PRESENT WORK
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STUDIES IN NATURAL PRODUCTS

Chemical investigation of the various parts of the following medicinal plants was carried out, and a number of constituents were obtained and chemically examined. The research work submitted in the present thesis has been described separately plant-wise in two sections:

1. Seseli indicum, W. & A., and
2. Zataria multiflora, Boiss.

Besides the new compound, Indosterol (from S. indicum), a large number of known products have been isolated and identified on the basis of physico-chemical methods and modern techniques.
SESELI INDICUM

Seseli indicum\(^1\), Wight & Arnold (Fam. Umbelliferae) grows generally in the plains of India. It is an annual herb having white or pinkish flowers. The seeds are often pale yellow and are medicinally important. They act as an anthelmintic for round-worms and are also stimulant, carminative and stomachic. They are also used as a medicine for cattle. The drug (Sans. - Vanayamani) is commonly called "Banjowan" and the physicians presume it to be "Ajmod", while in the market it is called as "Ajmoda".

Bose and Guha\(^2\) isolated two coumarins: seselin and bergaptene, while the third one, \(\beta\)-isopimpinellin, was obtained by Spath et al.\(^3\) The seeds also contain fatty oil\(^4\). Later, essential oil (2.5\%) was collected from the seeds by steam-distillation in these laboratories and its physico-chemical properties and composition were studied\(^5\). The oil contained limonene, isovaleric acid, an unidentified acid (b.p. 227-31\(^\circ\)), a crystalline acid (m.p. 110-11\(^\circ\)C), seselin, and bergaptene. The presence of selinene was indicated in the higher fractions. The identity of the sesquiterpene was later confirmed by Bhattacharya et al.\(^6\) and the above acid (m.p. 110-11\(^\circ\)C) was identified to be \(\beta\)-cyclolavandulic acid. The presence of
cyclolavandulal also was reported in the essential oil by Dev et al. 7

In view of the medicinal importance and utility of the drug it appeared desirable, and of considerable interest, to carry out systematic chemical examination of the plant material since only a small amount of the work, as described above, has been done so far. The dried seeds were taken up for thorough study in the light of the modern aids and techniques of research.

The dried and powdered seeds were subjected to solvent-extraction with a number of solvents (petroleum ether, benzene and alcohol respectively) and the respective extracts were then examined separately. A number of products have been isolated in pure form and characterised in the following manner.

The petroleum ether extract was divided into neutral (I) and alkali-soluble (II) parts by treatment with aqueous solution (20%) of potassium hydroxide. The neutral part (I) on saponification, steam-distillation and chromatography (alumina) yielded a product (A), m.p. 68-70°; 从业者 max 2900, 2830, 1470, 1375, 720 and 710 cm⁻¹. It appeared to be a saturated hydrocarbon, and most probably hentriacontane and/or dotriacontane according to literature 8. For final confirmation the product was subjected to gas - liquid - chromatographic analysis and the product was found to be a mixture composed of a series of
n-alkanes ($C_{23}-C_{35}$): mainly nonacosane ($C_{29}$, 41.2%); hentriacontane ($C_{31}$, 24.8%); tritriacontane ($C_{33}$, 16.9%); heptacosane ($C_{27}$, 6.4%); triacontane ($C_{30}$, 3.3%); and octacosane ($C_{28}$, 3.3%) accompanied by traces of the rest. The odd numbered hydrocarbons predominated as usual. This mixture was compared (GLC) with a standard mixture of authentic specimens of n-alkanes.

Another product (B) was obtained in crystalline form, m.p. 137-38°, $\alpha_{D}^{30}$ = 33.3°, from the neutral part on further elution with petroleum-ether and benzene (1:1, v/v). The compound gave positive Liebermann-Burchard test and yellow colour with tetranitromethane, and was identified as $\beta$-sitosterol by mixed-melting point determination, co-TLC with an authentic specimen of the compound, and also by IR spectrum ($\gamma_{\text{max}}$ 3570 & 1653 cm$^{-1}$). It yielded $\beta$-sitosteryl acetate, m.p. 126-7°, which did not show any depression in melting-point on admixture with an authentic specimen of $\beta$-sitosteryl acetate.

On further elution with benzene and crystallisation from methanol and chloroform another crystalline compound (C; TLC: $\text{AgNO}_3$-homogeneous), m.p. 153-55°, $\alpha_{D}^{30}$ + 30°, mol. wt. 412 (mass), was obtained. On studying its physico-chemical properties, by preparation of its acetate and other derivatives and certain chemical reactions etc. it was found to be a new sterol (positive Liebermann-Burchard test). Hence, it was subjected to extensive
studies for structural elucidation. The sterol has been found to belong to the stigmastane series and has been named "Indosterol" (from Seseli indicum). Its structure has been discussed separately at the end of the description of the results regarding this plant, (vide: Page 65).

The gummy alkali-soluble part (II) was taken in benzene and then subjected to chromatography over silica gel. Elution with petroleum-ether (60-80°) afforded a solid which gave shining crystals from methanol and chloroform, m.p. 83-85°; $\lambda_{\text{max}}$ 300, 297sh, 254, 245sh and 224nm. This was identified as suberosin by its properties, m.p., m.m.p. and co-TLC with an authentic specimen of the coumarin.

Further elution of the column with benzene gave a compound, m.p. 187-88°; $\lambda_{\text{max}}$ 310, 268, 259, 250, 243 and 222nm. It was identified as bergaptene by co-TLC. The coumarin did not show any depression in mixed melting point with an authentic specimen of the compound.

Fractions with chloroform yielded another crystalline product, m.p. 119-20°, which was confirmed as seselin by direct comparison (m.m.p. and co-TLC) with an authentic specimen and U.V. ( $\lambda_{\text{max}}$ 330, 293, 283 and 218 nm). The latter fractions afforded a yellowish crystalline product, m.p. 151-53°; $\lambda_{\text{max}}$ 312, 269, 249, 241, and 223 nm. It was characterised as
isopimpinellin (m.p., m.m.p., co-TLC). The extract contains some more coumarins also in the form of inseparable mixture.

The benzene extract of the powdered seeds was thoroughly examined and it gave all the four coumarins and Indosterol mainly. No additional product could be isolated in this case.

The entire ethanolic extract of the seeds, obtained after exhausting them with petroleum ether, was examined for the presence of free sugars by paper chromatography. Glucose and rhamnose were found to be present in the extract.

This extract was also used for detecting the presence of carboxylic acids by ascending paper chromatography. n-Butanol:formic acid:water (4:1:5, v/v) were used for developing and bromophenol-blue solution as spraying reagent. Standard samples of a number of acids were used simultaneously for comparison. Oxalic, malic and citric acids were characterised in the extract.

The powdered seeds of Seseli indicum were also extracted with sodium chloride solution for amino-acids. The extract was refluxed with hydrochloric acid and the hydrolysate was then taken in a porcelain dish and the mineral acid was completely removed. The residue was then extracted with absolute alcohol and the alcoholic extract was examined by descending paper chromatography using n-butanol:acetic acid:water as
solvent system and authentic specimens of a number of amino-acids for comparison. Valine, Serine, alanine, leucine, asparagine, and threonine were identified as amino-acids with the help of ninhydrin.

**COMPOUND C INDOSTEROL**

The crystalline compound (C) having m.p. 153-55°, \( \leftarrow \infty \rightarrow J_D^{30} + 30° \), gave yellow colour with tetranitromethane and positive Liebermann-Burchard (red \( \rightarrow \) green) and Noller's tests (red colour; Cf: cholesterol). By perbenzoic acid titration it indicated the presence of two olefinic linkages and a crystalline product, m.p. 123-25°, was obtained. Infrared spectrum of the compound (vide: Spectrum A) indicated the presence of OH group (3325 & 1050 cm\(^{-1}\)) and olefinic linkages (1650 & 830 cm\(^{-1}\)); one of the ethylenic linkages may be tri-substituted. Mass spectrum of the compound (vide: Spectrum B) indicated and molecular ion and base peak at m/e 412 and other significant peaks at m/e 369, 351, 314, 300, 273, 271, 255, 213, 159, 81 and 43 etc. Nuclear magnetic resonance spectrum (vide: Spectrum C) showed three vinyl protons (at \( \delta 5.12 \& 5.35 \)) and methyl protons\(^{12} \) (at \( \delta 0.7, 0.75, 0.8, 0.85, 0.9, 0.967, 1.00 \) and 1.07). The fragmentation pattern (mass spectrum) indicated
the compound to be a sterol with an unsaturated side chain.

Found: C, 84.00% \( \pm \) H, 11.89%
Calcd. for \( C_{29}H_{48}O \): C, 84.40% \( \pm \) H, 11.72%

The compound contains one hydroxyl group since it yielded a monoacetate, m.p. 135-38°, \( \left[ \alpha \right]_D^{22} + 20^\circ \), \( M^+ 454 \); \( \nu_{\text{max}} \) 1732 & 1264 cm\(^{-1}\). Nuclear magnetic resonance spectrum of the acetate also indicated the presence of three vinyl protons (\( \delta \) 5.11 and 5.35), and methyl signals (at \( \delta \) 0.7, 0.75, 0.8, 0.83, 0.9, 0.95, 1.00, 1.03) (vide: Spectra D, E and F). The compound also yielded a benzoate, m.p. 160-63°; and a 3:5-dinitrobenzoate, m.p. 231-33°; (N, 4.09%; Calcd. for \( C_{36}H_{50}O_6N_2 \): N, 4.62%).

According to the literature available no sterol of the composition \( C_{29}H_{48}O \) consisting of two double bonds, one hydroxyl group, and the above data appears to be known for its structure so far. This compound may, therefore, be given the name "Indosterol" (I) (from Seseli indicum). Some of the physical constants of this compound could be compared to an extent with those of \( \gamma \)-spinasterol as given in the Table I. The structure of the so-called \( \gamma \)-spinasterol does not appear to have been defined as yet. Indosterol showed depression in m.p. when mixed with \( \alpha \)-spinasterol (II) as well as with stigmasterol (III) separately.
C) INDOSTEROL (NMR)

D) INDOSTERYL ACETATE (MASS)
INDOSTERYL ACETATE (IR)

INDOSTERYL ACETATE (NMR)
### Table - I

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Indosterol m.p. (°C)</th>
<th>α-Spinasterol m.p. (°C)</th>
<th>Stigmasterol m.p. (°C)</th>
<th>γ-Spinasterol m.p. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. Benzoate</td>
<td>160-63</td>
<td>201-02; [-22.5]</td>
<td>160</td>
<td>118-19; [-10.3]</td>
</tr>
<tr>
<td>4. 3:5 DNB</td>
<td>231-33</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5. Tetra-hydro-sterol</td>
<td>137-38; [+29]</td>
<td>-</td>
<td>136-37; [+25]</td>
<td>-</td>
</tr>
</tbody>
</table>

Specific rotations are given in parentheses in degrees measured in sodium light.

The above facts prompted the author to study this compound in more detail with a view to elucidate its structure which has now been inferred to be as (I) on the basis of the following observations.

The nuclear magnetic resonance spectrum (60 MHz.) of the compound (C) indicated the chemical shifts for methyl groups at $\delta$ 0.7, 0.75, 0.8, 0.85, 0.90, 0.967, 1.00 and 1.07 which may be assigned possibly due to the presence of methyl protons respectively at carbon atoms 18; 29; 26 & 27; 29; 26, 27 & 29; 21; 19 and 21. The value of $\delta$ 5.12 (dt, 2H) may be assigned.
to the protons at the C_{22}-C_{23} double bond, while the value \( \delta 5.35 \) (dt, 1H) for the second olefinic linkage; possibly \( \triangle^{9(11)} \). Further, the position of the double bond in the side-chain may also be inferred at C_{22} by NMR spectrum due to the downfield shift of the C-21 methyl signals \(^{12a}\) (\( \delta 0.967 \) & 1.07 cf: \( \beta \)-sitosterol \(^{12a}\); \( \delta 0.893 \) & 0.952; and cholesterol: \( \delta 0.875 \) & 0.93). The chemical shift at \( \delta 3.6 \) (m, 1H) in the case of the compound, and at \( \delta 4.6 \) (m, 1H) in the case of Indosteryl acetate indicates \(^{17}\) trans-fusion of rings A and B and hydroxyl group at C_{3} position.

On hydrogenation (PtO_{2}/AcOH) indosterol yielded a crystalline product, m.p. 136-38°C; \( \int \alpha \int_D + 29° \). It gave an acetate, m.p. 129-30°C; \( \int \alpha \int_{D}^{24} + 18° \). The hydrogenated product gave negative test with tetranitromethane and did not indicate any vinyl proton in the NMR spectrum (vide: Spectrum G), but methyl signals at \( \delta 0.65, 0.683, 0.765, 0.783, 0.83, 0.85, 0.93 \) and 1.00; \( \int \) max 3400 & 1040 cm\(^{-1}\). This product (which may be shown as IV) and its acetate did not show any depression in melting points on admixture respectively with authentic specimens of stigmastanol (V, — obtained from \( \beta \)-sitosterol — VI by hydrogenation) and the acetate of the latter (V). Co-IR (vide: Spectrum H), co-TLC and m.m.p. of (IV) and stigmastanol (V) and their acetates suggest that indosterol has stigmastanol carbon skeleton (IV).
TETRAHYDROINDOSTEROL (NMR)

TETRAHYDROINDOSTEROL & STIGMASTANOL (IR)
One olefinic linkage may be located at $\text{C}_9-\text{C}_{11}$ position (as indicated above) further because the sterol was readily hydrogenated in acetic acid solution using platinum catalyst$^{18}$. It has been reported that the hydrogenation with palladium in ether$^{19}$, or over platinum catalyst in acetic acid$^{20}$ affected saturation of the $\triangle^{22(23)}$ double bond and isomerisation of $\triangle^7$ to $\triangle^{8(14)}$ (e.g. $\alpha$-spinasterol $\rightarrow \triangle^{8(14)}$ stigmasterol $\rightarrow$ VII). In the present case hydrogenation yielded tetrahydro- product by platinum catalyst.

On oxidation with chromium trioxide indosterol gave two products: The major product, m.p. 156-57$^\circ$; $\int \alpha \int_D^{24} \int_D^{50}$ had $\alpha,\beta$-unsaturated ketonic system$^{21a}$; $\lambda_{\text{max}}$ 252 nm ($\epsilon$ 11,000) (vide: Spectrum I); $\mu_{\text{max}}$ 1690 and 1660 cm$^{-1}$; $\text{M}^+$ 424. The NMR spectrum (vide: Spectrum J) showed methyl signals at $\delta$ 0.75, 0.8, 0.85, 0.9, 0.98, 1.10 and olefinic signals at $\delta$ 5.12 (t, 2H) and $\delta$ 6.18 (s, 1H). The downfield shift ($\delta$ 6.18) of the one vinyl proton in the NMR spectrum suggested the presence of $\alpha,\beta$-unsaturated ketonic system$^{21b}$. The formation of $\alpha,\beta$-unsaturated ketone on oxidation suggested allylic oxidation possibly occurring$^{21c}$ at $\text{C}_{12}$. This compound could be formulated as (VIII). It gave positive Zimmermann test. The mass spectrum of this ketone indicated peaks at m/e 424, 381, 312, 285, 137, 81 & 43 etc. The formation of the product (VIII) indicates the presence of
KETONE VIII (UV)

252 nm
nuclear $\triangle^{9(11)}$ unsaturation. The second oxidation product (minor), m.p. 157-58°; $\int^{\infty} \alpha \int^{24}_{D} + 30^\circ$, $\nu_{\text{max}} 1722 \text{ cm}^{-1}$ (vide: Spectrum K), does not absorb UV light and is formulated as (IX). This product also showed positive Zimmermann test (C$_3$ ketone).

The intense peak at m/e 271 in the mass spectrum (Scheme I) of Indosterol indicates apparently that the formation of this fragment takes place due to the interesting cleavage associated with the presence of a double bond in the side chain as described by Djerassi et al. This peak appears due to the formation of the fragment (m/e 271) left after the loss of the side chain (m/e 139) together with two hydrogen atoms from the molecule. Formation of another ion, m/e 273, is also indicated (peak less intense than m/e 271 peak) as a result of competing allylic cleavage of C$_{17}$-C$_{20}$ bond. Thus, the presence of $\triangle^{22(23)}$ double bond in the side chain is indicated by the above peaks and also due to the strong peak at m/e 300 as a result of the allylic cleavage (Schemes II & III).

The position of the trisubstituted double bond in the nucleus of this compound at C$_9$-C$_{11}$ may also be concluded by excluding the rest of the possibilities of the presence of such olefinic linkage in the nucleus. It has been reported that the nuclear double bond has some influence on the fragmentation induced by the side chain double bond. Generally the m/e 271 peak is more intense than that at m/e 314 if the sterol has a
KETONE VIII (NMR)

KETONES VIII & IX (IR)
\( \triangle 7(8) \) double bond, while the reverse situation exists in the case of the \( \triangle 5(6) \) isomers\(^{13} \) (e.g. stigmasteryl - III). Further, it has also been reported\(^{22} \) that the m/e 271 peak is the base peak in the \( \triangle 7(8) \) steroids (e.g. \( \alpha \)-spinasterol - II). But, in the present case the base peak is the molecular ion peak, m/e 412, although m/e 271 peak is more intense than the m/e 314 peak. It may, therefore, be concluded that the double bond in indosterol is not present either at C\(_5\) or C\(_7\) in the ring B of the steroidal nucleus.

The possibility of the presence of unsaturation in the ring D is also eliminated since there seems to be no evidence of the formation of cyclopentenone in the sterol on oxidation when the major product (VIII) is obtained (\( \nu \)\(_{\text{max}} \) 1690 & 1660 cm\(^{-1} \)).

It was recognised\(^{23} \) that one of the most general fragmentations of sterols and related C-17-substituted steroids is formed due to the loss of 42 mass unit (by loss of carbon atoms 15, 16, 17 of ring D) together with the C-17 side chain. In the present case the peak at m/e 231 from 412 — (42 + 139) is formed accordingly. The fragment of mass 231 as an allylic carbonium ion is formed by transfer of an hydrogen atom from C-8 (Scheme I). It has also been mentioned\(^{13} \) that more than one fragmentation is involved in the formation of the fragment m/e 231. However, the retention of C-18 methyl function is proposed and is supposed to be more general (Scheme II). Further, the rupture of the highly
SCHEME I
SCHEME II
SCHEME III
substituted 13-17 bond releases the strain. Formation of the ion \( \text{M}^+ \ 412 \) has been supposed to be of the major significance in the fragmentation of side chain bearing sterols when there is no unsaturation in the ring D. Thus, the possibility of unsaturation in the ring D may also be ruled out.

The above evidence may be considered adequate for the structure of Indosterol carrying the important feature of the \( \triangle 9(11) \) double bond in the steroidal nucleus. Any further work in this regard could not be carried out due to paucity of the material and time.

However, some more experiments may be designed towards strengthening the evidence for the above structure (I), and a few are given below for the purpose. They (and/or others) are proposed to be carried out during the programme of post-doctoral work in these laboratories.

Indosterol may be partially hydrogenated \( \text{Pd/C} - 10\% \) and then acetylated. The properties of dihydroindosterol and its acetate may be studied and those of the former may be compared with the characteristics of \( \beta \)-sitosterol (VI) and other such products, and then some conclusions may be drawn on the basis of the nature of partial hydrogenation in the light of the observations of Barton et al. Further, dihydroindosteryl acetate \( (X) \) may be oxidised by chromic acid. The formation of \( (XI) \) and
its study by various spectra is likely to establish further the presence of C₉–C₁₁ olefinic linkage in the sterol.

The retro-aldol condensation of (XI), the formation of the cleavage product and the detailed study of the physico-chemical properties and spectra of the same are expected to prove conclusively the structure of indosterol as (I).
ZATARIA MULTIFLORA

Zataria multiflora, Boiss (Saatar), is a small herbaceous plant, and a reputed and extensively used drug in the Unani system of medicine. The drug consists of small leaves mixed with portions of slender woody stems and numerous minute flowers. It is much used in India in the form of infusion as an agreeable aromatic stimulant, diaphoretic, diuretic and for several other purposes. This fragrant drug has been mentioned to contain an aromatic essential oil with a minty odour; a red and tasteless acid resin, and some tannic acids giving a green precipitate with ferric chloride. No chemical studies seem to have been carried out on this plant so far except its essential oil. The plant material was subjected to steam-distillation in these laboratories a few years back and a golden yellow essential oil (0.6%) was obtained. The physico-chemical properties of the oil have been studied and it has been found to contain mostly phenolic constituents (69%): mainly carvacrol accompanied by traces of thymol. The non-phenolic portion (31%) consists of p-cymene as the major constituent.

In view of its importance as a reputed drug it was considered desirable to carry out the systematic chemical investigation of the fragrant plant-material since its only
essential oil\textsuperscript{28} has been examined to some extent so far.

The dried and powdered drug (consisting of stems, flowers and seeds) was subjected to solvent extraction with a number of solvents (petroleum ether, benzene and alcohol successively) and the different extracts thus obtained have been examined separately. A number of different products have been isolated\textsuperscript{29} in pure form from these extracts through chromatography and crystallization, and then thoroughly studied.

The petroleum ether extract of the drug was divided into neutral (I) and acidic (II) parts by treatment with alkali. The neutral part (I) on saponification, steam-distillation and repeated chromatography (alumina) yielded a product (A), m.p. 65-66\textdegree. It was found to be identical with triacontane (C\textsubscript{30}) on the basis of its elemental analysis, infrared spectrum and mixed melting point determination with an authentic specimen of the hydrocarbon (C\textsubscript{30}H\textsubscript{62}). But, according to mass spectrum the molecular weight of the product was found to be 464.53 corresponding to the formula C\textsubscript{33}H\textsubscript{66}. But the melting point of tri-triacontane recorded in the literature\textsuperscript{8} is 71.9\textdegree. Hence, finally to clarify this anomaly the product (A) was then subjected to gas-liquid chromatographic analysis for verification and confirmation. As a result of this analysis the product (A) was found to be a mixture of n-alkanes (C\textsubscript{27}-C\textsubscript{35}) containing mainly n-C\textsubscript{33} (62\%); n-C\textsubscript{31} (17\%); n-C\textsubscript{29} (5\%); n-C\textsubscript{32} (4\%); and
n-C_{35} (3.5\%) accompanied by the minor quantities of n-C_{34}, n-C_{28}, and n-C_{27} alkanes and traces of 2-methyl and 3-methyl branched paraffins. Odd numbered n-alkanes predominated as usual in this case also.

Another product \((B)\) was obtained in the crystalline form (m.p. 136-37°; \(\alpha D = -32.5°\)) from the neutral part on further elution with benzene and repeated crystallisation (methanol and chloroform). It gave positive Liebermann-Burchard test and yellow colour with tetranitromethane. The compound has been identified as \(\beta\)-sitosterol by infrared spectrum and mixed melting point with an authentic specimen of the compound. This compound afforded \(\beta\)-sitosteryl acetate, m.p. 123-24°; benzoate, m.p. 144-46°, and 3:5-dinitrobenzoate, m.p. 203-06°. These derivatives were characterised by melting point determination, I.R. or elemental analysis etc. The acetate did not show any depression in m.p. on admixture with the authentic specimen of \(\beta\)-sitosteryl acetate.

On elution with benzene and chloroform another colourless crystalline (ethyl acetate) product \((C)\), m.p. 254-56°, was obtained (TLC-homogeneous). It gave positive Liebermann-Burchard and Noller's tests and yellow colour with tetranitromethane, and also yielded a diacetate, m.p. 212-13°, \(\nu_{\text{max}} 1245 \text{ cm}^{-1}\) (acetate), and 890 cm\(^{-1}\) (\(=\text{CH}_2\)). The compound was identified as betulin on
the basis of its co-TLC with an authentic specimen of the triterpene. The IR \( \nu_{\text{max}} \) 3380 cm\(^{-1}\) (OH) and 880 cm\(^{-1}\) (\(=\text{CH}_2\)); and the mass spectrum \(^{30}\) \(M^+ 442, C_{30}H_{50}O_2\) were also found to be comparable with those of the authentic sample of betulin.

The acidic part (II) of the petroleum-ether extract was found to contain a product (D), m.p. 74-75°, which gave a methyl ester with diazomethane having m.p. 56-57°. This compound was supposed to be behenic acid on the basis of m.p., elemental analysis and infrared spectrum: \( \nu_{\text{max}} \) 3340, 1705, 725 cm\(^{-1}\).

Also, its methyl ester showed no depression on mixed-melting point determination with an authentic specimen of methyl behenate. But, by GLC analysis the product was found to be a mixture of four components: behenic (C\(_{22}\), 9%); lignoceric (C\(_{24}\), 56%), cerotic (C\(_{26}\), 31%); and montanic (C\(_{28}\), 4%) acids.

On further elution with benzene and ether two more acids (E & F), m.p. 285-90°; \( \angle \alpha \angle \)D\(_{20}^0 + 85°\); \( \angle \alpha \angle \)D\(_{20}^0 + 94°\), were also isolated from the alkali soluble part. Both of them gave Liebermann-Burchard and Noller's tests and yellow colour with tetranitromethane. The infrared spectra (vide-experimental section) indicated them to be triterpenic acids belonging to the oleanolic acid series\(^{31}\). Although the spectra were almost identical but they (acids) showed significant depression on mixed-melting point determination.
The major component (E), m.p. 285-90°, $\int_\infty \int_{20} + 83°$, gave an acetate, m.p. 279-81°; $\int_\infty \int_D + 68.5°$; a methyl ester, m.p. 197-98°; $\int_\infty \int_{25} + 47.8°$; and a methyl ester acetate, m.p. 213-15° (TLC—all homogeneous). A lactone, m.p. 244°, was prepared from this acid on treatment with dry hydrogen chloride in chloroform (10 minutes); $\gamma_{\text{max}}$ 1765 cm$^{-1}$ (lactone). The acid was also oxidised by Jones' method yielding a ketone (IR and 2,4-dinitrophenyl hydrazone, m.p. 256-59°) having m.p. 162-64° and giving positive Zimmermann test. The acid (E) absorbed perbenzoic acid equivalent to one ethylenic linkage and indicated the presence of one carboxylic acid group on titration with standard alkali. The above products were examined by physico-chemical methods, and the compound (E) was finally identified as oleanolic acid on the basis of the above evidence, nuclear magnetic resonance & mass spectra, and the superimposable infrared spectrum with that of the authentic specimen of oleanolic acid (through the courtesy of Prof. Dr. R. Tschesche, Bonn), although melting points of the various products (as noted above) did not compare well with those as recorded in literature; (Table 5, p. 117).

The minor component (P), m.p. 280-85°, $\int_\infty \int_{20} + 94°$, also yielded an acetate, m.p. 262-63°; methyl ester, m.p. 214-16°; and a methyl ester acetate, m.p. 203-06°. On the basis of the infrared spectrum of the acid itself and those of its derivatives the acid was found to be belonging to the oleanolic acid series.
But, on further careful examination (co-TLC, silica-gel, AgNO₃, etc.) the product was found to be a mixture of Oleanolic, Ursolic and epi-oleanolic acids.

The benzene extract obtained after exhausting the drug with petroleum-ether was also divided into alkali-soluble and neutral parts in the usual manner. The neutral part did not yield any pure compound and the alkali-soluble part on chromatography and crystallisation afforded only one pure product (significant yield) which was identified to be oleanolic acid in the above manner.

The drug, after treatment with petroleum ether and benzene successively, was extracted with rectified spirit. The alcoholic extract was triturated with petroleum ether and benzene and then the residue was taken in water and divided into ether-soluble and ether-insoluble parts.

The ethereal solution on concentration yielded two components (G & H) through t.l.c. Both the products gave positive Mg/HCl and ferric chloride tests and were later obtained in pure form in some quantity by preparative thin-layer chromatography (benzene:pyridine:formic acid - 36:9:5, v/v).

The product G, m.p. 223-30°, indicated \( \lambda_{\text{max}} \) 345, 266(sh), 245(sh) nm; \( \text{AlCl}_3 \): 420, 301, 270 nm; \( \text{AlCl}_3\text{HCl} \): 345, 294, 270 nm; \( \text{NaOAc} \): 410, 310, 279, 264 nm; \( \text{NaOAc/H}_3\text{BO}_3 \): 370, 303, 290, 255 nm;
and NaOMe: 400, 315, 275 nm. It was identified as luteolin supported by m.p., m.m.p. and co-TLC with an authentic specimen. It gave an acetate, m.p. 234-35°; NMR: $\delta$ 2.35 (d, acetate protons); 6.65 (d, 6-H); 6.9 (d, 8-H); 7.4 (d, 5-H); 7.72 (s, 2'-H) and 7.94 (s, 6'-H). Its methyl ether had m.p. 191-93°, NMR: $\delta$ 3.95 (d, methoxyl protons), 6.35 (d, 6-H); 6.55 (d, 8-H); 6.9 (d, 5'-H, $J=8.0$ cps); 7.3 (2'-H) and 7.4 (6'-H).

The other component (H, minor), m.p. 288-90°, was identified as 6-methyl quercetin by m.p., m.m.p. and co-TLC with an authentic specimen of the compound. Its identity was further confirmed by preparation of its methyl ether, m.p. 165-66°, NMR: $\delta$ 1.85 (s, 3H); 4.00 (s, OCH$_3$); 6.6 (s, 8-H); 7.00 (d, 5'-H); 7.4 (s, 2'-H); and 7.5 (s, 6'-H).

The residue obtained from the ether-insoluble part of the alcoholic extract was subjected to preparative paper chromatography using n-butanol:acetic acid:water (4:1:5, v/v). Three main components (I, J & K) were obtained in pure form. They also gave positive ferric chloride and Shinoda tests for flavonoidal compounds.

The product (I) had m.p. 250-55°; $\lambda_{max}$ 343, 268, 250 nm; AlCl$_3$: 410, 350, 330, 297, 272 nm; AlCl$_3$/HCl: 390, 345, 296, 278, 262 nm; NaOAc: 405, 266, 252 nm; NaOAc/H$_3$BO$_3$: 370, 264, 252 nm; and NaOMe: 395, 268, 257 nm; IR: $\nu_{max}$ 3500, 3120, 1675, 1570,
1555, 1500 and 840 cm\(^{-1}\). This was subjected to the acid-
hydrolysis and the aglycone obtained had m.p. 320-25\(^0\). It was
identified as luteolin by m.p., m.m.p. and co-TLC. The aqueous
solution after working up as usual was tested for the sugar
moiety which was found to be glucose by paper chromatography
in the usual manner. Methyl ether of the product (I) was also
prepared, and then hydrolysed. It gave the partially methylated
aglycone having m.p. 285\(^0\); \(\lambda_{\text{max.}}\) 326, 285, 262 nm; \(\text{AlCl}_3\): 326,
280, 264 nm; \(\text{AlCl}_3/\text{HCl}\): 325, 285, 263 nm; \(\text{NaOAc}\): 320, 276 nm;
\(\text{NaOAc/H}_3\text{BO}_3\): 329, 281, 266 nm; and \(\text{NaOMe}\): 328, 276 nm. These
values indicated the linkage of glucose at C\(_7\). Thus, the
product (I) was identified as luteolin-7-O-D-glucoside.

The compound (J) showed UV absorption at \(\lambda_{\text{max.}}\) 340, 268,
256 nm; \(\text{AlCl}_3\): 415, 323sh, 297sh, 278, 257 nm; \(\text{AlCl}_3/\text{HCl}\): 399,
345, 296sh, 277, 260 nm; \(\text{NaOAc}\): 405, 350, 267sh, 257sh nm;
\(\text{NaOAc/H}_3\text{BO}_3\): 375, 256 nm; and \(\text{NaOMe}\): 408, 298 and 274 nm. It
was hydrolysed (mineral acid - 10\%) and the aglycone obtained
possessed m.p. 312-16\(^0\). It showed no depression in melting point
on admixture with an authentic specimen of quercetin. The sugar
part was also analysed by paper chromatography and found to
contain glucose and rhamnose. Methyl ether, m.p. 153-55\(^0\) (TLC-
homogeneous), and acetate, m.p. 190-95\(^0\), of the above aglycone
were also prepared which further confirmed its identity.
The diglycoside was converted into methyl ether and the product was then hydrolysed. The resulting partially methylated aglycone was also studied. It showed $\lambda_{\text{max}}$: 330, 282, 265 nm; $\text{AlCl}_3$: 330, 282, 265 nm; $\text{AlCl}_3$/HCl: 330, 282, 265 nm; NaOAc: 340, 310, 274 nm; NaOAc/$H_3BO_3$: 330, 281, 266 nm; and NaOMe: 380, 345, 309, 274 nm. Finally, the compound J was acetylated and the acetyl derivative was purified by chromatography (silica gel). The acetate (semi-solid) showed NMR signals at $\delta$ 1.2 (d, CH$_3$, rhamnose); 2.0 and 2.4 (d, acetate protons); 4.7 (1 H, rhamnosyl protons) and 5.3 (m, 1H, glucosyl proton). The above facts indicated the product (J) to be quercetin-7-0-D-glucoside-3-rhamnoside.

The product K had m.p. 172-73°: $\lambda_{\text{max}}$: 330, 270 nm; $\text{AlCl}_3$: 384, 335, 301, 270 nm; $\text{AlCl}_3$/HCl: 387, 335, 301, 372 nm; NaOAc: 398, 311, 270 nm; NaOAc/$H_3BO_3$: 328, 270 nm; and NaOMe: 400, 305sh, 279 nm. It afforded an acetate, m.p. 207-08° (TLC homogeneous). The compound K was hydrolysed by sulphuric acid (10%) and the aglycone, m.p. 347-48°, was identified$^{37}$ as apigenin by m.p., m.m.p. and co-TLC with an authentic specimen. The sugar moiety was confirmed as glucose by co-paper chromatography (n-butanol: acetic acid:water 4:1:5, v/v - organic layer). On the basis of the above the product (K) has been identified as apigenin-7-0-D-glucoside.
Another product (L) was also isolated from the above alcoholic extract. It gave positive Liebermann-Burchard test and yellow colour with tetranitromethane, m.p. 297-99°, $\gamma_{\text{max}}$ 3500, 1055 (Broad) cm$^{-1}$. It afforded an acetate, m.p. 175-77°; $\gamma_{\text{max}}$ 1750 cm$^{-1}$ (C=O), 1245 cm$^{-1}$; NMR: δ 0.70, 0.80, 0.88, 1.00 (CH$_3$ protons); 2.03 (q, acetate protons), 5.25 (1 vinyl proton). The infrared spectrum of L and the NMR spectrum of its acetate were found to be identical with those of $\beta$-sitosterol-D-glycoside and its acetate respectively.

The above saponin was hydrolysed (mineral acid 10%) into the aglycone and sugar parts. Glucose has been identified as the sugar moiety by co-paper-chromatography. The aglycone, m.p. 131-34°, has been found to be $\beta$-sitosterol by m.p., m.m.p. and co-TLC with an authentic sample of the sterol. The compound (L) was characterised further as $\beta$-sitosterol-D-glycoside by m.p., m.m.p. and co-TLC with an authentic specimen of the saponin (vide: Spectra Co-IR-L).

The entire alcoholic extract of the drug was also examined for the presence of free sugars by co-paper chromatography. Galactose, glucose and rhamnose were identified by comparison.

The drug was extracted by aqueous ethanol (50%) at the room and boiling temperatures as well. These extracts were then separately examined by ascending paper-chromatography for non-volatile carboxylic acids using n-butanol:formic acid:water, and
SITOSTEROL-\(\beta\)-D-GLUCOSIDE (CO-IR)
the bromophenol-blue solution as the spraying reagent. Authentic specimens of a number of acids were used for comparison simultaneously. The alcoholic extract contained succinic and gluconic acids, while the aqueous-alcoholic extract, however, indicated the presence of succinic, malic and oxalic acids.

The drug was also extracted with sodium chloride solution (2%) for amino-acids. The extract was then hydrolysed with hydrochloric acid (6N), and the mineral acid was totally removed. The residual gummy matter was dissolved in absolute alcohol, and alcoholic extract was then examined for amino-acids by descending paper chromatography using n-butanol:acetic acid:water (4:1:5, v/v, upper layer), and ninhydrin (in acetone) as a detecting reagent. Authentic samples of a number of amino-acids were used for comparison simultaneously. Glycine, Arginine, Valine and Asparagine were found to be present in the above extract.