EXPERIMENTAL

The melting points (mp) reported are uncorrected. The UV spectra were recorded in methanol on Perkin-Elmer Hitachi 200 spectrophotometer. The IR spectra were recorded in KBr using Perkin-Elmer 1430 ratio recording infrared spectrophotometer. The $^1$H NMR spectra were taken in CDCl$_3$ and CDCl$_3$-DMSO-d$_6$ on Varian EM 390 spectrometer using tetramethylsilane as internal standard. $^{13}$C NMR spectra were recorded in CDCl$_3$ on a Bruker WP 400 NMR spectrometer operating at 100 MHz. The EI mass spectra were obtained with Jeol D-300 mass spectrometer attached to a JMA-2CO system. Optical rotations were measured in chloroform and methanol in Perkin-Elmer 241 polarimeter.

For column chromatography silica gel (BDH and Acme, 100-200 mesh) was used. TLC plates were prepared with silica gel G (E.Merck) and activated at 110° for 30 min. The plates were sprayed with 60% sulphuric acid. Anhydrous sodium sulphate was used as drying agent.

Albino porton strain male rats (non-fasted) weighing between 100-160 g were used for determining antiinflammatory activity. The animals were procured from Central Animal House, Panjab University, Chandigarh. The animals were maintained at standard diet (Lipton India Ltd., Bangalore) and tap water ad libitum.
Collection of *Vitex negundo* was done in June-July, 1988 during flowering, from outskirts of Chandigarh. The flowering spikes (entire inflorescence) were separated and dried. The seeds of *V. negundo* were procured from the local market and their identity was confirmed by comparison with the authentic reference sample preserved at the Herbarium cum Museum of the Department of Pharmaceutical Sciences, Panjab University, Chandigarh. *Vanda roxburghii* roots were obtained from the Director, Tropical Botanic Garden and Research Institute Trivandrum (Kerala). *Acampe papillosa* (roots, stem and leaves) was collected in March, 1986 from the surroundings of Dehradun (U.P.). The identity of these plants was confirmed by comparison with the reference herbarium sheets preserved at Herbarium cum Museum of the Department of Botany, Panjab University, Chandigarh. A representative specimen of each of the plants has been deposited in the Herbarium cum Museum of the Department of Pharmaceutical Sciences, Panjab University, Chandigarh. The plant material was dried under shade and reduced to a moderately coarse powder.

3.1 PRELIMINARY EXTRACTION AND SCREENING OF PLANTS FOR ANTIARTHRITIC ACTIVITY

The powdered plant material 100 g each in case of *Vitex negundo* (flowers), *Vanda roxburghii* (roots), *Acampe papillosa* (roots), *A. papillosa* (stem and leaves) and 200 g in case of *Vitex negundo* (seeds) was extracted sequentially with petroleum ether (60-80°), chloroform and methanol in a
Soxhlet unit till complete exhaustion. The solvent from each extract was recovered under reduced pressure to obtain various extracts (Table 7) for preliminary antiinflammatory screening.

Table 7: Extractives Obtained (in g) from Different Plants

<table>
<thead>
<tr>
<th>Plant (Part)</th>
<th>Petroleum ether extract</th>
<th>Chloroform extract</th>
<th>Methanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitex negundo (Seeds)</td>
<td>5.5</td>
<td>0.7</td>
<td>14.0</td>
</tr>
<tr>
<td>V. negundo (Flowers)</td>
<td>4.6</td>
<td>2.2</td>
<td>10.0</td>
</tr>
<tr>
<td>Vanda roxburghii (Roots)</td>
<td>1.5</td>
<td>2.3</td>
<td>4.8</td>
</tr>
<tr>
<td>Acampe papillosa (Roots)</td>
<td>3.4</td>
<td>5.6</td>
<td>25.0</td>
</tr>
<tr>
<td>A. papillosa (Stem and Leaves)</td>
<td>7.3</td>
<td>5.7</td>
<td>13.9</td>
</tr>
</tbody>
</table>

All the extracts were subjected to screening for antiinflammatory activity using carrageenan-induced rat paw oedema model. In all sets of experiments the rats were divided into control, standard and test groups each comprising of five animals. Oedema was induced by subcutaneous injection of 0.1 ml of 1.0% solution of carrageenan in normal saline in the subplanter region of hind paw of all the animals of different groups. Each extract (500 mg/kg) suspended in normal saline-tween 20 (95:5) was administered orally to the animals of the test group 1 h before the injection of carrageenan (0.1 ml of 1.0% solution in normal saline). Ibuprofen (50 mg/kg, p.o.) suspended in normal saline-tween 20 (95:5) was given to the rats of the standard group. The animals of control group were given normal...
Table 8: Per cent Inhibition of Oedema (at the end of 3.5 h) by the Various Extracts of *Vitex negundo* Seeds.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg, p.o.</th>
<th>Mean increase in paw volume ml ± S.D.</th>
<th>% Inhibition of oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Control</td>
<td>-</td>
<td>0.49 ± 0.063</td>
<td>-</td>
</tr>
<tr>
<td>B Standard (Ibuprofen)</td>
<td>50</td>
<td>0.18 ± 0.058</td>
<td>63.3</td>
</tr>
<tr>
<td>C Petroleum ether extract</td>
<td>500</td>
<td>0.38 ± 0.066</td>
<td>21.5</td>
</tr>
<tr>
<td>D Chloroform extract</td>
<td>500</td>
<td>0.31 ± 0.043</td>
<td>34.8</td>
</tr>
<tr>
<td>E Methanol extract</td>
<td>500</td>
<td>0.52 ± 0.048</td>
<td>-</td>
</tr>
</tbody>
</table>

F ratio: 52.17 (calculated): 1.47 (critical)

Significant difference among groups, p = 0.05

* A and B - D
* B and A, C - E
* C and A, B, D, E
* D and A - C, E
* E and B - D

*To be interpreted as - Group A differs significantly from groups B, C and D; group B differs from groups A, C, D and E; group C differs from groups A, B, D and E; and so on.
Table 9: Percent Inhibition of Oedema (at the end of 3.5 h) by the Various Extracts of *Vitex nequndo* Flowers.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg, p.o.</th>
<th>Mean increase in paw volume ml ± S.D.</th>
<th>% Inhibition of oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Control</td>
<td>-</td>
<td>0.49 ± 0.065</td>
<td>-</td>
</tr>
<tr>
<td>B Standard</td>
<td>50</td>
<td>0.16 ± 0.057</td>
<td>66.3</td>
</tr>
<tr>
<td></td>
<td>(Ibuprofen)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C Petroleum ether extract</td>
<td>500</td>
<td>0.38 ± 0.069</td>
<td>21.5</td>
</tr>
<tr>
<td>D Chloroform extract</td>
<td>500</td>
<td>0.29 ± 0.049</td>
<td>39.7</td>
</tr>
<tr>
<td>E Methanol extract</td>
<td>500</td>
<td>0.52 ± 0.063</td>
<td>-</td>
</tr>
</tbody>
</table>

F ratio: 52.17 (calculated); 1.47 (critical)

Significant difference among groups, p = 0.05
- A and B-D
- B and A, C-E
- C and A, B, D, E
- D and A-C, E
- E and B-D
Table 10: Per cent Inhibition of Oedema (at the end of 3.5 h) by the Various Extracts of *Vanda roxburghii* Roots.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg, p.o.</th>
<th>Mean increase in paw volume ml ± S.D.</th>
<th>% Inhibition of oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Control</td>
<td>-</td>
<td>0.57 ± 0.113</td>
<td>-</td>
</tr>
<tr>
<td>B Standard (Ibuprofen)</td>
<td>50</td>
<td>0.19 ± 0.084</td>
<td>66.6</td>
</tr>
<tr>
<td>C Petroleum</td>
<td>500</td>
<td>0.26 ± 0.063</td>
<td>54.3</td>
</tr>
<tr>
<td>D Chloroform extract</td>
<td>500</td>
<td>0.33 ± 0.063</td>
<td>42.1</td>
</tr>
<tr>
<td>E Methanol extract</td>
<td>500</td>
<td>0.44 ± 0.055</td>
<td>21.9</td>
</tr>
</tbody>
</table>

F ratio: 52.17 (calculated); 1.47 (critical)

Significant difference among groups, p = 0.05

A and B - E
B and A, C - E
C and A, B, D, E
D and A - C, E
E and A - D
Table 11: Percent Inhibition of Oedema (at the end of 3.5 h) by the Various Extracts of *Acampe papillosa* Roots.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg, p.o.</th>
<th>Mean increase in paw volume ml ± S.D.</th>
<th>% Inhibition of oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Control</td>
<td>-</td>
<td>0.51 ± 0.051</td>
<td>-</td>
</tr>
<tr>
<td>B Standard</td>
<td>50</td>
<td>0.19 ± 0.122</td>
<td>62.7</td>
</tr>
<tr>
<td></td>
<td>(Ibuprofen)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C Petroleum</td>
<td>500</td>
<td>0.36 ± 0.096</td>
<td>33.0</td>
</tr>
<tr>
<td>ether extract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D Chloroform</td>
<td>500</td>
<td>0.39 ± 0.087</td>
<td>23.3</td>
</tr>
<tr>
<td>extract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E Methanol</td>
<td>500</td>
<td>0.49 ± 0.065</td>
<td>3.9</td>
</tr>
<tr>
<td>extract</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F ratio: 52.17 (calculated); 1.47 (critical)

Significant difference among groups, p = 0.05
- A and B-D
- B and A, C, D, E
- C and A, B, D, E
- D and A, B, E
- E and B-D
### Table 12: Percent Inhibition of Oedema (at the end of 3.5 h) by the Various Extracts of *Acamoe oscillosa* Stem and Leaves.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Mean increase in paw volume (ml ± S.D.)</th>
<th>% Inhibition of oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Control</td>
<td>-</td>
<td>0.51 ± 0.09C</td>
<td>-</td>
</tr>
<tr>
<td>B Standard (Ibuprofen)</td>
<td>50</td>
<td>0.16 ± 0.039</td>
<td>68.6</td>
</tr>
<tr>
<td>C Petroleum ether extract</td>
<td>500</td>
<td>0.29 ± 0.045</td>
<td>43.1</td>
</tr>
<tr>
<td>D Chloroform extract</td>
<td>500</td>
<td>0.33 ± 0.053</td>
<td>35.2</td>
</tr>
<tr>
<td>E Methanol extract</td>
<td>500</td>
<td>0.48 ± 0.048</td>
<td>5.8</td>
</tr>
</tbody>
</table>

F ratio: 52.17 (calculated); 1.47 (critical)

Significant difference among groups, p = 0.05

- A and B-D
- B and A, C-E
- C and A, B, E
- D and A, B, E
- E and B-D
3.2.1.1 Study of the Oil

The physical characteristics of the oil were determined.

Specific gravity (25°C) = 0.9393
Refractive index (25°C) = 1.4679

The chemical characteristics of the oil were found. 362

These are given in Table 13.

Table 13: Chemical Characteristics of V. negundo Seed Oil.

<table>
<thead>
<tr>
<th>Obs. No.</th>
<th>Iodine value (ICl method)</th>
<th>Acid value</th>
<th>Saponification value</th>
<th>% Unsaponifiable matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>98.0</td>
<td>9.1</td>
<td>187.1</td>
<td>9.683</td>
</tr>
<tr>
<td>2.</td>
<td>97.9</td>
<td>9.1</td>
<td>187.6</td>
<td>9.647</td>
</tr>
<tr>
<td>3.</td>
<td>98.1</td>
<td>9.2</td>
<td>187.1</td>
<td>9.695</td>
</tr>
<tr>
<td>Mean</td>
<td>98.0</td>
<td>9.1</td>
<td>187.2</td>
<td>9.674</td>
</tr>
</tbody>
</table>

The oil (20 g) was saponified by refluxing with 0.5N ethanolic potassium hydroxide (200 ml) for 1 h and the unsaponifiable matter was separated. 362 The aqueous layer was acidified with dilute hydrochloric acid and extracted with ether (3x200 ml). The solvent was removed to get mixed fatty acids (17.3 g). The iodine value 362 and the saponification equivalent 363 were determined. These are given in Table 14.
Table 14: Chemical Characteristics of Mixed Fatty Acids from *V. negundo* Seed Oil.

<table>
<thead>
<tr>
<th>Obs. No.</th>
<th>Iodine value</th>
<th>Saponification equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>105.8</td>
<td>279.2</td>
</tr>
<tr>
<td>2</td>
<td>105.8</td>
<td>279.9</td>
</tr>
<tr>
<td>3</td>
<td>105.7</td>
<td>279.8</td>
</tr>
<tr>
<td>Mean</td>
<td>105.8</td>
<td>279.6</td>
</tr>
</tbody>
</table>

**Preparation of Methyl Esters**: The fatty acids (2.3 g) were converted into methyl esters by boiling with methanol (10 ml) containing 1% sulphuric acid for 2 h, and removing the unesterified acids by washing the ether solution of esters with potassium carbonate solution. The ether solution was washed with water, dried and evaporated to yield methyl esters (0.9 g).

**TLC of Methyl Esters**: Petroleum ether solution of methyl esters was spotted on silica gel G plates and developed using petroleum ether–ether–acetic acid (70:30:1) system. The plates were sprayed with concd sulphuric acid and heated at 150° for 10 min. The TLC profile was comparable to the methyl esters obtained from ground nut oil.
The unsaturated fatty acids were detected by TLC as silver ion complexes. A petroleum ether solution of methyl esters was spotted on plates prepared with silica gel G containing 12.5% silver nitrate and developed using petroleum ether-ether-acetic acid (90:10:1) system. For comparison, methyl esters prepared from linseed oil were run side by side. The spots were detected by spraying with concd sulphuric acid and heating at 150° for 10 min. The TLC profile was comparable to the methyl esters obtained from linseed oil.

**GLC of Methyl Esters**: The methyl esters of mixed fatty acids were analyzed by GLC on a polyester column (20% diethylene glycol succinate on chromosorb W, 2M x 1/8", 190°C) with a flame ionization detector. The peak areas were measured by triangulation. The results of the calculated composition are given in Table 1 (under Results and Discussion).

### 3.2.1.2 Study of the Unsaponifiable Matter

The oil (417 g) obtained from *V. negundo* seeds (14 kg) was refluxed with 1 N ethanolic potassium hydroxide (2L) in four batches and the unsaponifiable fraction (40.0 g) was separated. It was chromatographed over silica gel (800 g) column. The elution was carried out with a successive series of solvents in increasing order of polarity. The eluate was collected in 500 ml fractions in tared flasks, the solvent removed under reduced
pressure and the residue weighed. The eluates were monitored by TLC and the fractions with identical TLC pattern were combined. Elution with petroleum ether (5 x 500 ml) gave a residue (6.0 g) which was crystallized from n-hexane, mp 67° (4.83 g, 'Compound A').

Elution with petroleum ether-benzene (7:3, 5 x 500 ml) afforded a residue (0.5 g) which was crystallized from n-hexane, mp 87-88° (0.09 g, 'Compound B'). Further elution with petroleum ether-benzene (3:7, 5 x 500 ml) and benzene (7 x 500 ml) gave a residue (1.5 g) which was crystallized from n-hexane to give an entity, mp 82° (0.29 g, 'Compound C'). The elutions with benzene-chloroform (9:1, 8 x 500 ml) gave a sticky mass which on repeated chromatographic resolution over silica gel and crystallization from petroleum ether afforded needles, mp 129-130° (0.15 g, 'Compound D'). The eluates obtained with benzene-chloroform (1:1, 5 x 500 ml) gave a semisolid mass (0.15 g), which on further chromatographic resolution over silica gel and crystallization from chloroform-methanol mixture gave white needles, mp 155-156° (0.06 g, 'Compound E'). The residue (2.0 g) obtained on elution with chloroform (10 x 500 ml) was sticky in nature. This on crystallization from methanol gave white needles, mp 135-136° (1.02 g, 'Compound F'). Elution with chloroform-methanol (49:1) gave a sticky dark brown mass (10.19 g). This on repeated chromatography over silica gel and crystallization from petroleum ether (60-80°)-ethyl acetate gave yellow needles (0.13 g, 'Compound G'), mp 154-155°.
'Compound A' 

The IR spectrum showed bands at 2962, 2925, 2857 cm\(^{-1}\) (C-H stretching), 1472, 1464, 1378 cm\(^{-1}\) (C-H bending), 720 and 730 cm\(^{-1}\) \(-\left(\text{CH}_2\right)_X\). The IR spectrum and mp are in close agreement with the alkane fraction already isolated from the seeds of *V. nequundo*. 330

'Compound B' 

The IR spectrum showed bands at 2842 cm\(^{-1}\) (C-H stretching), 1460, 1370 cm\(^{-1}\) (C-H bending), 728 and 720 cm\(^{-1}\) \(-\left(\text{CH}_2\right)_X\). GLC resolution on a 6 ft x 1/8 in. column of 1.5% OV-101 on 100/200 HP chrom G, linear temperature programme 90-240° at 4°/min showed peaks corresponding to alkanes 28:0 (46.8%) and 30:0 (53.2%).

'Compound C' 

The IR spectrum showed absorption at 3300 cm\(^{-1}\) (O-H stretching), 720 and 730 cm\(^{-1}\) \(-\left(\text{CH}_2\right)_X\). GLC analysis on an SE-30 column (3%, 237°) revealed it to be a mixture of alcohols 26:0 (54.0%) and 28:0 (46.0%).

'Compound D' 

TLC on silica gel G with chloroform (R\(_f\) 0.36) and petroleum ether-ethyl acetate (8:2, R\(_f\) 0.62) gave single spot.

Anal.:

Yield : 0.001%

[\(\alpha\)]\(_D\)\(^{26}\) : +30.8° (c 0.003, CHCl\(_3\))
UV $\lambda_{\text{max}}^{\text{MeOH}}$: 276 nm ($\log \epsilon < 2.91$) and 267 nm ($\log \epsilon < 2.92$)

IR (Nujol): 3270 (br, O-H), 1040, 825 and 725 cm$^{-1}$.

$^1$H NMR (90 MHz, CDCl$_3$): $\delta$ 7.21 (d, $J = 8.5$ Hz, 1H, 11-H), 7.12 (dd, $J = 8.5$ Hz, 2.5 Hz, 1H, 12-H), 6.96 (d, $J = 2.5$ Hz, 1H, 14-H), 3.30 (dt, $J = 7.0$ Hz, 3.0 Hz, 1H, 6$\beta$-H), 2.90 (m, 3H, 7-CH$_2$ and 15-H), 1.46 (s, 1H, O-H), 1.25 (d, $J = 7.0$ Hz, 6H, 16-CH$_3$ and 17-CH$_3$), 1.22 (s, 3H, 20-CH$_3$), 1.08 (s, 3H, 19-CH$_3$), 0.91 (s, 3H, 18-CH$_3$).

$^{13}$C NMR (22.5 MHz, CDCl$_3$): Assignments of all the carbons are given in Table 2 (under Results and Discussion).

MS m/z (%): 286 ($M^+$, 51), 271 ($M^+-\text{CH}_3$, 67), 253 ($M^+-\text{CH}_3-\text{HOH}$, 100), 56 (74) and 43 (75).

Preparation of Acetate: The acetate was prepared by stirring 'Compound D' (0.08 g) with dry pyridine (0.6 ml) and acetic anhydride (0.3 ml) for 12 h at room temperature. The contents were poured in water. The separated solid was filtered, washed with water and dried. It was crystallized from methanol. The crystalline product (0.07 g) showed mp 108$^\circ$. TLC of the acetate gave single spot ($R_f$ 0.49, petroleum ether-ethyl acetate, 9:1). The IR spectrum of the acetate showed bands at 1738 cm$^{-1}$ (C=O stretching of esters) and 1250 cm$^{-1}$ (C—O stretching of acetate). Its $^1$H NMR (60 MHz) spectrum in CDCl$_3$ gave signals at $\delta$ 7.06 (m, 3H), 4.61 (m, 1H, CHOAc), 2.90 (m, 3H, 7-CH$_2$ and 15-H), 2.07 (s, 3H, OAc), 0.96 (s, 6H, 18-CH$_3$ and
19-CH$_3$), 1.20 (s, 3H, 20-CH$_3$), 1.21 (d, $J = 7.0$ Hz, 6H, 16-CH$_3$
and 17-CH$_3$). The EI mass spectrum gave peaks at m/z 328 (M$^+$, 69%),
and 253 (M$^+$ - CH$_3$ - CH$_3$COOH, 100%).

**Oxidation Product of 'Compound D'**: A solution of
'Compound D' (0.06 g) in acetone (1.5 ml) was stirred with
Jones reagent (0.003 g CrO$_3$ in 1.3 ml acetic acid) for 1 h at
room temperature. The mixture was poured in excess of water
and extracted with ether (4 x 25 ml). The ether extract was
washed with water, dried and evaporated under reduced pressure
to yield the residue (0.05 g). It was first passed through
a column of silica gel and then crystallized from methanol to
give plates (0.03 g), mp 89-90°. TLC on silica gel gave a
single spot (R$_f$ 0.62, chloroform). The IR spectrum of oxidation
product showed bands at 1720 cm$^{-1}$ (C = O stretching of ketone),
1508, 1470, 1440 and 837 cm$^{-1}$. In the EI mass spectrum the
peaks appeared at m/z 284 (M$^+$, 11%), 269 (M$^+$-CH$_3$, 20%), 268 (97%)
and 226 (100%).

The 'Compound D' has been characterized as 5β-hydro-8,11,
13-abietatrien-6β-ol (60).

**'Compound E'**

TLC on silica gel with chloroform (R$_f$ 0.34) and
petroleum ether-ethyl acetate (8:2, R$_f$ 0.59) gave single spot.

**Anal.**

**Yield**: 0.0004%
$[\alpha]_{D}^{25} = +18.51$ (c 0.0027, CHCl₃).

IR (KBr): 3390 (br O-H stretching), 1639, 1630 (C=C stretching), 1372, 1355 (gem-dimethyl) and 822 cm⁻¹ (C=CH₂).

¹H NMR (60 MHz, CDCl₃): δ 5.30 (br s, 2H, C=CH₂), 3.15 (m, 1H, CHOH). Signals for C-methyl groups appeared at δ 1.69 (s, 3H), 1.11 (s, 3H), 1.04 (s, 3H), 0.99 (d, J = 3.0 Hz, 3H), 0.93 (s, 3H), 0.83 (s, 3H) and 0.56 (s, 3H).

MS m/z (%): 426 (M⁺, 8), 326 (34) and 284 (86).

**Preparation of Acetate**: The acetate was prepared by stirring a mixture of 'Compound E' (0.025 g) with dry pyridine (0.2 ml) and acetic anhydride (0.1 ml) for 12 h at room temperature, following the procedure outlined under 'Compound D'. It was crystallized from chloroform-methanol to give an entity (0.014 g), mp 129-130°, exhibiting single spot on TLC (Rf 0.81, chloroform). The IR spectrum of the acetate gave bands at 1727 cm⁻¹ (C=O stretching of esters) and 1256 cm⁻¹ (C—O stretching of acetate). Its ¹H NMR spectrum in CDCl₃ gave signals at δ 5.31 (br, 2H, C=CH₂), 4.53 (m, 1H, CHOAc), 2.09 (s, 3H, OAc). The signals due to C-methyl groups appeared at 1.69 (s, 3H), 1.11 (s,3H), 1.07 (s, 3H), 0.99 (s, 3H), 0.94 (s, 3H), 0.87 (s, 3H), 0.56 (s, 3H). The EI mass spectrum of the acetate gave peaks at m/z 468 (M⁺,5%), 368 (21%), 325 (74%) and 44 (100%).

This compound has been identified as lanostane-8,25-C dien-3β-ol (61).
'Compound F'

'Compound F' gave positive Liebermann-Burchard test for sterols. The IR spectrum showed bands at 3390 cm\(^{-1}\) (O-H) and 980 cm\(^{-1}\) (trans-olefinic C-H out-of-plane bending). It showed no depression in mixed melting point with authentic sample of \(\beta\)-sitosterol. A mixture with the latter was inseparable on TLC. The IR spectra of 'Compound F' and \(\beta\)-sitosterol were identical.

'Compound G'

It gave positive ferric chloride test for phenols. TLC on silica gel gave single spot in two different solvent systems viz. benzene-acetone (9:1, \(R_f\) 0.50) and toluene-chloroform-acetone (4:0.25:3.5, \(R_f\) 0.83).

Anal.:

Yield : 0.001%

\(\text{UV}_{\lambda}^{\text{MeOH}}\) : 256 nm (log \(\varepsilon\) 4.26), 273 nm (log \(\varepsilon\) 4.18), 348 nm (log \(\varepsilon\) 4.21).

\(\text{IR (KBr)}\) : 3460 (O-H), 1668 (C=O), 1646, and 1559 cm\(^{-1}\) (C=C).

\(^1\text{H NMR (60 MHz, CDCl}_3\) : \(\delta\) 12.73 (s, 1H, OH), 7.78 (m, 2H, 2'-H and 6'-H), 7.02 (d, \(J = 9.0\) Hz, 1H, 5'-H), 6.52 (s, 1H, 8-H), 3.97 (s, 9H, 3 \times OCH\(_3\)), 3.94 (s, 3H, OCH\(_3\)) and 3.88 (s, 3H, OCH\(_3\)).

\(^{13}\text{C NMR (100 MHz, CDCl}_3\) : The assignments of all the carbons are given in Table 3 (under Results and Discussion).
**Preparation of Acetate**: The acetate was prepared by stirring a mixture of 'Compound G' (0.05 g) with dry pyridine (0.6 ml) and acetic anhydride (0.3 ml) at room temperature for 12 h and worked up by usual method. It was crystallized from methanol to give an entity (0.03 g), mp 156-157°. TLC of the acetate in benzene-acetone (9:1) gave single spot (Rf 0.47). The UV spectrum showed absorption at 240 nm (logε 3.45), 250 nm sh (logε 3.40), 335 nm (logε 3.50). The IR spectrum of the acetate showed bands at 1765 cm⁻¹ (C==O stretching of esters) and 1258 cm⁻¹ (C—O stretching of acetate). Its ¹H NMR spectrum (90 MHz) in CDCl₃ gave signals at δ 7.72 (m, 2H, 2'-H and 6'-H), 6.94 (d, J = 9.0 Hz, 1H, 5'-H), 6.86 (s, 1H, 8-H), 3.92 (s, 9H, 3 x OCH₃), 3.81 (s, 3H, OCH₃), 3.74 (s, 3H, OCH₃) and 2.53 (s, 3H, OAc).

The IR spectrum of 'Compound G' was superimposable to the IR spectrum of authentic artemetin. These data characterized the 'Compound G' as artemetin (65).
which on repeated chromatography and crystallizations did not yield anything conclusive. Elutions with chloroform-methanol (99:1, 2 x 500 ml) afforded a sticky residue (0.9 g). It was taken up in methanol. The methanol-soluble part was mostly the colouring matter and nothing could be isolated from this. However, the methanol-insoluble part on crystallization from ethyl acetate yielded an entity, mp 82° (0.028 g, 'Compound H'). Further elution with chloroform-methanol (99:1, 3 x 500 ml) gave a dark brown sticky mass (8.0 g). It could not be purified. However, it was acetylated with acetic anhydride and pyridine at room temperature. The acetate thus obtained, on chromatography and crystallization from methanol gave a crystalline entity, mp 195-196° (0.025 g, 'Compound I'). Further, a few eluates of the column with chloroform—methanol (98:2, 10 x 500 ml) afforded a dark green mass (3.2 g, 'Fraction J'). It was found to be a mixture of two entities. Resolution of the mixture on silica gel column gave two compounds. These were crystallized from benzene-acetone mixture and methanol, respectively. The former was labelled as 'Compound J-1', mp 204-205° (0.035 g) and the latter as 'Compound J-2', mp 222-224° (0.35 g). Subsequent elutions with the same solvent (15 x 500 ml) yielded a dark brown residue (7.0 g). It was picked up with acetone. The acetone-soluble part (3.0 g) on chromatography over silica gel and crystallization from methanol afforded yellow needles, mp 126-127° (0.53 g, 'Compound K'). The acetone-insoluble sticky mass (4.0 g) could not be purified. It was treated with acetic anhydride (15.0 ml) and pyridine (30.0 ml) at
room temperature and the product thus obtained was labelled as 'Fraction L'. TLC showed it to be a mixture of two entities. Both the entities were separated by column chromatography over silica gel. These were crystallized from petroleum ether-chloroform and chloroform-methanol, respectively. These were designated as 'Compound L-1', mp 182-183° (0.91 g) and 'Compound L-2', mp 205° (0.25 g). The eluates obtained with chloroform-methanol (95:5, 15 x 500 ml) yielded dark brown residue (7.5 g) which on rechromatography and crystallization from chloroform-methanol afforded an entity, mp 283° (0.42 g, 'Compound M'). Finally, elution with chloroform-methanol (90:10, 15 x 500 ml) gave dark brown sticky mass (4.0 g) which did not yield any pure entity even after repeated chromatography of the residue.

'Compound H'

The IR spectrum showed bands at 2928, 2859 cm⁻¹ (C-H stretching), 1710 cm⁻¹ (C = O stretching of acid), 1468 , 1435 cm⁻¹ (C-H bending), and 732, 722 cm⁻¹ [-(CH₂)ₓ⁻]. The Compound was methylated with diazomethane and its GLC resolution on an SE-30 column (3%, 237°) showed peaks corresponding to aliphatic acids 18:0 (trace), 18:1 (trace), 22:0 (1%), 23:0 (trace), 24:0 (16%), 25:0 (4%), 26:0 (54%), 27:0 (2%), 28:0 (19%) and 30:0 (2%).

'Compound I'

The compound gave positive Liebermann-Burchard test for triterpenoids. TLC on silica gel with chloroform-methanol (9.5:0.5, Rᶠ 0.32) and petroleum ether-ethyl acetate (7:3,Rᶠ 0.43)
gave single spot.

\textbf{Anal.}:

\begin{itemize}
  \item \textbf{Yield} : 0.0002\%
  \item \([\alpha]_D^{20} : +48.6^\circ \text{ (c 0.0109, CHCl}_3\text{)}
\end{itemize}

\textbf{IR (KBr)} : 2932, 2950 (C-H stretching), 1735 (C \equiv O stretching of esters), 1697 (C \equiv O stretching of carboxyl group), 1382, 1368 (gem-dimethyl) and 1245 cm\(^{-1}\) (C – O stretching of acetate).

\(1^H\text{NMR (90 MHz, CDCl}_3\text{)} : \delta 5.19 \text{ (t, J = 2.2 Hz, 1H, 12-H)}, 4.46 \text{ (t, J = 7.5 Hz, 1H, 3\textsuperscript{-}H)}, 1.98 \text{ (s, 3H, OAc)}, 1.02 \text{ (s, 3H)}, 0.91 \text{ (s, 6H)}, 0.81 \text{ (s, 6H)}, 0.72 \text{ (s, 3H)} \text{ and } 0.59 \text{ (s, 3H)}.

\(13^C\text{NMR (100 MHz, CDCl}_3\text{)} : \) The assignments of all the carbons are given in Table 4 (under Results and Discussion).

\textbf{MS m/z (\%)} : 498 (M\(^+\), 1), 438 (M\(^+\) – AcOH, 3), 248 (100), 203 (45) and 189 (28).

\textit{Hydrolysis of 'Compound I':} A solution of potassium hydroxide (0.035 g) in ethanol (1.5 ml) was added to 'Compound I' (0.012 g) and the reaction mixture was refluxed for 4 h. The contents were cooled and poured in water (10 ml). The resulting solution was acidified with dilute hydrochloric acid and extracted with ethyl acetate (4 x 15 ml). The ethyl acetate extract was washed with water and dried. Removal of solvent under reduced pressure yielded a white amorphous compound (0.008 g) mp 226-228\(^\circ\).
TLC of the hydrolyzed product with chloroform-methanol (9.5:0.5) gave single spot ($R_f$ 0.41). Its IR spectrum gave bands at 3434 cm$^{-1}$ (O-H stretching) and 1691 cm$^{-1}$ (C=O stretching of acid).

The study of spectral data of 'Compound I' and its derivative identified it to be $3\beta$-acetoxyolean-12-en-27-oic acid (66).

'Compound J-1'

The compound gave positive test for phenols. TLC on silica gel with chloroform-methanol-acetic acid (90:10:0.01 , $R_f$ 0.42) gave single spot.

**Anal.** :

Yield : 0.0003%

IR (KBr): 3500 (O-H), 1690 (C=O stretching of acid), 1600, 1580, 1575 and 1480 cm$^{-1}$ (C=C).

$^1$H NMR (60 MHz, CDCl$_3$-DMSO-d$_6$) : $\delta$ 7.54 (dd, $J = 9.0$ Hz, 2.0 Hz, 1H), 7.51 (d, $J = 2.0$ Hz, 1H), 6.84 (d, $J = 9.0$ Hz, 1H), 3.87 (s, 3H, OCH$_3$).

MS m/z (%) : 168 ($M^+$, 100), 153 ($M^+ -$ CH$_3$, 70), 125 (22) and 97 (31).

**Preparation of Acetate** : The acetate was prepared by stirring a mixture of 'Compound J-1' (0.015 g) with dry pyridine (0.2 ml) and acetic anhydride (0.1 ml) at room temperature.
The acetate was crystallized from petroleum ether-chloroform mixture to give an entity (0.008 g), mp 125°. TLC of the acetate with chloroform-methanol-acetic acid (95:5:0.01, Rf 0.37) showed single spot. The \( ^1 \)H NMR spectrum in CDC\(_3\) gave signals at \( \delta \) 7.79 (m, 2H), 7.17 (d, \( J = 8.6 \) Hz, 1H), 3.92 (s, 3H, OCH\(_3\)), 2.35 (s, 3H, OAc). The EI mass spectrum showed molecular ion peak at m/z 210 (17%).

The 'Compound J-1' showed no depression in mixed melting point with authentic vanillic acid\(^{366}\) prepared by oxidation of vanillin with silver oxide. The compound was found to be identical in all respects (co-TLC, IR and \( ^1 \)H NMR) with authentic vanillic acid.

'Compound J-2'

The compound gave positive Liebermann-Burchard test for triterpenoids. TLC on silica gel with chloroform-methanol (9:1, Rf 0.59) and petroleum ether-ethyl acetate (4:6, Rf 0.55) gave single spot.

**Anal. :**

Yield : 0.003%

\[ \begin{align*}
[\alpha]^{26}_D & : + 61.9^\circ \ (c \ 0.0021, \ MeOH). \\
\text{UV } \lambda_{\text{max}}^{\text{MeOH}} & : 224 \text{ nm (log } \varepsilon 2.61) \\
\text{IR (KBr) } & : 3200 \ (\text{br, O-H stretching}), 1700 \ (\text{C = O stretching, carboxyl group}), 1390, \ 1375 \ (\text{gem-dimethyl}) \text{ and } 1040 \text{ cm}^{-1}.
\end{align*} \]
$^1$H NMR (60 MHz, CDCl$_3$-DMSO-d$_6$) : $\delta$ 5.27 (br m, 2H, 6-H and 12-H), 3.87 (m, 1H, 2/3-H) and 3.34 (d, $J = 3.0$ Hz, 1H, 3β-H). The C-methyl groups appeared at 1.14 (3H), 1.09 (3H), 0.98 (3H), 0.96 (3H), 0.92 (3H), 0.84 (3H) and 0.81 (3H).

MS m/z (%) : 470 (M$^+$, 1), 452 (M$^+$ - HOH, 1), 434 (M$^+$ - 2 x HOH, 2), 425 (M$^+$ - COOH, 2), 248 (100), 203 (65), 189 (20) and 133 (39).

**Preparation of Acetate** : The acetate was prepared by stirring a mixture of 'Compound J-2' (0.025 g) with dry pyridine (0.4 ml) and acetic anhydride (0.2 ml) and worked up in the usual way. TLC of the acetate with chloroform-methanol (9.5:0.5, $R_f$ 0.31) showed single spot. Its $^1$H NMR spectrum in CDCl$_3$ gave signals at $\delta$ 5.28 (br m, 2H, 6-H and 12-H), 4.98 (br s, 1H, 2-H), 4.70 (br s, 1H, 3-H), 2.11 (s, 3H, OAc) and 1.96 (s, 3H, OAc). Signals due to C-methyl groups were observed at $\delta$ 1.25 (3H), 1.18 (3H), 1.12 (3H), 1.04 (3H), 0.97 (3H), 0.87 (3H) and 0.76 (3H).

**Preparation of Methyl Ester** : An icq cold solution of 'Compound J-2' (0.06 g) in methanol was treated with an excess of ethereal solution of diazomethane. The solvent was distilled. The residue (0.06 g) thus obtained was purified by passing through a silica gel column. This on crystallization from methanol gave needle-shaped crystals (0.035 g), mp 285-286°. TLC on silica gel with petroleum ether-ethyl acetate (7:3, $R_f$ 0.28) gave single spot. The IR spectrum gave bands at 1722 cm$^{-1}$ (C=O stretching of esters) and 1238 cm$^{-1}$ (C-O stretching of acetate). The EI mass spectrum gave peaks at m/z 484
Preparation of Acetonide: A solution of 'Compound J-2' (0.025 g) in dry acetone was refluxed with p-toluene-sulphonic acid (0.18 g) for 6 h. The reaction mixture was poured into sodium bicarbonate solution (10%, 20 ml) and extracted with dichloromethane (4 x 15 ml). The combined organic layer was washed with water, dried and purified by passing through a silica gel column. The acetonide (0.016 g) thus obtained gave single spot ($R_f$ 0.62) on TLC in chloroform-methanol (9:1). The EI mass spectrum of the acetonide gave peaks at $m/z$ 510 (1%), 464 ($M^+ - H - COOH$, 1%), 452 ($M^+ - CH_3COCH_3$, 1%), 406 (5%), 248 (100%) and 203 (48%).

These spectral characteristics of 'Compound J-2' and its derivatives identified it to be $2\alpha, 3\alpha$-dihydroxyolean-12,5-dien-28-oic acid (70).

'Compound K'

This compound gave positive test for phenols. TLC on silica gel with chloroform-methanol (9:1) and petroleum ether-ethyl acetate (4:6) gave single spot ($R_f$ 0.53 and 0.2 respectively).

Anal.: Yield: 0.005 %.

$[\alpha]_{D}^{26}$: $-176.0^\circ$ (c 0.005, MeOH).

UV $\lambda_{\text{max}}^{\text{MeOH}}$: 359 nm ($\log \epsilon$ 4.24), 255 nm ($\log \epsilon$ 4.31) and 207 nm ($\log \epsilon$ 4.44).
IR (KBr) : 3390 (br, O-H stretching), 2840 (C-H stretching of aldehyde), 1650 (C==O stretching of \( \alpha, \beta \)-unsaturated aldehyde), 1620, 1565 and 1515 cm\(^{-1}\) (C==C stretching).

\( ^1H \) NMR (90 MHz, CDCl\(_3\) — DMSO — d\(_6\) ) : \( \delta \) 9.5 (s, 1H, CHO), 7.32 (s, 1H, 1-H), 6.96 (s, 1H, 8-H), 6.70 (s, 1H, 5-H), 6.59 (br s, 1H, 2'-H), 6.54 (d, \( J = 8.0 \) Hz, 1H, 5'-H), 6.23 (dd, \( J = 8.0 \) Hz and 2.7 Hz, 1H, 6'-H), 4.32 (s, 1H, 4-H), 3.82 (s, 3H, OCH\(_3\)), 3.64 (s, 3H, OCH\(_3\)), 3.45 (d, \( J = 5.0 \) Hz, 2H, -OCH\(_2\)-), 3.28 (s, 1H, OH), 3.21 (s, 1H, OH), 3.12 (s, 1H, OH).

MS m/z (%) : 356 (M\(^+\), 62), 338 (M\(^+\) — HOH, 23), 325 (M\(^+\) — CH\(_2\)OH, 75).

\textbf{Preparation of Acetate (72) :} The acetate was prepared by stirring a mixture of 'Compound K' (0.1 g) with dry pyridine (1.0 ml) and acetic anhydride (0.5 ml). The acetate (0.091 g) thus obtained was purified by passing through a silica gel column. TLC of the acetate on silica gel with petroleum ether-ethyl acetate (4:6) gave single spot (R\(_f\) 0.69). The IR spectrum showed bands at 1760 cm\(^{-1}\) (C==O stretching of esters) and 1240 cm\(^{-1}\) (C-O stretching of acetate). The \( ^1H \) NMR spectrum in CDCl\(_3\) gave signals at \( \delta \) 9.67 (s, 1H, CHO), 7.43 (s, 1H, 1-H), 7.11 (s, 1H, 8-H), 7.03 (s, 1H, 5-H), 6.92 (d, \( J = 8.0 \) Hz, 1H, 5'-H), 6.65 (br s, 1H, 2'-H), 6.52 (dd, \( J = 8.0 \) Hz and 2.7 Hz, 1H, 6'-H), 4.28 (s, 1H, 4-H), 4.08 (d, \( J = 7.2 \) Hz, 2H, -CH\(_2\)O-), 3.96 (s, 3H, OCH\(_3\)), 3.68 (s, 3H, OCH\(_3\)). The signals for O-acetyl protons appeared at \( \delta \) 2.36 (3H), 2.32 (3H) and 2.01 (3H). The assignments
of all the carbons of $^{13}$C NMR spectrum (22.5 MHz) of the acetate (72) are given in Table 5 (under Results and Discussion). The EI mass spectrum gave the molecular ion peak at m/z 482 (1%).

The study of spectral data of 'Compound K' and its derivative characterized it to be 6-hydroxy-4-[(4-hydroxy-3-\(\text{-}\)) methoxyphenyl]-3-hydroxymethyl-7-methoxy-3,4-dihydronaphthalen -2-al (71).

'Compound L-1'

This compound also gave positive test for triterpenoids. TLC on silica gel with chloroform-methanol (9.5:0.5, Rf 0.57) and petroleum ether-ethyl acetate (7:3, Rf 0.30) gave single spot.

\textbf{Anal.:}

\begin{itemize}
  \item \textbf{Yield} : 0.008\%
  \item \text{[\(\alpha\)]$_D^{26}$} : +10.76° (c 0.0065, MeOH).
  \item UV $\lambda_{\text{max}}^{\text{MeOH}}$: 225 nm (log\(\epsilon$ 2.62).
  \item IR (KBr): 3355 (br, O-H stretching), 1745 (C\(\equiv\)O stretching of esters), 1698 (C\(\equiv\)O stretching, carboxyl group), 1250 (C-O stretching of acetate), 1380, 1367, 1030 and 1042 cm$^{-1}$.
  \item $^1$H NMR (60 MHz, CDCl$_3$): 5.28 (m, 2H, 6-H and 12-H), 4.72 (br s, 1H, 2\(\alpha\)-H), 4.64 (br s, 1H, 3\(\beta\)-H), 2.05 (s, 3H, OAc), 1.99 (s, 3H, OAc). The chemical shifts of C-methyl groups were observed at $\delta$ 1.26 (3H), 1.07 (6H), 0.90 (9H) and 0.76 (3H).
\end{itemize}
$^{13}$C NMR (CDCl$_3$, 22.5 MHz): The assignments of all the carbons are given in Table 6 (under Results and Discussion).

MS m/z (%): 554 ($M^+$, 1), 434 ($M^+ - 2 \times CH_3COOH$, 4), 419 (20), 248 (100), 203 (66), 189 (23) and 133 (44).

**Hydrolysis of 'Compound L-1':** A solution of potassium hydroxide (0.45 g) in ethanol (15 ml) was added to 'Compound L-1' (0.150 g) and the reaction mixture was refluxed for 4 h. The contents were cooled and worked up following the procedure outlined under 'Compound I'. This was obtained as a white amorphous compound (74), mp 245-246° (dec), (0.130 g). TLC of hydrolyzed product gave single spot ($R_f$ 0.57) in chloroform-methanol (9:1). Its IR spectrum gave bands at 3420 cm$^{-1}$ (O-H), 1700 cm$^{-1}$ (C = O stretching), 1460, 1367, 1380 and 1052 cm$^{-1}$.

The hydrolyzed product did not form the acetonide.

**Preparation of Methyl Ester of Hydrolyzed Product:** The hydrolyzed product (0.06 g) obtained above was converted to methyl ester with diazomethane. This on crystallization with methanol afforded needle-shaped crystals (0.03 g), mp 205-206°. TLC gave single spot ($R_f$ 0.64) in chloroform-methanol (9:1). Its IR spectrum showed bands at 3420 cm$^{-1}$ (O-H stretching), 1730 cm$^{-1}$ (C = O stretching of esters), 1265 cm$^{-1}$ (C-O stretching). The EI mass spectrum gave peaks at m/z 484 ($M^+$, 1%), 203 (100%) and 189 (24%).

These data identified the 'Compound L-1' as 2β, 3α,6-diacetoxyoleana-5,12-dien-28-oic acid (73).
'Compound L-2'

This compound gave positive test for triterpenoids. TLC on silica gel gave single spot with chloroform-methanol (9.5:0.5; Rf 0.51) and petroleum ether-ethyl acetate (7:3, Rf 0.22).

**Anal.:**

Yield : 0.002%

\[[\alpha]_D^{26}\] : -13.33° (c 0.0052, MeOH).

UV \(\lambda_{\text{max}}^{\text{MeOH}}\) : 225 nm (log \(\varepsilon\) 2.81).

IR (KBr) : 3250 (O-H stretching), 1760 (C==O stretching of esters), 1710 (C==O stretching of carboxyl group), 1390 and 1380 (gem-dimethyl), 1265 (C—O stretching of acetate), 1169, 1058, 1048 and 980 cm\(^{-1}\).

\(^1\)H NMR (60 MHz, CDCl\(_3\) ) : \(\delta\) 5.41 (m, 2H, 6-H and 12-H), 5.13 (dd, \(\delta\) = 10.0 and 4.0Hz, \(\text{H}, 2\beta\text{-H}\)), 4.76 (d, \(\delta\) = 10.0 Hz, 1H, 3\(\alpha\)-H), 2.55 (s, 1H, OH), 2.06 (s, 3H, OAc), 1.98 (s, 3H, OAc). The C-methyl groups appeared at \(\delta\) 1.24 (s, 3H), 1.21 (s, 3H), 1.06 (s, 3H), 0.99 (s, 3H), 0.96 (s, 3H), 0.90 (s, 3H) and 0.73 (s, 3H).

**MS m/z (%):** 57C (M\(^+\), 1), 552 (M\(^+\) - HOH, 1), 305(1), 264(2), 246(6) and 219(8).

The above spectral data spotted the compound as 2\(\alpha\), 3\(\beta\)-diacetoxy-18-hydroxyoleana-5,12-dien-28-oic acid (77).
'Compound M'

TLC of this compound on silica gel with chloroform-methanol (8:2) gave single spot (Rf 0.57). It was identified as β-sitosterol-D-glucoside.

3.3 INVESTIGATION OF VITEX NEGUNDO FLOWERS

3.3.1 STUDY OF THE PETROLEUM ETHER EXTRACT

The powdered flowers (280 g) were extracted with petroleum ether for 24 h in a Soxhlet apparatus. The solvent was distilled and the last traces removed under reduced pressure to yield a residue (12.8 g). It was chromatographed over silica gel (360 g) column. Elution with petroleum ether (6 x 200 ml) gave a white residue (1.6 g) which was crystallized from ethyl acetate, mp 62-63° (0.60 g). It was identified as an alkane. Elutions with petroleum ether-benzene mixtures (57 x 200 ml) and benzene-chloroform (95:5, 31 x 200 ml) gave yellowish sticky mass (3.0 g) which on repeated column chromatography and crystallization did not yield anything conclusive. Further elution with benzene-chloroform (9:1, 28 x 200 ml) yielded a light brownish residue (0.4 g), which was crystallized from ethyl acetate to afford a white amorphous entity, mp 75-78° (0.08 g). The IR spectrum of this fraction identified it to be an aliphatic alcohol. Elutions with different proportions of benzene-chloroform (53 x 200 ml) yielded a residue (1.1 g) which on crystallization from methanol afforded white crystals, mp 134-135° (0.02 g). It was identified as β-sitosterol. Elutions with chloroform-methanol (99:1, 10 x 200 ml)
and (98:2, 5 x 200 ml) afforded a green sticky mass (3.0 g). This on purification by passing through a silica gel column and crystallization from petroleum ether-ethyl acetate mixture gave yellow needle-shaped crystals, mp 154-155° (0.05 g). It was identified as artemetin (65). Finally, elutions with various proportions of chloroform-methanol gave a residue (2.0 g). This on column chromatography and crystallization did not yield anything substantial for further study.

3.3.2 STUDY OF THE CHLOROFORM EXTRACT

The marc left after extraction of flowers (950 g) with petroleum ether was exhausted with chloroform in a Soxhlet apparatus. The solvent was removed under reduced pressure to yield a dark green residue (20.0 g). It was chromatographed over silica gel (400 g). Elutions with chloroform-methanol (95:5, 17 x 200 ml) gave an entity (0.1 g), mp 281-282°. It was identified as β-sitosterol-D-glucoside.

3.4 INVESTIGATION OF VANDA ROXBURGHII ROOTS

3.4.1 STUDY OF THE PETROLEUM ETHER EXTRACT

The powdered roots (2.1 kg) of V. roxburghii were extracted exhaustively with petroleum ether (60-80°) by hot extraction in a Soxhlet apparatus. The solvent was distilled under reduced pressure to yield the residue (31.5 g). It was chromatographed over silica gel (750 g) column. Elution with petroleum ether (3 x 500 ml) gave a residue (2.4 g) which was crystallized from
ethyl acetate, mp 62-63° (2.0 g). The IR spectrum spotted this to be an alkane. Elutions with petroleum ether-benzene (9:1, 5 x 500 ml; 8:2, 10 x 500 ml; 1:1, 8 x 500 ml) gave a residue (0.6 g) which could not be purified. The later elutions with petroleum ether-benzene (1:1, 4 x 500 ml) gave a sticky residue (1.5 g) which was crystallized from ethyl acetate to yield an amorphous material, mp 69-70° (0.5 g). IR spectrum of the compound identified it to be a wax ester. The residue (3.1 g) obtained on elution with petroleum ether-benzene (2:8, 30 x 500 ml) and benzene (17 x 500 ml) was sticky in nature. This on repeated column chromatography and crystallization did not yield anything conclusive. The later elutions with benzene (20 x 500 ml) gave a residue (1.4 g) which was crystallized from ethyl acetate to yield an entity, mp 86-87° (0.14 g). IR spectrum spotted it to be an aliphatic alcohol. The mother liquor left after the removal of aliphatic alcohol gave a yellowish semi-solid mass (1.26 g). It was refluxed with methanol for 30 min and kept overnight at room temperature. The methanol-soluble part (0.36 g) did not yield any pure entity. The methanol-insoluble part (0.9 g) was treated with pyridine (4 ml) and acetic anhydride (2 ml) mixture at room temperature. The acetylated product (0.91 g) thus obtained on repeated column chromatography and crystallization from petroleum ether afforded white crystals, mp 83-84° (0.08 g, 'Compound N'). The eluates obtained from different proportions of benzene-chloroform mixtures (22 x 500 ml) and chloroform (17 x 500 ml) gave a residue (2.1 g) which did not yield any
pure entity. The residue (12.8 g) obtained on elutions with chloroform-methanol (99:1, 12 x 500 ml; 98:2, 10 x 500 ml) was a dark brown sticky mass. It was refluxed with methanol for 1 h and kept at room temperature for 12 h. The methanol-soluble part (5.2 g) was rechromatographed over silica gel to get two crystalline entities. These were identified as wax acid (0.04 g), mp 66-67° and β-sitosterol (0.43 g), mp 134-135°. The methanol-insoluble part (7.6 g) was resinous in nature and it could not be purified. Finally, the elution was done with chloroform-methanol (95:5, 10 x 500 ml). The residue (3.0 g) thus obtained did not yield anything worthwhile for further studies.

*Compound N*

TLC of this compound on silica gel gave single spot with chloroform (Rf 0.46) and petroleum ether-ethyl acetate (9:1, Rf 0.41).

**Anal.**

Yield : 0.004 %

\[ \text{UV}_{\text{max}}^{\text{MeOH}} : 309 \text{ nm (log ε 4.00)}, 280 \text{ nm (log ε 4.28)}, 232 \text{ nm (log ε 4.06) and 214 } \text{nm (log ε 4.08).} \]

\[ \text{IR(KBr)} : 2935, 2860 (C-H stretching), 1768 (acetoxy carbonyl), 1710 (\alpha, \beta-unsaturated ester carbonyl), 1634, 1602 (C = C stretching), 1245, 1230 (C - O stretching of acetate and ester) \]

and 725 cm\(^{-1}\) \(\text{[-(CH}_2)_x\text{]-}.\)
$^1$H NMR (60 MHz, CDCl$_3$): $\delta$ 7.73 (d, $J = 16.0$ Hz, 1H, Phenyl-$CH$), 7.25 (br s, 3H), 6.45 (d, $J = 16.0$ Hz, $=CH-COOR$), 4.22 (t, $J = 6.0$ Hz, 2H, $-OCH_2$), 3.88 (s, 3H, OCH$_3$), 2.27 (s, 3H, OAc), 1.29 (br s, 44H, $-(CH_2)_{22}$), 0.85 (t, $J = 5.5$ Hz, 3H, CH$_3$).

MS m/z (%): 572 (M$^+$, 1), 530 (6), 502 (100), 219 (4), 194 (39), 177 (37) and 137 (13).

Hydrolysis of 'Compound N' : A solution potassium hydroxide (0.12 g) in ethanol (4.0 ml) was added to 'Compound N' (0.05 g) and the reaction mixture was refluxed for 1 h. The contents were cooled and poured in water (30 ml) and extracted with chloroform (3 x 25 ml). The chloroform extract was washed with water, dried and removal of solvent under reduced pressure afforded a residue (0.03 g). It was crystallized from petroleum ether to yield an entity, mp 78° (0.019 g). It was identified as tetracosanol. The aqueous portion was acidified with dilute hydrochloric acid. It was extracted with ethyl acetate (4 x 25 ml). The ethyl acetate extract was washed with water, dried and removal of solvent under reduced pressure gave a residue (0.011 g). It was identified as ferulic acid from the spectral data and comparison with authentic sample.

These spectral data and chemical characteristics identified the 'Compound N' as tetracosylferulate (78).
3.4.2 STUDY OF THE CHLOROFORM EXTRACT

The defatted roots (Sect. 3.4.1) were extracted exhaustively in a Soxhlet apparatus to yield a residue (48.3 g) which on chromatographic separation yielded $\beta$-sitosterol-D-$\beta$-glucoside (0.52 g).

3.5 INVESTIGATION OF ACAMPE PAPILLOSA

3.5.1 STUDY OF THE ROOTS

The powdered roots (1.3 kg) of *A. papillosa* on hot extraction with petroleum ether yielded the residue (45 g). It was chromatographed over silica gel (800 g) column. Elution with petroleum ether (3 x 500 ml) afforded a residue (0.9 g) which was crystallized from ethyl acetate, mp 56-58° (0.56 g). It was identified as $n$-alkane. The eluates obtained with petroleum ether-benzene (1:1, 11 x 500 ml) gave a sticky residue (1.0 g) which on crystallization from ethyl acetate gave an entity, mp 72-73° (0.20 g), characterized as $n$-alkanol. Further, elution with chloroform (14 x 500 ml) gave a sticky residue (5.5 g), which afforded $\beta$-sitosterol on chromatographic resolution over silica gel.

The chloroform extract (44 g) from defatted roots (790 g) on column chromatography over silica gel yielded $\beta$-sitosterol-D-$\beta$-glucoside (0.08 g).

3.5.2 STUDY OF THE STEM AND LEAVES

The powdered stem and leaves (500 g) of *A. papillosa* on hot extraction with petroleum ether yielded a residue
(36.5 g). This on column chromatography over silica gel afforded a \( \text{n-alkane} \) (mp 55-56°, 0.50 g), \( \text{n-alkanol} \) (mp 84°, 0.32 g) and \( \beta \)-sitosterol (0.02 g).

Chloroform extract (18.5 g) of defatted stem and leaves gave \( \beta \)-sitosterol-D-glucoside (0.053 g).

3.6 ANTIARTHRITIC ACTIVITY OF 'COMPOUNDS D, G, J-2, K' AND HYDROLYZED PRODUCT OF 'COMPOUND L-1'.

The 'Compounds D (60) and G (65), obtained through the chromatographic fractionation of unsaponifiable matter of the petroleum ether extract and the 'Compounds J-2 (70), K (71)' and the hydrolyzed product (74) of 'Compound L-1', obtained from chromatographic separation of chloroform extract of \textit{V. negundo} seeds were subjected to screening for antiinflammatory activity using the procedure mentioned earlier (Sect. 3.1). The doses used for all these compounds were 50 mg/kg, p.o. and the results are expressed in Tables 15 and 16.
Table 15: Percent Inhibition of Oedema (at the end of 3.5 h) by 'Compound D and G'.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg, p.o.</th>
<th>Mean increase in paw volume ml ± S.D.</th>
<th>% Inhibition of oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Control</td>
<td>-</td>
<td>0.61 ± 0.061</td>
<td>-</td>
</tr>
<tr>
<td>B Standard (Ibuprofen)</td>
<td>50</td>
<td>0.23 ± 0.044</td>
<td>62.1</td>
</tr>
<tr>
<td>C 'Compound D' (60)</td>
<td>50</td>
<td>0.50 ± 0.078</td>
<td>18.1</td>
</tr>
<tr>
<td>D 'Compound G' (65)</td>
<td>50</td>
<td>0.41 ± 0.077</td>
<td>32.4</td>
</tr>
</tbody>
</table>

F ratio: 52.17 (calculated); 1.47 (critical)

Significant difference among groups, p = 0.05
- A and B - D
- B and A, C, D
- C and A, B, D
- D and A - C
Table 16: Per cent Inhibition of Oedema (at the end of 3.5 h) by 'Compounds J-2, K' and the Hydrolyzed Product of 'Compound L-1'.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose mg/kg, p.o.</th>
<th>Mean increase in paw volume ml ± S.D.</th>
<th>% Inhibition of oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td>A  Control</td>
<td>-</td>
<td>0.66 ± 0.091</td>
<td>-</td>
</tr>
<tr>
<td>B  Standard (Ibuprofen)</td>
<td>50</td>
<td>0.24 ± 0.045</td>
<td>63.2</td>
</tr>
<tr>
<td>C  'Compound J-2' (70)</td>
<td>50</td>
<td>0.54 ± 0.087</td>
<td>18.7</td>
</tr>
<tr>
<td>D  'Compound K' (71)</td>
<td>50</td>
<td>0.39 ± 0.092</td>
<td>40.6</td>
</tr>
<tr>
<td>E  Hydrolyzed Product of 'Compound L-1' (74)</td>
<td>50</td>
<td>0.43 ± 0.071</td>
<td>34.3</td>
</tr>
</tbody>
</table>

F ratio: 52.17 (calculated); 1.47 (critical)

Significant difference among groups, p = 0.05

A and B - E
B and A, C - E
C and A, B, D, E
D and A - C
E and A - C.