (5 ml) and pyridine (1 ml) and was stirred mechanically with slight warming for 8 hours. The mixture was kept over-night. 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 (Na_2SO_4) and evaporated to dryness. The residue was crystallised with ethanol, m.p. 75^{o}-76^{o}C.

Thin layer chromatography of the acetyl derivative of BC

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^{o}C for 15 minutes gave a single black spot (Rf 0.65).

Elemental analysis of the acetyl derivative of BC

Found	Calculated for $C_{33}H_{66}O_2$
C = 80.25 %	C = 80.16 %
H = 13.21 %	H = 13.36 %

IR spectrum of acetyl derivative of BC (in CCl_4)

The observed peaks and their assignments are as follows:

Peaks cm^{-1}	Assignment
2925	C-H stretching
2852	C-H stretching
1740	Acetate - CO
1465	C-H bending
1362	C-H bending
1235	$O-COCH_3$
1020	C-O stretching

Thin layer chromatography of the compound BD

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.

Rf (a) 0.24
(b) 0.42

Elemental analysis of the compound BD

Found	Calculated for $C_{29}H_{50}O$
C = 83.98%	C = 84.05%
H = 12.15%	H = 12.07%

Reactions of the compound BD

(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 ethanol) was treated with ethanolic solution of digitonin a precipitate was formed.

IR spectrum of the compound BD (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
950	

Acetylation of the compound BD

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 (58 mg), m.p. 128°C, $[\alpha]_D$ - 38° (chloroform).

Elemental analysis of the acetyl derivatives of the compound BD

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

IR spectrum of the acetyl derivative of the compound BD (in CCl_4)

The observed peaks and their assignments are as follows:

Peaks cm^{-1}	Assignment
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 BD

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 2 hours and cooled when the digitonide separates out. It was filtered and crystallised with absolute alcohol, m.p. 227°-228°C.

Benzoylation of the compound BD - The compound (50 mg) was treated with benzoylchloride (10 ml) and two drops of pyridine. The mixture was heated on water bath and allowed to stand over-night at room temperature. The mixture was then poured in ice cold water. The solid

was filtered off, washed with 2% aqueous KOH and then thoroughly with water. It was crystallised from a mixture of methanol:acetone (1:1), m.p. 144°C.

Nuclear magnetic resonance spectrum of the compound BD

Signals τ	Assignment
9.27	Methyl protons
9.02	Methyl protons
9.10	Methyl protons
9.18	Methyl protons
8.90 to 7.62	Methylene protons
4.66	Olefinic protons
6.33	Hydroxyl proton

Treatment of the alcoholic extract of B. diffusa

The defatted roots of Boerhaavia diffusa (8 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 and ethylacetate.

Etherial solution was washed with little water, dried (Na_2SO_4), and extracted with 10% sodium bicarbonate solution. Sodium bicarbonate extract on acidification gave a brown coloured precipitate. The 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 (Na_2SO_4), and the chloroform was evaporated. A light brown coloured solid (0.52 g) was obtained.

Etherial solution was then washed with 2N sulphuric acid (4 x 30 ml). Combined acidic extracts were extracted with chloroform (4 x 30 ml).

The acidic extract was then made alkaline with sodium bicarbonate and extracted with chloroform (6 x 50 ml). Chloroform extracts gave a positive spot test with Dragendorff's reagent for alkaloids. The chloroform solution was then washed thoroughly with water, dehydrated (Na_2SO_4), and evaporated under reduced pressure to give a light brown solid.

Treatment of acidic fraction

tlc of the crude acidic compound on activated silica gel G plate showed four spots of which only one was prominent.

The acidic compound (0.52 g) was dissolved in alcohol-chloroform mixture, adsorbed on 10 g of silica gel and placed on top of a dry packed silica gel column (30" x 1"). The column was eluted with solvents of

increasing polarity, with benzene, chloroform, chloroform:methanol (9:1), and finally with methanol. Elutes from benzene and chloroform gave no worthwhile residue. Elutes from chloroform:methanol (9:1) were collected on tlc pattern, and the solvent was evaporated under reduced pressure. A light yellow solid (0.35 g) was obtained. The solid on repeated crystallisations with ethanol gave a white crystalline compound BE (0.24 g), m.p. 297°-298°C.

Thin layer chromatography of the compound BE

Activated silica gel G plate was spotted with methanol solution of the compound and developed in a tank saturated with chloroform:methanol (95:5). The air-dried plate was sprayed with concentrated sulphuric acid and then heated at 120°C for 15 minutes. A single black spot developed (Rf 0.54).

Another plate developed in the identical conditions was sprayed with chlorosulphonic acid reagent (10 ml of chlorosulphonic acid in 20 ml of glacial acetic acid). The plate on heating at 130°C for 15 minutes developed a single reddish violet spot (Rf 0.54).

Elemental analysis of the compound BE

Found	Calculated for $C_{30}H_{48}O_3$
C = 78.50 %	C = 78.94 %
H = 10.58 %	H = 10.52 %

Solubility of the compound BE - The compound is highly soluble in carbondisulphide and is sparingly soluble in chloroform, solvent ether, alcohol and acetone. The compound is insoluble in water.

Reactions of the compound BE

(i) Liebermann-Burchard reaction: - A small amount of 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 violet colour developed.

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

(iii) Noller reaction

(a) When a little of the compound was treated with pure thionyl chloride a yellow colour was obtained which changed to red.

(b) When a dilute solution of anhydrous ferric chloride in thionyl chloride was added to a

small quantity of the compound, the following colour sequence was observed -

violet ⟶ purple ⟶ red

(c) When a solution of antimony trichloride in thionyl chloride was added to the compound, a violet colour developed which changed to red.

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

(v) Brieskorn reaction - When a little of the compound was treated with a 30% solution of chlorosulphonic acid in glacial acetic acid, a reddish violet colour was observed.

(vi) Tschugajew reaction - A solution of the compound in chloroform was mixed with an excess of acetyl chloride and a little of zinc chloride. On boiling a red colour with a greenish-yellow fluorescence was observed.

(vii) When the compound in ethanol was treated with ethanolic solution of digitonin, no precipitate was formed.

Acetylation of the compound BE: - The compound (50 mg) was acetylated with a mixture of acetic anhydride (5 ml)

in the usual manner and the acetylated product was crystallised with ethanol to give a crystalline compound (52 mg), m.p. 287°C, $[\alpha]_D$ + 64° ($CHCl_3$).

Esterification of the compound BE - Diazomethane was prepared by the action of aqueous KOH on p-toluenesulphonyl-methynitrosamide (Diazald).

The compound (50 mg) was taken in ether (10 ml) and an etherial solution of diazomethane was added till the solution acquired a definite yellow colour. It was kept for 24 hours. Excess of diazomethane was destroyed by adding a few drops of acetic acid and the solvent was distilled under reduced pressure. Methyl ester was crystallised with ethanol:chloroform (1:1) mixture, m.p. 168°-169°C, $[\alpha]_D$ + 61° ($CHCl_3$)

Methyl ester on acetylation with acetic anhydride and pyridine gave acetyl methyl ester, m.p. 241°C , $[\alpha]_D$ + 79° ($CHCl_3$).

Oxidation of the compound BE with chromic acid (Bowers)

The compound was dissolved in pure acetone (distilled over pot. permanganate). To this was added chromic acid solution, dropwise, from a microburette until a persistent orange-brown colouration appeared, which indicated that the oxidation was complete. After keeping

at room temperature for some time, the crystalline product was filtered. The compound gave a positive Zimmermann colour test for 3-keto group. The IR spectrum showed that the peak at 3640 cm^{-1} in the original compound had disappeared and a new peak at 1735 cm^{-1} had appeared.

Zimmermann colour test (Barton)

The compound (3 mg) obtained from chromic acid oxidation of the compound BE was dissolved in 1 ml of 2N-KOH in absolute alcohol. A violet colour developed which faded on dilution.

IR spectrum of the compound BE (in KBr)

The observed peaks and their assignments are as follows:

Peaks cm^{-1}	Assignment
3624	O-H stretching (alcoholic)
3535	O-H stretching (carboxylic)
2980	C-H stretching
2940	C-H stretching
2862	C-H stretching
1715	C=O (COOH)
1650	ethylenic double bond
1386	CH_3-bending oscillations
1372	of C_{19} and C_{20}
1360	

Peaks cm^{-1}	Assignment
1315 1280 1245	Oscillations of the tertiary - COOH
1200 1178 1162 1118	Triterpenic acid
1025 990 962	Tertiary - COOH and O-H
835 800-820	Ethylenic double bond

Treatment of the basic fraction

Thin layer chromatography

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 spotted with chloroform solution of the basic fraction was developed in a tank saturated with chloroform:methanol (99:1). The developed plate was air-dried and sprayed with Dragendorff's reagent. Two orange coloured spots developed (Rf 0.96 and 0.40).

Column chromatography

The base mixture was dissolved in a minimum volume of chloroform and was applied at top of a chromatography column ($1\frac{1}{2}$" x 36") containing a benzene slurry of neutral alumina (140 g). Elution of the column was carried out utilising a discontinuous gradient elution technique, beginning with bezene and followed by chloroform and chloroform:methanol (98:2). 25 ml fractions were collected. Later ten elutes from chloroform were combined on tlc pattern and evaporated to dryness under reduced pressure to give compound BF.

Earlier elutes of chloroform:methanol (98:2) gave a mixture of the two compounds. Later six fractions were combined on tlc pattern and evaporated to dryness under reduced pressure to give compound BG. Fractions containing mixture of two compounds were combined and rechromatographed.

Compound BF and BG were purified by sublimation under vacuum (0.1 mm at 70°C), m.p. 279°C-280°C and 292°C-293°C, respectively.

Thin layer chromatography of compound BF

Two activated neutral alumina plates were spotted with a chloroform solution of the compound and were

developed in a tank of chloroform. One of the developed plates was air-dried and sprayed with Dragendorff's reagent. A single pink coloured spot was observed (Rf 0.45). The second developed plate was sprayed with concentrated sulphuric acid and heated at 120°C for 15 minutes. A single black colour spot developed (Rf 0.45).

The compound gave a positive test for nitrogen.

Solubility of the compound BF - It is soluble in ethyl alcohol, chloroform, solvent ether and benzene but insoluble in water.

UV spectrum of the compound BF (in methanol)

The compound gave the following absorption bands

λ_{max} 215 nm
λ_{max} 273 nm

IR spectrum of the compound BF (in $CHCl_3$)

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}, 1525 cm^{-1}, 1470 cm^{-1}, 1375 cm^{-1}
1265 cm^{-1}, 1185 cm^{-1}, 1040 cm^{-1}, 970 cm^{-1} and 865 cm^{-1}.

Methiodide of the compound BF - The compound (15 mg) was taken in ethanol (2 ml) and methyl iodide (1 ml) was added. It was then refluxed on water bath for an hour and was kept over-night in a refrigerator. The crystalline derivative was filtered, recrystallised with ethanol, and dried at 80°C in vacuum, m.p. 211°C-212°C.

Picrate of the compound BF - The compound (20 mg) was dissolved in ethanol and added to an alcoholic solution of picric acid. The mixture was heated on water bath and cooled. A reddish yellow coloured crystalline compound separated out. The picrate was filtered and crystallised with ethanol, m.p. 119°C (decomposition).

Thin layer chromatography of compound BG

Two activated neutral alumina plates were spotted with a chloroform solution of the compound and the plates were developed in a tank saturated with chloroform: methanol (98:2).

One of the developed plates was air-dried and sprayed with Dragendorff's reagent. A single pink coloured spot was observed (Rf. 0.52). The second developed plate was sprayed with concentrated sulphuric acid and heated at 120°C for 15 minutes. A single black colour spot developed (Rf 0.52).

Solubility of the compound BG - It is soluble in ethanol and chloroform, sparingly soluble in benzene and ether, and is insoluble in water.

Methiodide of the compound BG - Methiodide was prepared as in the case of compound BF, m.p. 216°-217°C (decomposition).

Picrate of the compound BG - The picrate prepared by the usual method showed, m.p. 213°-214°C.

UV spectrum of the compound (in methanol)

The compound gave the following absorption bands:

λ_{max} 225 nm

λ_{max} 290 nm

λ_{max} 275 nm (shoulder)

IR spectrum of compound BG

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

3645 cm^{-1}, 3525 cm^{-1}, 3400 cm^{-1}, 2925 cm^{-1}, 2960 cm^{-1}
1722 cm^{-1}, 1625 cm^{-1}, 1510 cm^{-1}, 1320 cm^{-1}, 1310 cm^{-1},
1170 cm^{-1}, 1025 cm^{-1}, 1100 cm^{-1}, 970 cm^{-1} and 820 cm^{-1}.

Treatment of the ethyl acetate extract

The ethyl acetate extract was concentrated under reduced pressure and was kept in a refrigerator. After 24 hours

a crystalline compound started separating out. It was then left for 10 days and filtered. The residue was recrystallised twice with ethanol, to give a white crystalline compound BH (0.35 g), m.p. 289°C-290°C (decomposition) $\left[\alpha\right]_D$ - 40° (pyridine).

Thin layer chromatography of the compound BH

Activated plate of silica gel G was spotted with ethanol solution of the compound and developed in a tank saturated with chloroform:methanol (95:5). 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 BH

Found	Calculated for $C_{35}H_{60}O_6$
C = 73.12 %	C = 72.91 %
H = 10.50 %	H = 10.41 %

Reaction of the compound BH

(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 BH (in KBr)

Peaks cm^{-1}	Assignment
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, 1250	gem-dimethyl
1150-1015	various C-O-C
800-825	

Acetylation of the compound BH - 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 10 hours. The reaction mixture was then left overnight 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 (Rf 0.65).

Elemental analysis of the acetate

Found	Calculated for $C_{43}H_{68}O_{10}$
C = 69.45 %	C = 69.35 %
H = 9.15 %	H = 9.27 %

Determination of the acetyl percentage

The acetyl percentage in the acetyl derivative of the compound was determined by the method of Weisenberger as described by Belcher and Codbert.

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 BH (in CCl_4)

The observed peaks and their assignments are as follows:

Peaks cm^{-1}	Assignment
2930	C-H stretching
2854	C-H stretching
1762	Acetate-CO
1464	C-H bending
1365	gem-dimethyl
1375	
1248	$O-COCH_3$
1215	C-O-C stretching
1025	

NMR spectrum of the compound BH

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

Hydrolysis of the compound BH and identification of sugars

The compound (0.1 g) and 5% aqueous 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 (Na_2SO_4). The solvent was distilled off. The residue thus obtained was crystallised with absolute alcohol, m.p. 136°-137°C, $[\alpha]_D$ -35° ($CHCl_3$).

The compound was readily soluble in chloroform, benzene, solvent ether and hexane but sparingly soluble in hexane and ethanol. The aqueous solution left after extraction with solvent ether was neutralised with barium carbonate and filtered. The filtrate 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 (Rf 0.17). This indicated the presence of only one sugar which from its Rf value appeared to be glucose. This was confirmed by Co-chromatography with an authentic

sample of D(+)-glucose. The identity of the sugar was further confirmed by preparation of osazone; m.p. 199°-200°C.

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, a single black spot developed (Rf 0.44).

Elemental analysis of aglycone

Found	Calculated for $C_{29}H_{50}O$
C = 84.54 %	C = 84.05 %
H = 11.60 %	H = 12.07 %

Reactions of the aglycon

(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 some time.

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 = 81.21%	C = 81.57%
H = 11.72%	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
950	

Detection of sugars in B.diffusa

The alcoholic extract of B. diffusa 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 sugar. Paper chromatography of the sugar mixture showed the presence of three sugars. These sugars were identified by co-paper chromatography (descending) on whatman paper 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 (Rf 0.18) fructose (Rf 0.24) and sucrose (Rf 0.12).

Quantitative estimation of sugars

Air-dried and powdered roots (100 g) of B.diffusa were extracted with 75% ethanol in a soxhlet apparatus for 48 hours. Alcohol 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^{++} was 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 Whatman No.1 chromatographic paper. Two sheets (8" x 18") were taken, one sheet was used as blank. A base line was drawn. The paper was spotted along the base line at five places each with 10 λ sugar solution. Chromatograms were developed in a chamber saturated with ethyl-acetate: 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 three spots, and the sections were cut out. Corresponding blanks were cut from the blank paper. The strips containing glucose, fructose, sucrose 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 the 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 spectrophotometer. The same procedure was followed with blank.

A standard absorption curve between micrograms of sugar and corresponding absorbance was plotted for glucose, fructose, and sucrose. A straight line was obtained in each case. The amount of sugar was then determined by referring to the standard curve and thus the percentage of glucose, fructose and sucrose was calculated in the roots of B. diffusa. The procedure was repeated thrice under identical conditions. The percentage of glucose, fructose and sucrose 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 = 1.66%

Fructose = 2.87%

Sucrose = 1.29%

-x-

CHAPTER III

ISOLATION AND STUDY OF HENTRIACONTANE, HENTRIACONTOL, NONACOS-1-ene-4-one AND MIXTURE OF β-SITOSTEROL-D(+)-GLUCOSIDE AND STIGMASTEROL-D(+)-GLUCOSIDE FROM THE STEMS AND ROOTS OF TRIANTHEMA PETANDRA (SYN-ZALEYA GOVINDIA) N.C. NAIR

Trianthema Pentandra roots and stems were extracted with petroleum ether and the neutral fraction of the extract was chromatographed on a neutral alumina column. Elution of the column with petroleum ether, petroleum ether: benzene (9:1), and with pure benzene gave compounds TA, m.p. 67°C, TB, m.p. 75°C, and TC m.p. 86°C successively.

Examination of TA, TB, and TC on tlc plates showed them to be homogeneous.

Study of compound TA

The elemental analysis of the compound TA corresponds to the molecular formula $C_{31}H_{64}$. This molecular formula is supported by the molecular ion peak m/e 436 in the mass spectrum. Spectrum showed no $(M-15)^+$ peak but an intense molecular ion peak and fragments of 14 mass units were observed. This shows that compound is a straight chain aliphatic hydro-carbon[67].

The IR spectrum of the compound showed characteristic bands at 725 cm^{-1} and 714 cm^{-1} indicating a long n-alkane chain[68,69]. NMR spectrum of the compound gave only two signals, one at 9.12 τ assigned to methyl protons and another strong signal at 8.75 τ assigned to methylene protons. This further indicates that the compound is a straight chain saturated aliphatic

hydrocarbon[71]. The hydrocarbon on the basis of the above observations has been identified as hentriacontane and can be represented as

$$CH_3 \cdot CH_2(CH_2)_{27}CH_2 \cdot CH_3$$

TA

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

Study of compound TB

The elemental analysis of the compound corresponds to the molecular formula $C_{29}H_{56}O$. This molecular formula is supported by the molecular ion peak m/e 420 in mass spectrum. The compound gave yellow colouration with tetranitromethane indicating its unsaturated character[73]. It gave negative test for steroids and triterpens.

The IR spectrum of the compound (in KBr) showed a strong peak at 1733 cm^{-1} indicating that compound has a carbonyl function. The peak at 1685 cm^{-1} indicates C=C and that at 722 cm^{-1} and 715 cm^{-1} indicates a n-alkane chain. The compound TB appears to be a unsaturated aliphatic long chain ketone.

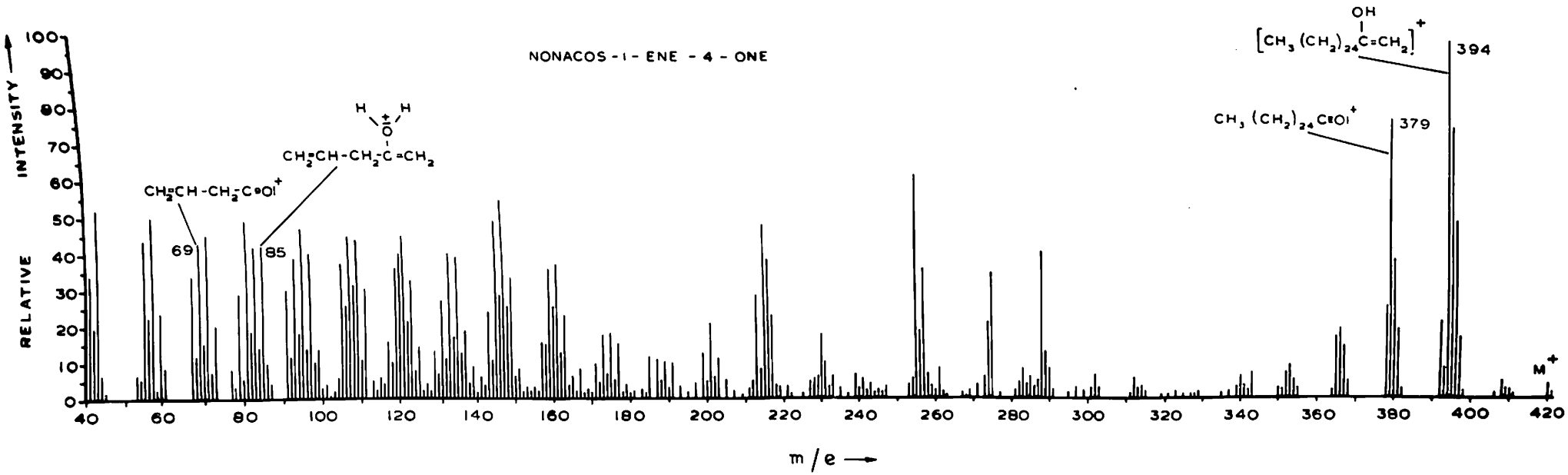
With 2:4 dinitrophenylhdrazine the compound formed an orange yellow crystalline hydrazone, m.p. 89°-90°C.

Lithium aluminium hydride reduction of TB in dry ether gave a compound of m.p. 85°-87°C. In IR spectrum of the reduction product, the peak at 1735 cm^{-1} had disappeared and a new peak at 3625 cm^{-1} had appeared, indicating that the carbonyl group has been reduced to an alcoholic group.

Peaks at 3625 cm^{-1}, 1095 cm^{-1}, 1060 cm^{-1}, 1015 cm^{-1}, characteristic of secondary alcoholic group[109], indicate that the carbonyl function in TB is a ketonic group. The peak at 1665 cm^{-1} characteristic of C=C is also present in the IR spectrum of the reduction product.

The alcohol on acetylation gave an acetyl derivative, m.p. 65°C. The IR spectrum of the acetyl-derivative showed a peak at 1735 cm^{-1} (acetate CO) and a peak at 1252 cm^{-1} ($O-COCH_3$). The peak at 3625 cm^{-1} had disappeared. The peak at 1685 cm^{-1} is assigned to C=C. The acetyl derivative also gave a yellow colouration with tetranitromethane[73].

On the basis of the above observations it appears that compound TB is an unsaturated aliphatic long chain ketone. The UV spectrum of TB in hexane showed absorp-



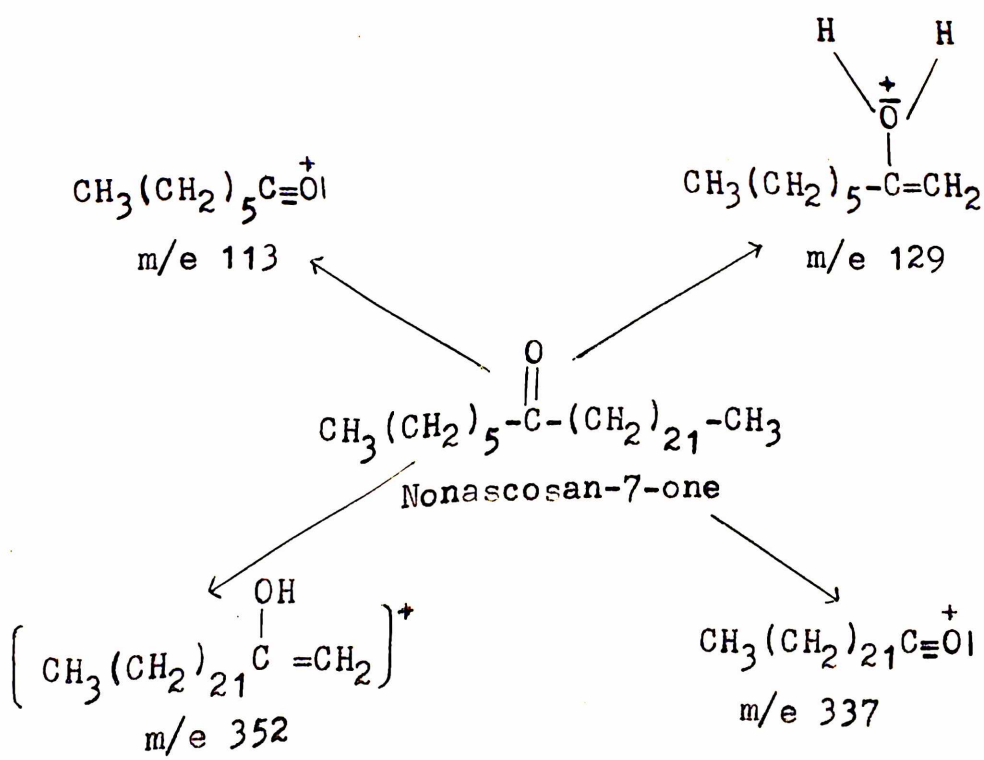
MASS SPECTRUM OF NONACOS-1-ENE-4-ONE OCCURRING IN TRIANTHEMA PENTANDRA

tion maxima at 227 nm which rules out the possibility of the double bond being in conjugation with the keto group.

Mass spectral study of ketone TB

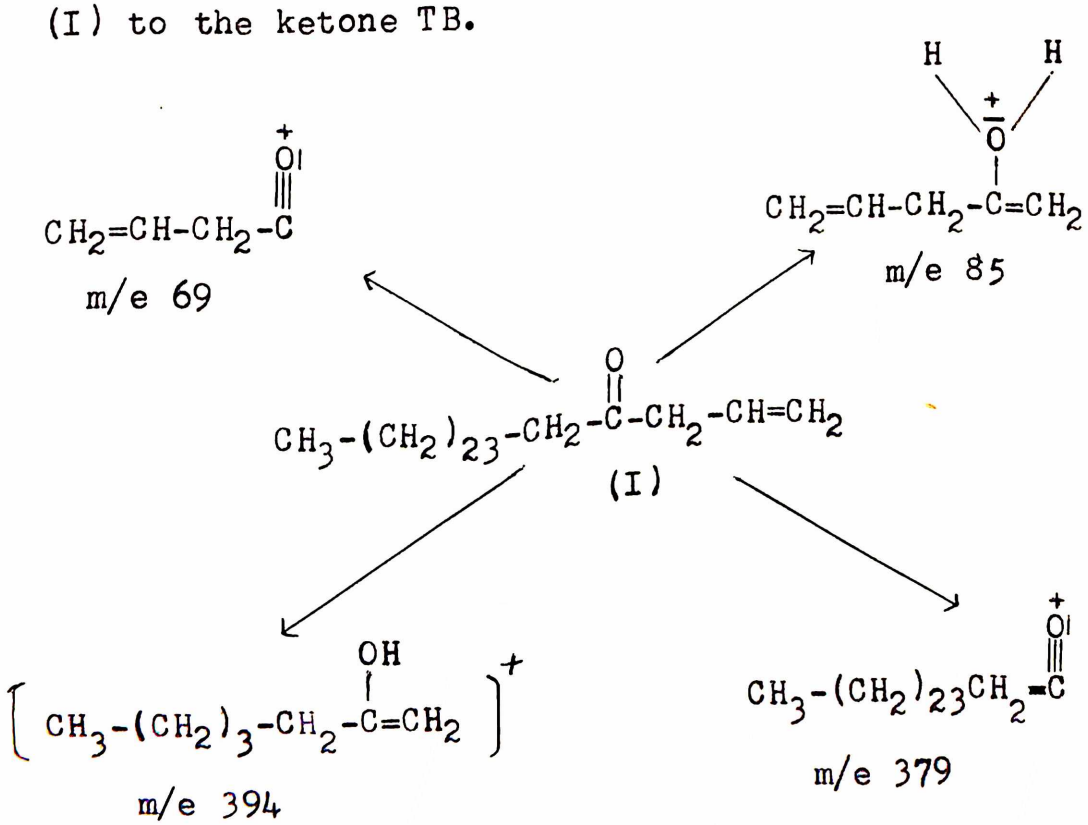
Ketones are known to undergo frequent rearrangements in mass spectrum through a six membered ring intermediate, followed by fragmentation with retention of the positive charge on the oxygen containing fragments.

V. Wollrab studied the mass spectrum of a mixture of nonacosan-7-one and nonacosan-10-one, derived from the secondary alcohols occurring in wax of flower Rosa damascena[109]. Fragmentation pattern of nonacosan-7-one as observed by V. Wollrab in mass spectrum is as follows:



In the mass spectrum of the ketone TB, no significant $(M-15)^+$ peak was observed and the ratio $(M-15)^+/M^+$ may be considered as close to zero. This indicates that ketone TB has a straight chain[110]. Mass spectrum showed $(M+1)^+$ peak characteristic of asymmetrical ketones[111]. The ratio of M^+ to $(M+1)^+$ is two.

The most intense peak obtained in the spectrum of TB is at m/e 394. The next most intense peak is at m/e 379. Other significant peaks are at m/e 85 and m/e 69. These mass fragments can be explained on the basis of the fragmentation pattern given by V. Wollrab for Nonacosan-7-one, by assigning the following structure (I) to the ketone TB.



NMR study of ketone TB

The proposed structure for the ketone TB is further supported by the Nuclear Magnetic Resonance spectrum of the compound. This spectrum shows signal at 9.12 τ, assigned to three methyl protons and a strong signal at 8.72 τ due to methylene protons. A multiplet centred at 7.60 τ is assigned to methylenic protons next to the carbonyl function. Signal at 5.80 τ is assigned to the olefinic protons in the molecule. On the basis of spectral datas and chemical studies the following structure is therefore proposed for the ketone TB

$$CH_3-(CH_2)_{23}-CH_2-\overset{\overset{O}{\|}}{C}-CH_2-CH=CH_2$$

TB

It should be noted that the mass spectra of C_{29} ketones have been studied for the first time in 1968, Laseter et al[112] used mass spectral data for establishing the structure of 15-nonacosane. Later V. Wollrab[109] studied mixture of ketones derived from secondary alcohols occurring in wax of flower of Rosa damacena. B. Stoianova-Ivanova and K. Mladenova[113] have identified mixture of ketones (C_{26} to C_{30}) in Rose bud and Rose flower waxes.

Saturated C_{29} ketones are known to occur in

plants[110,112,113]. But as far as our knowledge goes, it is for the first time that the occurrence of an unsaturated C_{29} ketone with a terminal double bond and a carbonyl group at C_4 is being reported.

Study of compound TC

The elemental analysis of the compound TC corresponds to molecular formula $C_{31}H_{62}O$. The compound gave negative tetranitromethane test showing this to be a saturated compound. The compound TC gave negative test for steroids and terpens.

IR spectrum of the compound TC in KBr showed 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 was further supported by the triplet at 6.34 τ in NMR spectrum, which also had 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 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 showed 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 TC has been identified as Hentriancontal. 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. Hentriacontol can be written as

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

TC

The occurrence of a C_{31} hydrocarbon (Hentriacontane), a C_{31} alcohol (Hentriacontol), and a C_{29} ketone (noncos-1-ene-4-one) in the wax of the plant T.pentandra is of much significant biogenetically.

Treatment of the alcoholic extract of T. pentandra

The residue from the alcoholic extract of the defatted roots and stems of T. pentandra was extracted with solvent ether. The ether was completely evaporated under reduced pressure. The residue upon repeated crystallisations with chloroform:ethanol (1:1) and with absolute alcohol gave a white crystalline compound TD (150 mg), m.p. 302°-303°C (decomposition), $[\alpha]_D$ - 38° (pyridine). Homogeneity of the compound was established by thin layer chromatography on silica gel G plate.

The elemental analysis of the compound TD corresponds to the formula $C_{35}H_{60}O_6$. The compound gave yellow colouration with tetranitromethane indicating unsaturation in the compound. In Liebermann-Burchard test the compound first gave a pink colour which changed to green immediately. Compound gave red colour in Salkowaski test. The alcoholic solution of the compound did not give precipitate with digitonin. The compound gave positive Molish's test for sugar but did not give the characteristic colour with aniline phthalate reagent.

On the basis of the elemental analysis and colour reactions, the compound TD under study appears to be a phytosterolin in which the reducing group of the sugar is involved in the glycosidic linkage.

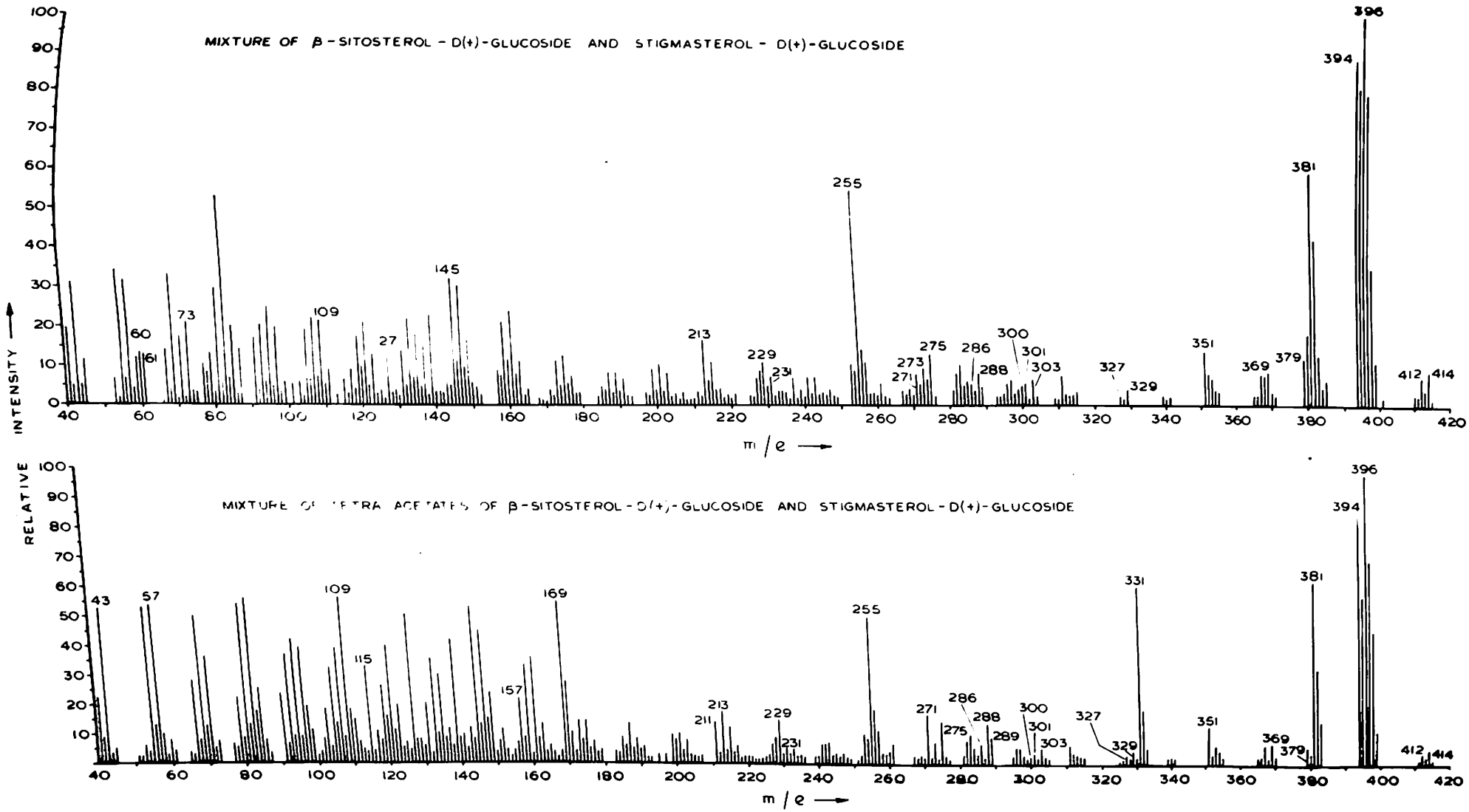
IR spectrum of the compound showed strong broad absorption at 3400-3425 cm^{-1} indicating the hydroxyl functions in the compound. Absorption bands between 1150 cm^{-1} and 1010 cm^{-1} indicated various C-O-C groups. Peak at 1650 cm^{-1} indicates the presence of C=C in the compound. Peaks at 1362 cm^{-1} and 1375 cm^{-1} indicated the presence of gem-dimethyl group.

The phytosterolin on acetylation with a mixture of acetic anhydride and pyridine gave an acetate, m.p.

157°C , $[\alpha]_D$ - 30° (chloroform).

The determination of acetyl group percentage by the method of Wiesenberger as described by Belcher[106], indicates the presence of four hydroxyl groups in the compound. IR spectrum of the acetate showed peaks at 1765 cm^{-1} (acetate CO) and 1222 cm^{-1} ($O-COCH_3$).

Nuclear Magnetic Resonance spectrum of the tetra-acetate showed strong triplet at 7.98 τ assigned to the protons $CH_3-\underset{\underset{O}{\|}}{C}-$(one signal superimposed with the other). Signals at 9.28 τ, 9.12 τ, 9.19 τ and 9.14 τ are assigned to the methyl protons of the steroid part of phytosterolin. Signal at 5.81 τ is assigned to the protons of CH_2CAC. A deformed multiplet envelop between 4.98 τ to 4.78 τ and between 8.94 τ to 7.66 τ is assigned to the protons of CHOAC and methylene protons of steroid ring and side chain respectively. A deformed envelop between 4.50 τ to 4.75 τ is assigned to the ethylenic protons.



MASS SPECTRA OF GLUCOSIDES OCCURRING IN TRIANTHEMA PENTANDRA AND THEIR TETRA ACETATES

Mass spectral studies of phytosterolin

Phytosterolins and their tetracetates are unstable when run in a mass spectrophotometer even using direct inlet probe. Towards the end of the spectrum of the phytosterolins an intense peak at m/e 396 was observed. As discussed in Chapter II the peak at m/e 396 may arise from the fragment obtained after elimination of the sugar from β-sitosterol-D(+)-glycoside. Peaks at m/e 397, 398, 414, 381, 329, 303, 288, 275, 273, 255, 231, 229, 213 and in lower region of mass spectrum peaks at m/e 145, 127, 109, 73, 61, and 60, were also present in the mass spectrum. These mass peaks are identical with the mass peaks in the spectrum of β-sitosterol-D(+)-glucoside obtained from B. diffusa (Chapter II, plate 1).

Besides those peaks the mass spectrum also has the following set of peaks at m/e 394, 395, 412, 379, 369, 351, 327, 301, 300, 286, 273, 227.

These peaks can be explained if we assume that phytosterolin under study is a mixture of β-sitosterol -D(+)-glucoside and stigmasterol-D(+)-glucoside. Mass peak at m/e 300 is the characteristic peak of steroids having double bond between C_{22} and C_{23} [114,115].

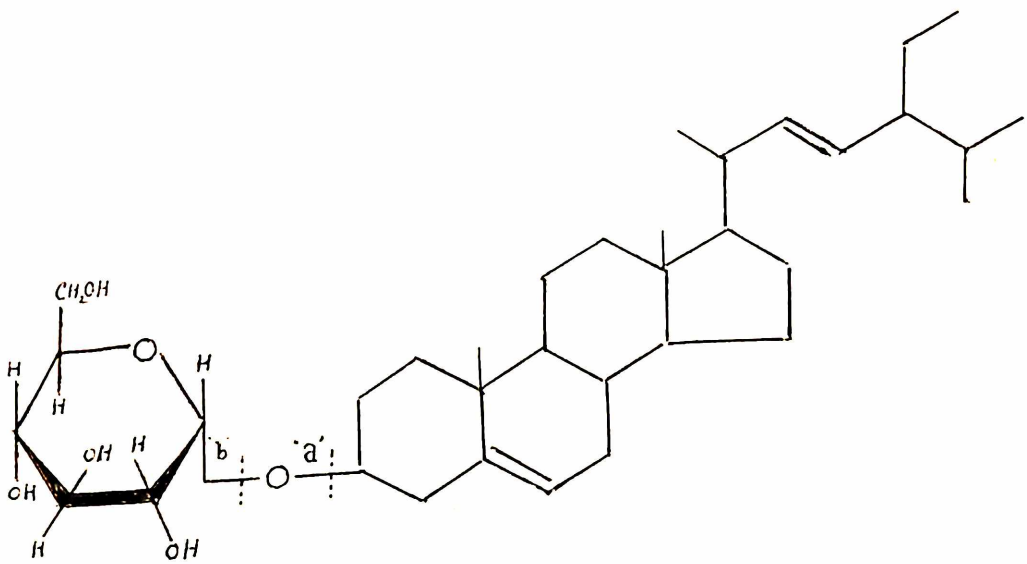
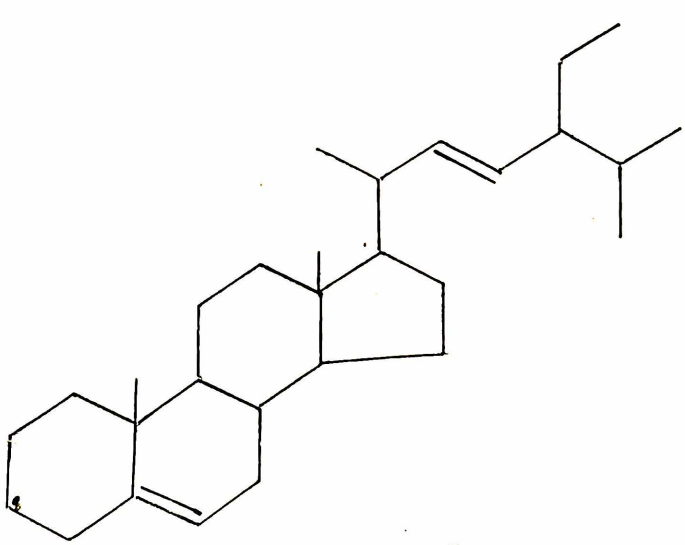


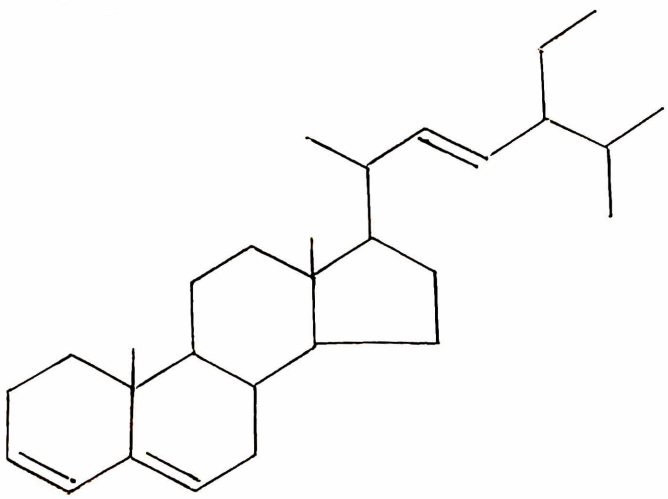
Fig.II. Stigmasterol-D(+)-glucoside

Peak at m/e 395 may arise by the cleavage of carbon oxygen bond at 'a' (Fig. II)



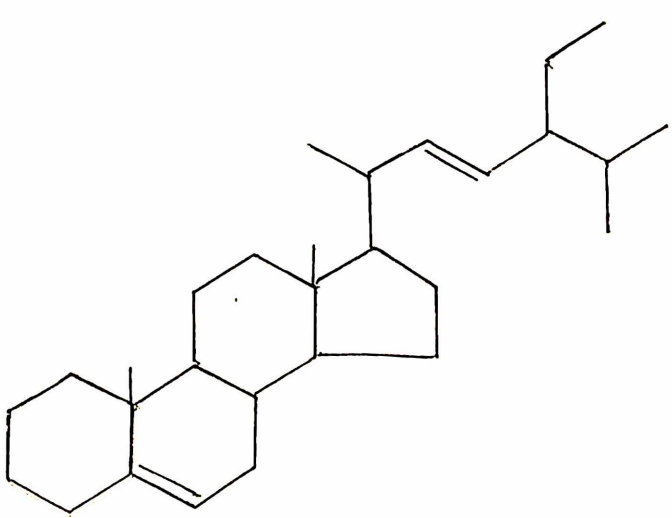
m/e 395

Peak at m/e 394 corresponds to the fragment obtained after elimination of sugar moiety (Fig.II).



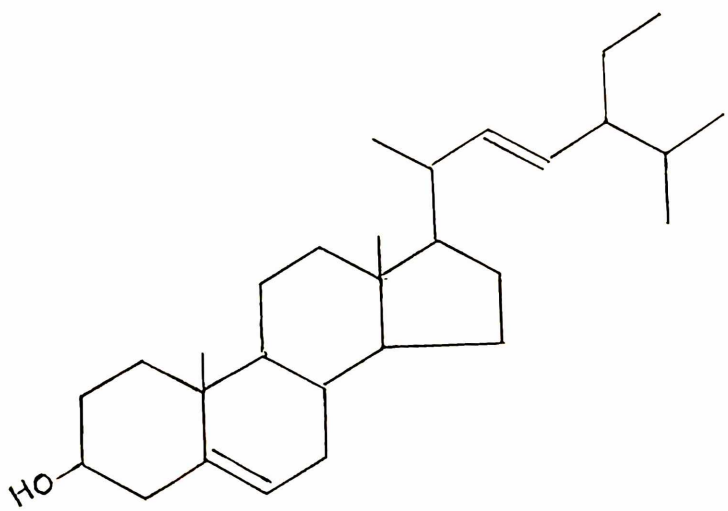
m/e 394

A peak at m/e 396 is also possible which may arise by the cleavage of carbon oxygen bond at 'a' (Fig.II) and a hydrogen rearrangement.



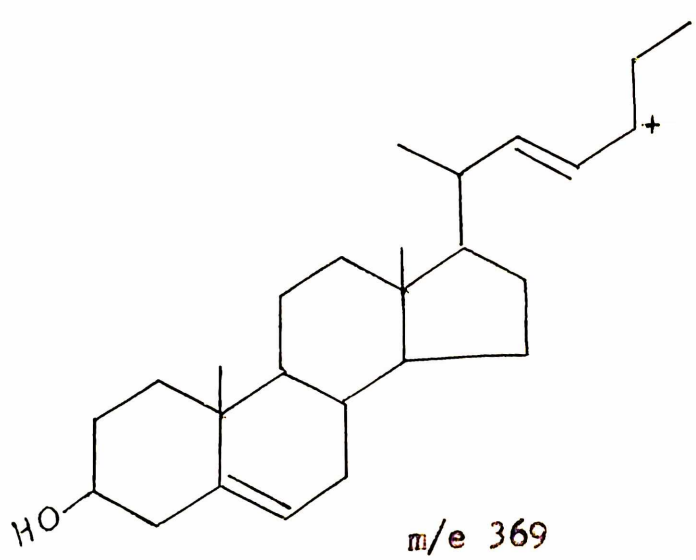
m/e 396

Peak at m/e 412 which corresponds to the mass of stigmasterol may arise by the cleavage of carbon-oxygen bond at 'a' and a hydrogen rearrangement.



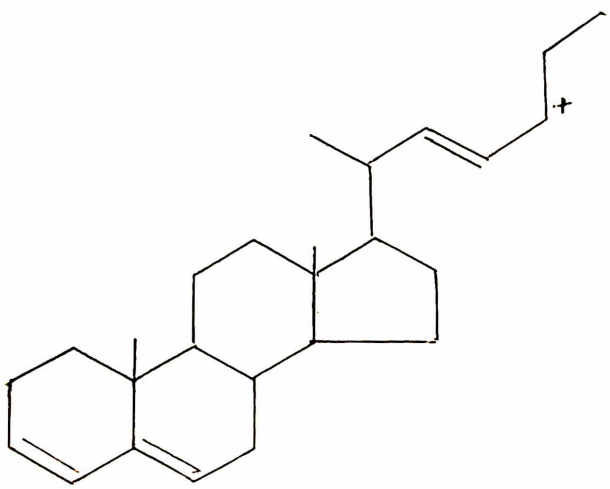
m/e 412

The other significant peaks in the mass spectrum are at m/e 369 and m/e 351. Peak at 369 may arise by the elimination of isopropyl terminal group from stigmasterol[116]



m/e 369

Peak at m/e 351 may arise by the loss of the terminal isopropyl group followed by a molecule of water from stigmasterol.



m/e 351

Peaks at m/e 327, 301, 286, 273 are expected fragments from stigmasterol indicating a double bond between C_5-C_6 [117].

Peak at 273 may arise by the elimination of the side chain and a loss of molecule of water followed by loss of two hydrogen also from the tetracyclic fragment.

Mass spectrum of tetra-acetate of phytosterol TD

Mass spectrum of tetraacetate shows expected peaks from mixture of β-sitosterol -D(+)-glucoside and stigmasterol-D(+)-glucoside

m/e 414, 399, 398, 397, 396, 381, 329, 303, 288, 275 and 273.

m/e 412, 397, 395, 394, 379, 327, 301, 300, 286 and 273.

Besides these peaks the spectrum shows a strong peak at m/e 331 which may arise by the cleavage of carbon-oxygen bond at 'b' (Fig. III).

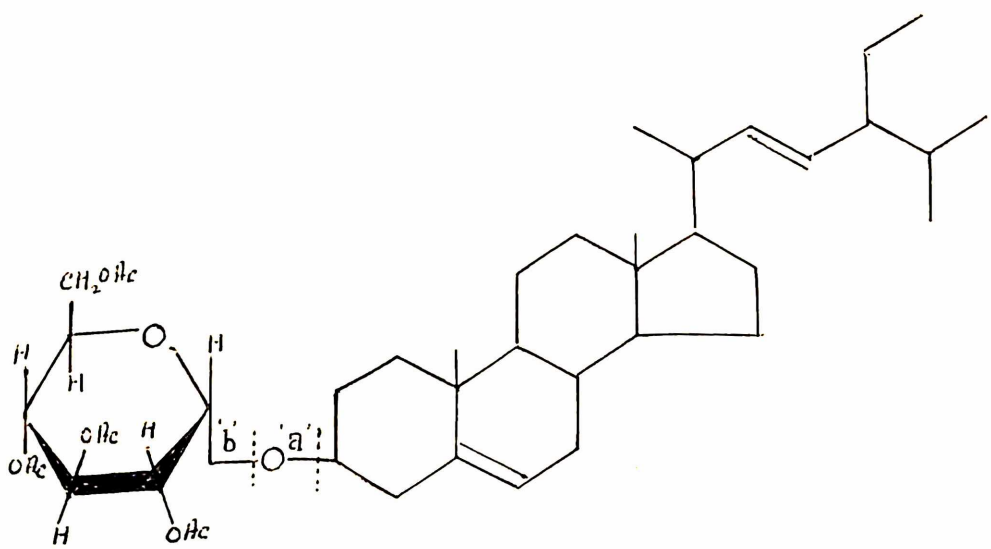
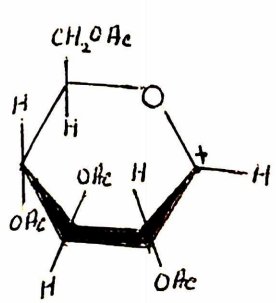


Fig. III



m/e 331

The spectrum also shows the following peaks at m/e 289, 271, 229, 187, 160, 157, 115, 109 and at m/e 57.

These fragments are identical for glucose tetra-acetate.

From the mass spectrum of phytosterolin it is established that phytosterolin under study is a mixture of β-sitosterol-D(+)-glucoside and stigmasterol-D(+)-glucoside.

-x-

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

Air-dried and powdered stems and roots of Trianthema pentandra (4.5 Kg), collected locally, were extracted in a soxhlet apparatus with petroleum ether (b.p. 60°-80°C) for 76 hours. Solvent was distilled under reduced pressure, and the dark green residue so obtained was dissolved in hot methanol-acetone (1:1) mixture and kept in a refrigerator, the greenish 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 the previously obtained solid. The process was repeated thrice. The solid material so obtained was collected and the filterate which contains mainly chlorophyll was discarded.

The greenish solid (19.15 g) was dissolved in solvent ether and separated into acidic, basic and neutral fractions. Acidic and basic fraction gave negligible residues which were discarded. The neutral, ether soluble fraction was washed thoroughly with water, dried (Na_2SO_4) and evaporated to dryness to give a solid (18.50 g). Thin layer chromatography of this crude solid showed four main spots in different solvent

systems. Plates were sprayed with concentrated sulphuric acid and heated for 15 minutes at 120°C.

Treatment of neutral material

The solid (14 g) was dissolved in minimum quantity of chloroform and was adsorbed on 50 g of neutral alumina. It was then placed on the top of a chromatographic column (36" x 3"), packed previously with 400 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 reduced pressure. These were divided into three portions designated alphabetically A, B, and C on the basis of tlc pattern. Each portion was rechromatographed on neutral alumina column of Brockmann activity 1.

Chromatography of portion A

Portion A (5.8 g) was chromatographed on neutral alumina column using petroleum ether for elution and finally the column was washed with benzene. 25 ml fractions were collected. First two fractions were discarded, fraction 3 to 8 were combined on tlc pattern. The residue on repeated crystallisations with hexane gave white shining flakes TA (4.2 g), m.p. 67°C.

Chromatography of portion B

Portion B (2.5 g) was adsorbed on 4 g of neutral alumina and placed on the top of a column (30" x 1") packed previously with neutral alumina (80 g). The column was washed with petroleum ether (100 ml) and the washings were discarded. It was then eluted with petroleum ether:benzene (9:1) mixture. 25 ml fractions were collected. Fraction 5 to 12 were combined on tlc pattern. The combined elute was evaporated to dryness under vacuum and crystallised twice with hexane to give a white crystalline compound TB (1.02 g), m.p. 75°C. Later fractions of petroleum ether:benzene (9:1) 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 (4.62 g) was adsorbed on 10 g of neutral alumina and placed on top of a column (30" x 1"), packed previously with neutral alumina (90 g). The column was washed with a mixture of 100 ml of petroleum ether: benzene (1:1), and the washings were discarded. Elutes from benzene were combined on tlc pattern. Finally the column was eluted with chloroform. The elutes from chloroform gave no worthwhile compound. Combined elutes

of benzene were evaporated under reduced pressure and crystallised with chloroform several times to give a white crystalline compound TC (2.25 g), m.p. 86°C.

Thin layer chromatography of compound TA

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

Elemental analysis of the compound TA

Found	Calculated for $C_{31}H_{64}$
C = 84.62%	C = 84.32%
H = 14.42%	H = 14.67%

Solubility of the compound TA

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

IRspectrum of compound TA (in KBr)

The observed peaks and their assignments are as follows:

Peaks cm^{-1}	Assignment
2920	C-H stretching
2852	C-H stretching
1460	C-H bending
1375	C-H bending
725 714	n-alkane chain

Nuclear magnetic resonance spectrum of the compound TA (in CCl_4)

Signals τ	Assignment
9.12	Methyl protons
8.75	Methylene protons

Thin layer chromatography of compound TB

The activated silica gel G plate was spotted with chloroform solution of the compound and developed in a tank saturated with petroleum ether:benzene (3:1). The developed plate was air-dried and sprayed with concentrated sulphuric acid. The chromatogram on heating at 120^{o} for 15 minutes developed a single black spot (Rf 0.77).

Elemental analysis of the compound TB

Found	Calculated for $C_{29}H_{56}O$
C = 82.45%	C = 82.85%
H = 13.34%	H = 13.57%

Solubility of the compound TB

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

IR spectrum of the compound TB

The observed peaks and their assignments are as follows:

Peaks cm^{-1}	Assignment
2915	C-H stretching
2855	C-H stretching
1733	CO stretching
1685	C=C
1460	C-H bending of CH_3 and CH_2
1470	in plane bending of $=CH_2$
1408	$-CH_2-CO-$
1375	C-H bending of CH3
1200	In plane bending of =CHR
1182	C-O stretching
1170	C-O stretching
722 715	n-alkane chain

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.

Preparation of 2:4 dinitrophenylhydrazone of compound TB

A solution of 2:4 dinitrophenylhydrazine was prepared in ethanol with a few drops of concentrated sulphuric acid. The compound TB (30 mg) was dissolved in ethanol (10 ml). The two solutions were heated separately on water bath. A hot solution of 2:4 dinitrophenylhydrazine reagent was added to the hot solution of the compound. The mixture was refluxed for 30 minutes. On cooling orange-yellow amorphous hydrazone separated out. The hydrazone (35 mg) was crystallised with absolute alcohol gave a yellow-orange coloured crystalline compound, m.p. 89°-90°C.

Reduction of the ketone TB with lithium aluminium hydride

The solution of the compound (60 mg) in dry ether was added dropwise to the suspension of Lithium aluminium hydride. When the addition was complete the mixture was stirred for another 4 hours. The excess of Lithium aluminium hydride was destroyed by adding water drop by drop. The clear etherial solution was decanted from the slurry. The slurry was washed twice with ether. The combined ether solution was washed with water, dried (Na_2SO_4) and the ether evaporated. The solid obtained was crystallised twice with chloroform:methanol (1:1) to give a white crystalline compound (43 mg), m.p. 86°-87°C.

Thin layer chromatography of reduction product of ketone TB

Activated silica gel plate was spotted with a chloroform solution of the compound and was developed in a tank saturated with benzene. Air-dried plate was sprayed with concentrated sulphuric acid and heated at 120°C for 15 minutes a single black colour spot developed (Rf = 0.40).

IR spectrum of the reduction product of ketone TB (in $CHCl_3$)

The observed peaks and their assignments are as follows:

Peaks cm^{-1}	Assignment (Szymanski)[118]
3625	OH stretching
2945	C-H stretching
2850	C-H stretching
1685	C=C
1465	Asymmetric bending of CH_3 and symmetric bending of CH_2
1450	In plane bending of $=CH_2$
1365	C-H bending of CH_3
1258	In plane bending O-H
1200	In plane bending of = CHR
1095	C-O stretching
1060	C-O stretching
1015	C-O stretching

Alcohol gave yellow colouration with tetranitromethane.

Acetylation of reduction product of TB

The compound (25 mg) was acetylated with a mixture of acetic anhydride (2 ml) and pyridine (0.5 ml). The reaction mixture was poured on crushed ice. The compound that separated out was extracted with ether (4 x 10 ml). The combined ether extracts were washed with water, dried (Na_2SO_4), and ether evaporated off. The solid on crystallisation with ethanol gave a white crystalline compound (20 mg), m.p. 65°C.

IR spectrum of above acetyl derivative (in CCl_4)

The observed peaks and their assignments are as follows:

Peaks cm^{-1}	Assignment
2925	C-H stretching
2852	C-H stretching
1735	Acetate (CO)
1680	C=C
1465	Asymmetric bending of CH_3 and symmetric bending of $-CH_2$
1362	C-H stretching of $-CH_2$
1252	$O-COCH_3$
1200	In plane bending of =CHR
1025	C-O stretching

Nuclear magnetic resonance spectrum of the ketone TB

Signals τ	Assignment
9.12	Methyl protons
8.72	Methylene protons
7.60 (multiplet)	$-CH_2-\overset{O}{\overset{\|\|}{C}}-CH-$
5.80	Olefinic protons

Thin layer chromatography of compound TC

Activated silica gel G plate was spotted with a chloroform solution of the compound and developed in a tank of hexane:chloroform (1:1) mixture. 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 (Rf 0.42).

Elemental analysis of the compound TC

Found	Calculated for $C_{31}H_{64}O$
C = 82.12%	C = 82.30%
H = 14.34%	H = 14.15%

Solubility of the compound TC

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

IR spectrum of the compound TC (in KBr)

The observed peaks and their assignments are as follows:

Peaks cm^{-1}	Assignment
3425	OH stretching
2925	C-H stretching
2852	C-H stretching
1472	C-H bending
1460	C-H bending
1370	C-H bending
1050	C-O stretching
725 715	n-alkane chain

Nuclear magnetic resonance spectrum of compound TC

Signals τ	Assignment
9.22	Methyl protons
8.68	Methylene protons
6.34 (triplet)	Proton of -OH group attached to a methylene group

Acetylation of the compound TC

The compound (100 mg) was acetylated with a mixture of acetic anhydride (10 ml) and pyridine (2 ml) and on usual treatment gave the acetyl derivative which on crystallisation with ethanol gave a white crystalline compound (80 mg), m.p. 76°C.

Thin layer chromatography of acetyl derivative of TC

Activated silica gel G plate was spotted with a chloroform solution of the compound and was developed in a tank saturated with petroleum ether:benzene (3:1). The developed plate was air-dried and sprayed with concentrated sulphuric acid. The chromatogram on heating at 120° for 15 minutes gave a single black spot (Rf = 0.41).

Elemental analysis of the acetyl derivative of TC

Found	Calculated for $C_{33}H_{66}O_2$
C = 79.89%	C = 80.16%
H = 13.53%	H = 13.36%

IR spectrum of acetyl derivative of TC (in CCl_4)

The observed peaks and their assignments are as follows:

Peaks cm^{-1}	Assignment
2925	C-H stretching
2853	C-H stretching
1742	Acetate CO
1465	C-H bending
1362	C-H bending
1235	O-$COCH_3$
1020	C-O stretching

Treatment of the alcoholic extract of T. pentandra

The roots and the stems of T. pentandra (4.5 Kg) after extraction with petroelum ether (40-60°C) were further extracted with ethanol in a soxhlet extractor for 48 hours. The ethanolic extract was concentrated under reduced pressure and 80 ml of distilled water was added to the residue. It was then subjected to liquid-liquid extraction with solvent ether. The ethanol solution was washed with little water, dried (Na_2SO_4), and evaporated to give a brown coloured solid. Upon repeated crystallisations (10 times) with absolute alcohol and with mixture of chloroform and absolute alcohol, the solid gave a white crystalline compound TD (150 mg), m.p. 302°-303°C (decomposition), $\left[\alpha\right]_D$ - 38° (pyridine).

Thin layer chromatography of compound TD

Activated silica gel G plate was spotted with ethanolic solution of the compound and the plate was developed in a tank saturated with ethylacetate:methanol (98:2). The air-dried plate was sprayed with concentrated sulphuric acid when a single pink colour spot developed. When the plate was heated at 120° for 15 minutes, the pink coloured spot turned black. No other spot was present.

Solubility of the compound TD

The compound is soluble in hot alcohol and ethyl acetate, sparingly soluble in chloroform and benzene. It is insoluble in water.

Reaction of the compound TD

1. Liebermann-Burchard reaction - The compound was taken in a few drops of acetic acid and 2 ml of acetic anhydride. To this were added a few drops of concentrated sulphuric acid. A pink colour developed which immediately changed to bluish green.

2. Salkowski reaction - When a drop of concentrated sulphuric acid was added to the compound in chloroform, a yellow colour developed which changed to red after some time.

3. 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.

4. Ethanolic solution of the compound did not give precipitate with an ethanolic solution of digitonin.

IR spectrum of the compound TD (in KBr)

The observed peaks and their assignments are as follows:

Peaks cm^{-1}	Assignment
3400-3425	O-H stretching
2930	C-H stretching
2848	C-H stretching
1454	C-H bending
1650	C=C stretching
1375 1362	gem-dimethyl
1152-1115	various C-O-C stretching
825-830	

Acetylation of the compound TD

The compound (50 mg) was acetylated with a mixture of acetic anhydride (5 ml) and pyridine (1 ml). Upon usual treatment the mixture gave acetyl derivative, m.p. 157°C, $\left[\alpha\right]_D$ - 30° (chloroform).

Thin layer chromatography of acetyl derivative of TD

Activated silica gel G plate was spotted with a chloroform solution of the compound. The plate was developed in a tank saturated with ethyl acetate: hexane (4:1). The air-dried plate was sprayed with concentrated sulphuric acid and the plate was heated at 120°C for 15 minutes, when a single black spot developed (Rf 0.58).

IR spectrum of the acetyl derivative of compound TD (in CCl_4)

The observed peaks and their assignments are as follows:

Peaks cm^{-1}:	Assignment
2925	C-H stretching
2852	C-H stretching
1765	Acetate CO
1465	C-H bending
1370, 1365	gem-dimethyl
1248	$O-CO-CH_3$
1222	$O-COCH_3$
1155	various C-O-C
1030	

NMR spectrum of the compound TD

Signals τ	Assignment
9.28	Methyl protons
9.12	Methyl protons
9.19	Methyl protons
9.14	Methyl protons
8.94 to 7.66	Methylene protons
7.98	Protons of $CH_3-\overset{\overset{O}{\|}}{C}-$
5.81	Protons of CH_2OAC
4.98 to 4.78	Protons of CHOAC
4.50 to 4.75	Ethylenic protons

REFERENCES

1. Gosselin, R.A., Lloydia, 25, 241 (1962).

2. De Laszlo, H.G., "Library of Medicinal Plants", W. Heffer and Sons Ltd., Cambridge, England (1958).

3. Dragendorff, G., "Die Heilplanzen der verschienden, Volker und Zeiten." Ferdinand Enke, Stuttgart, Germany (1898).

4. Biswas, K., "Common Medicinal Plants of Darjeeling" West Bengal Government Press, Alipore (1956).

5. Rao, R.S., Bull. Botan. Survey, India, 5, 165 (1963).

6. Kirtikar, K.R. and Basu, B.D., "Indian Medicinal Plants", 4, L.M. Basu, Allahabad (1933).

7. Rao, R.S., Pakistan J. Sci. Ind. Res., 4, 219 (1961).

8. Chopra, R.N., Nayer, S.L., and Chopra, I.C.,"Glossary of Indian Medicinal Plants", Council of Scientific and Industrial Research, New Delhi, India (1956).

9. Chopra, R.N. et al, "Indigenous Drugs of India", Dhur, Calcutta, India (1958).

10. Acharan, M.L., "La Medicine Natural al Alcance de Todas", Editorial Pax-Mexico, Carlos, Cesarman, Mexico, D.F., Mexico (1956).

11. Martinex, M., "Las Plants Medicinales de Mexico" 4th ed., Ediciones Botas. Mexico, D.F., Mexico (1959).

12. Rath, A., "Lecive Rostliny V domacin Uziti", Hejda and Zbroj, Mlade Boleslavi (1928).

13. Stopp, K., Econ. Botany, 17, 16 (1963).

14. Quisumbing, E., "Medicinal Plants of the Phillippines", Department of Agriculture and Natural Resources, Tech. Bull. 16, Manila Bureau of Printing, Manila, Republic of the Philippines (1951).

15. Oliver, B., "Medicinal Plants of Nigeria", Nigerian College of Arts, Science and Technology, Ibadan, Nigeria (1960).

16. Kit, S.M., Tr Leninger Farm. Inst., 8, 124 (1959).

17. Minaeva, V.G., "Medicinal Plants of Siberia", 3rd ed., Novosibir-Khizhnoe Izdatel, Novosibrisk (1960).

18. Schramm, G.,Wiss. Z. Karl. Marx-Uni. Leipzig Math., Naturwiss, Reihe, 6, 481 (1956).

19. Nikonov, G.K., et al. Aptechn Delo, 10, 71 (1961).

20. Roi J. Encyclopedia Biol., 47, 1, (1955), through Chem. Abstr., 53, 13293 (1959).

21. Nordal, A., "The Medicinal Plants and Crude Drugs of Burma, I, Collection of Research Material from Indigenous Sources During the year 1957-1961", Hellstron and Nordahls Boktrykkeri, A.S., also Norway (1963).

22. Melendez, E.N., "Plants Medicinals de Puerto Rico", University of Puerto Rico Exp, Agr., Sta., Bull 176 (1964).

23. Burkill, I.H., "A Dictionary of Economic Products of the Malay Peninsula", 2, Crown Agents, London, England (1935).

24. Watt. J.M., and Breyer Brandwijk, M.G., "Medicinal and Poisonous plants of Southern and Eastern Africa", 2nd ed., E. and S. Livingston, Ltd., Edinburgh and London (1962).

25. Lawrendiadis, G. Planta Med., 9, 164 (1961).

26. Webb, L.J., Council Sci. Ind. Res. Bull. 232, Melbourne (1948).

27. Brigg, L.H., J. Proc. Roy. Soc. N.S. Wales, 80,, 151 (1946).

28. Brooker, S.G., Cain, B.P., and Cambie, R.C., Trans. Roy. Soc., New Zealand, 1, 61 (1963).

29. Brooker, S.G., and Cooper, R.C., Econ. Botany, 15, 1 (1961).

30. Brooker, S.G., and Cooper, R.C., "New Zealand Medicinal Plants", United Press Ltd., Auckland, New Zealand (1961).

31. Hsu, C.M., J. Taiwan Pharm. Assoc., 8, 26 (1956).

32. Hsu, C.M., J. Taiwan Pharm. Assoc., 9, 38 (1957).

33. Pierre-Noel, A.V., "Les Plantes et les Legumes d'Haitiqui Guerissent", Port-Au-Prince, Haiti (1959).

34. Kerharo, J., and Bouquet, A., "Plants Medicinales et Toxiques de la Cote-D'lvoir-Haute-Volta" Vigot Freres, Paris, France (1950).

35. Gentry, H.S., "Riyo Mayo Plants," Carnegie Institute of Washington, Publ. 527, Washington (1942).

36. Haas, H., Arzneimittel-Forsch, 11, 49 (1961).

37. UNESCO, "Arid Zone Research XIII Medicinal Plants of the Arid Zone", Oberthur, Rennes (1960).

38. Farnsworth, N.R., J. Pharm., 3(4), 50 (1962).

39. Happieh, M.L., Beebe, C.W., and Rogers, J.S., J. Am. Leather Chemists Assoc., 49, 760 (1954).

40. Barclay, A.S., Gentry, H.S. and Jones, Q., Econ. Botany, 16, 95 (1962).

41. Earle, F.R. et al, J. Am. Oil Chemists, Soc., 36 304 (1959).

42. Gentry, H.S., and Miller, R.W., Econ. Botany, 19, 25 (1965).

43. Jones, Q., and Wolff, I.A., Econ. Botany, 14,56 (1960).

44. Goering, K.J., Eslick, R., and Brelsford, D.L., Econ. Botany, 19, 251 (1965).

45. Barclay, A.S. and Earle, F.R., Econ. Botany, 19, 33 (1965).

46. Earle, F.R. and Jones, Q., Econ. Botany, 16,221 (1962).

47. Tooky, H.L., and Jones, Q., Econ. Botany 19, 165(1965).

48. Correll, D.S., et al, Econ. Botany, 9, 307 (1955).

49. Hoppe-Seyler's Zeitschrift fur Physio-logische Chemie, Strassburg, 290 (1912); 270 (1913).

50. Piattelli, M., Minale, L., and Nicolous, R.A., (Univ.-Naples), Phytochemistry, 4(6), 817-23 (1965).

51. Ayhan Ububelen and Jack R. Cole, J. Pharm. Sci., 55 (12), 1368-70 (1966).

52. Kirtikar, K.R., and Basu, B.D., Rewritten by Blatter, E., Caius, J.F., and Mhaskar, K.S., "Indian Medicinal Plants", 3 Prabasi Press, Calcutta (1935).

53. Hoernle, A.F.R., The Bower Manuscript Supt. Govt. Printing, Calcutta (1893-1912).

54. National Cancer Institute, Central files.

55. Ghoshal, Foods and Drugs, 80 (1910).

56. Chopra, Ghosh, Ghosh and De, Ind. Med. Gaz., 58 203 (1923).

57. Agrawal, R.R., and Dutt, S., Acad. Sc., U.P, 4 73 (1934).

58. Agrawal, R.R., and Dutt, S., Proc. Acad. Sc., U.P. 5, 240 (1935).

59. Chopra, Chaterji and Ghosh, Ind. J. Med. Res., 28, 475 (1940).

60. Basu, N.K., Lal, S.B., and Sharma, S.N., Quarterly Journal of Pharmacy and Pharmacology, 20, No.1, pp.41-42 (1947).

61. Sankara, S. Subramanian and Ramakrishnan, S., Indian Journal of Pharmacy, 27, 2, 41-42 (1964).

62. Bhakuni, D.S., Dhar, M.L., Dhar, M.M., Dhawan,B.N., and Mehrotra, B.N., Indian Journal of Experimental Biology, 7, pp.250-262 Oct. (1969).

63. Nigam, B.C., Ph.D., Thesis, B.I.T.S.,Pilani,India(1970).

64. Agrawal, R.R., and Dutt, S., Proc. Acad. Sc., U.P. Part II, 240 (1934-35).

65. Basu, N.K., Lal, S.B., and Sharma, S.N., Quarterly Journal of Pharmacy and Pharmacology, 20, No.1, pp.39-40 (1947).

66. Dhar, M.L., Dhar, M.M., Dhawan, B.N., Mehrotra, B.N., and Ray, C., Indian Journal of Experimental Biology, 6, No. 4, pp. 232-247 (1968).

67. Biemann, K., Mass-Spectrometry-Organic Chemical Applications, p.78, McGraw Hill, New York (1962).

68. Bellamy, L.J., The Infra-red Spectra of Complex Molecules, pp. 27-28, Methuen, London (1962).

69. Misra, G., Nigam, S.K., and Mitra, C.R., Phytochem, 8, 2255 (1969).

70. Cross, A.D., "An Introduction of Practical Infra-red Spectroscopy", p.57 (1960).

71. JohnR. Dyer, "Application of Absorption Spectroscopy of Organic Compound, p.86, Prentice-Hall of India Private Ltd., New Delhi (1969).

72. Sen, A.B., Dhar, M.M., and Shukla, Y.N., Jour. Indian Chem. Soc., 45, 697 (1968).

73. Ruzicka, L., Leibigs, Ann. Chem. 25, 471 (1929).

74. Siddiqui, S.A. and Sen, A.B., Jour. Indian Chem. Soc., 46, 755-56 (1969).

75. Knapp, F.F. and Nicholas, H.J., Phytochemistry, 8, pp.212 (1969).

76. Liebermann, C., Ber, dtsch, Chem.Ges, 18, 1804 (1885).

77. Salkowski, E., Hoppe-Seylersz., 57, 521 (1908).

78. Gupta, D.R., and Garg, S.K., Jour. Indian Chem. Soc., 45 , 251 (1968).

79. Chatterji, A. (Mrs) and Roy, S.K., Jour. Indian Chem. Soc., 36, 268 (1959).

80. Adityachaudhury, N., and Pradip Kumar Gupta, Jour. Indian Chem. Soc., 46, 849 (1969).

81. Dixit, S.N., and Khosa, R.L., Indian J. Appl. Chem. 34, 1, 46-47 (1971).

82. Stahl, E., 'Thin Layer Chromatography A Laboratory Hand-book', Springer-Verlag, Berlin, p.201 (1965).

83. Tschugajew, Ref. Chem. Ztg. 24, 542 (1900).

84. Noller, C.R., Smith, R.A., Harris, G.H., and Walker, J.W., J. Amer. Chem. Soc., 64, 3047 (1942).

85. Brieskorn, C.H., and Briner, M., Pharm. Acta, Helvet, 28, 139 (1953).

86. Cole, A.R.H., and Michell, A.J., J. Chem. Soc., 2005-11 (1959).

87. Cole, A.R.H.,(Miss) G.T.A. Muller and (in part) Thornton, D.W., and Willix, R.L.X., J. Chem. Soc., 1219 (1959).

88. Cole, A.R.H. and Thornton, D.G., J. Chem. Soc., 1332 (1957).

89. Flett, J., 962 (1951).

90. Hadzi and Sheppard, Proc. Roy. Soc. A., 216, 274, (1953).

91. Shreve, Heether, Knight and Swern, Analyt. Chem., 1950, 22 (1948).

92. Freeman, J. Amer. Chem. Soc., 74, 2523 (1952).

93. Simonsen, J., and Ross, W.C.J., 'The Terpenes', 5, The University Press Cambridge, p.317 (1957).

94. Ruzicka, L., Geza Muller and Schellenberg, H., Helv. Chim. Acta, 22, 767 (1939).

95. Bowers, A., Halsale, T.G., Jones, E.R.H., and Lemin, A.J., J. Chem. Soc., 2548 (1953).

96. Barton, D.H.R., and Mayo, P.De, J. Chem. Soc., 887 (1954).

97. Snatzke, G., Lampert, F., und Tschesche, R., Tetrahedron, 18, pp.1417-1431 (1962).

98. Jones, R.N., und Sandorfy, C., Weissberger, A., Technique of Organic Chemistry, vol. 9, S.247, Interscience, New York (1956).

99. Bottomley, W., Cole, A.R.H., und White, D.E., J. Chem. Soc. 2624 (1955).

100. Shimanouchi, T., Tsuboi, M., Takanishi, T., und Iwata, N., Spectrochem, Acta, 16, 1328 (1960).

101. Vgl.dazu L.J. Bellamy, Ultrarotspectrum und Chemische Konstitution, S.137, Steinkopt (1955).

102. Bhattacharya, A.K., Ganguly, S.N., and Ganguli, G., Jour. Indian Chem. Soc., 46, 951-952 (1969).

103. Munier, R., and Macheboeuf, M., Bull. Soc. Chem. Biol. 31, 1144 (1949). and 33, 846(1951)

104. Basu, N.K., and Sharma, S.N., Quarterly Journal of Pharmacy and Pharmacology, 20, 41-42 (1947).

105. Koji, Nakanishi, "Infra-red Absorption spectroscopy", Holden-Day, Inc. Sen Francisco and Nankodo Company Limited, Tokyo, p.150.

106. Belcher, R., and Godbert, A.L., 'Semimicro Quantitative Organic Analysis' Longmans Green, Second Ed., 164 (1954).

107. Biemann, Klaus, "Mass spectrometry organic chemical Applications", McGraw-Hill, p.349 (1962).

108. Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, Fred., J. Ana. Chem. 28, 3, pp.350-356 (1956).

109. Wollrab, V., Phytochemistry, 8, pp.623-627 (1969).

110. Stoianova-Ivanova, B., Hadjieva, P., Phytochemistry, 8, pp.1549-52 (1969).

111. Beyon, J.H., Lester, G.R., Saunders, K.A., and Williums, A.E., Trans. Faraday Soc., 57, 1259 (1961).

112. Laster, J.L., Weber, D.J., and J. Ora, Phytochemistry, 7(6), 1605-8 (1968).

113. Stoianova-Ivanova, B., and Mladenova, K., and Popow, S., Phytochemistry, 10, pp. 1391-93 (1971).

114. Lenfant, M., Ellouz, R., Das, B.C., Zissmann, E., and Lederer, E., European J. Bio-chem. 7, 159 (1969).

115. Gerschengorn, M.C., Smith, A.R.H., Goulston, G., Goad, L.J., Goodwin, T.W., and Haines, T.H., Biochem. 7, 1698 (1968).

116. Lenfant, M., Lecompte, M.F., et al, Farrugia Phytochemistry, 9, 2529-2535 (1970).

117. Knight, B.P., J. Gas Chromatography 213 (1967).

118. Herman, A., Szyanski, 'Interpreted IR Spectra', 2 Plenum Press, p.157 (1966).

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