PART I. THE ISOLATION AND CHARACTERIZATION OF ALKALOIDS OF CAULOXYLUM THALICTROIDES (L.) MICHX.

PART II. THE ISOLATION AND CHARACTERIZATION OF ALKALOID AND NEUTRAL PRINCIPLES OF MAGNOLIA ACUMINATA L.

DISSEPTION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

by

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***

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Approved by

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Adviser
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PART I

THE ISOLATION AND CHARACTERIZATION OF ALKALOIDS OF

CAULOPHYLLUM THALICTROIDES (L.) MICHAEL.
INTRODUCTION AND HISTORICAL

A. Botanical Description (1)

Caulophyllum thalictroides (L.) Michx. (family, Berberidaceae) is commonly called blue-cohosh or squaw root. According to Lloyd (1), blue-cohosh is derived from the Algonkian word cohosh which literally means rough with hairs and its color from the bluish color of the plant and berries. The latter common name was introduced by Peter Smith who stated that Indian squaws used the rhizomes to facilitate childbirth and for certain uterine disorders. The genus name Caulophyllum is derived from two Greek words meaning stem and leaf while the species was so named by Linnaeus, because of the similarity of the plant leaves to those of the genus Thalictrum.

This North American plant, which grows to a height of two feet, is found in rich shady woods from New Brunswick and Southern Ontario to the Southern Alleghenies and between the Eastern coastal plain and the Mississippi Valley.

B. Prior Investigations

The plant was first examined by Mayer (2) who found it to contain a saponin and an alkaloid, while Elbert (3) later confirmed the presence of the
saponin but did not detect the alkaloid. In 1887 Lloyd (1) succeeded in isolating the crystalline saponin as well as the alkaloid hydrochloride.

A thorough chemical examination of *C. thalictroides* was carried out by Power and Salway (4) who isolated and identified the tertiary alkaloid as methylcytisine (I). Methylcytisine had been previously prepared as a derivative of the alkaloid cytisine by Partheil (5). Power and Salway likewise succeeded in crystallizing the saponin designated caulosaponin.

Acid hydrolysis of the saponin gave caulosapogenin, whose identity was confirmed by McShefferty and Stenlake (6) who found it to be identical to hederagenin (II). Power and Salway had isolated another saponin, caulophyllosaponin, but its presence could not be confirmed by McShefferty and Stenlake (6).

The Power and Salway investigation showed the presence of a phytosterol, *C₂₇H₄₆O* (m.p. 153⁰), citrullol (III) and the long-chained fatty acids oleic (IV), linoleic (V) and cerotic (VI).

The structure of citrullol was determined by Obata and co-workers (7) who isolated it from sugar beets.
C. **Occurrence of the Lupine Alkaloids**

In the main, lupine alkaloids have been isolated from numerous genera of the *Leguminosae* family (16). The presence of the lupine alkaloids, methylcystine (I), baptifoline (VII) and anagyrine (VIII) (8) in *C. thalictroides* represents one of the rare occurrences of this type of alkaloid in the family *Berberidaceae*. An examination of *Caulophyllum robustum* by Tomita and Takahashi (9) has revealed the presence of magnoflorine (IX) which was likewise observed in *C. thalictroides* (8). A later investigation (10) of *C. robustum* revealed the presence of methylcystine (I), d-lupanine (X) and taspine (XI).

The only other genus of the family *Berberidaceae* found to contain lupine alkaloids is *Leontice*. *Leontice albertii* has been shown to contain methylcystine, leontine (XII) and leontidine (C\(_{14}\)H\(_{18}\)ON\(_{2}\)) (11). *Leontice eversmannii* has been shown to contain leontamine (C\(_{14}\)H\(_{26}\)N\(_{2}\)) (12), taspine (XI), leontidine, d-sparteine (XIII), 1-lupanine (X), isoleontine (C\(_{15}\)H\(_{24}\)ON\(_{2}\)) and leontine (XII) (13).

Other isolated examples of lupine alkaloids have been found in *Anabasis aphylla* (family, *Chenopodiaceae*) (14) and *Chelidonium majus* (family, *Papaveraceae*) (15).

D. **Objective of Research**

In this laboratory there had been a concerted effort in the investigation of plants for potential therapeutic agents. One of the areas of effort was
to reinvestigate plants that had at one time been classified as an official drug but in recent times had been dropped from the official compendia. It was felt that if the older drugs were reinvestigated with modern methodology, that perhaps some of the pure compounds isolated might prove to be potential therapeutic agents. One such plant is *Caulophyllum thalictroides* (L.) Michx. which was in the "United States Pharmocopeia" from 1882-1905 and in the "National Formulary" from 1916-1950. Thus, *C. thalictroides* was chosen as a plant for investigation of possessing constituents of potential therapeutic activity.
EXPERIMENTAL

A. Source of Plant Material

The powdered roots and rhizomes of *Caulophyllum thalictroides* (L.) Michx. used in this investigation were obtained from S. B. Penick and Company (lot No. 668 BA W-80576).

B. Methodology—Chemical and Physical Analysis

1. Infrared spectra were taken in chloroform, Nujol mull or as a potassium bromide pellet on a Perkin-Elmer model 237 or 257 infrared spectrophotometer.\(^1\)

2. Ultraviolet spectra were determined in methanol or ethanol on a Cary Model 15 recording spectrophotometer.\(^2\)

3. Nuclear magnetic resonance (NMR) spectra were determined in deuterodimethylsulfoxide with tetramethylsilane as an internal standard on a Varian A-60 or A-60A Nuclear Magnetic Resonance Spectrometer.\(^3\) The NMR patterns were abbreviated as follows: singlets (s), doublets (d), triplets (t), quartets (q) and multiplets (m).

---

\(^{1}\) Perkin-Elmer Corporation, Norwalk, Connecticut

\(^{2}\) Allied Physics Corporation, Monrovia, California

\(^{3}\) Varian Associates, Palo Alto, California
4. Melting points were determined using a Thomas-Hööver Unimelt Apparatus in sealed evacuated capillaries and are corrected.

5. Microanalyses were determined by Scandinavian Micronanalytical Laboratory, Herlev, Denmark and Mr. J. Alicino, Metuchen, New Jersey.

6. The mass spectra were taken with an AEI MS-9 mass spectrometer.

7. Thin layer chromatography was performed on silica gel G with a thickness of 250 µ using 5 x 20 cm. or 20 x 20 cm. glass plates. Alkaloids were detected with Dragendorff's spray reagent (17).

8. Partition chromatographic analysis was carried out using a solvent system of Skellysolve B-ethylene dichloride-methanol-water (10:6:2.5:0.5) on Celite 545.

9. Ion exchange analysis was carried out using Amberlite IRA-410 (CI), 20-50 mesh.

---


5 Allied Electrical Industries

6 Made by E. Merck (Darmstadt, West Germany) Distributor: Brinkmann Instruments, Inc., Westburg, New York

7 Johns-Manville Corporation, New York, New York

8 Rohm and Haas, Philadelphia, Pennsylvania, Distributor: Mallinckrodt Chemical Works, St. Louis, Missouri
10. Absorption column chromatography was performed with Woelm neutral alumina\(^9\) or 100 mesh silicie acid\(^{10}\) – Celite 545 (4:1).

11. Optical rotations were determined using a Carl-Zeiss polarimeter.

C. Purity of Reagents

All reagents used in this investigation were of analytical purity, unless otherwise specified. The Skellysolve B was a petroleum ether fraction, b.p. 60–80\(^\circ\) and the ethylene dichloride was C.P. Extractions were carried out with U.S.P. ethanol.

D. Extraction and Initial Separation

The dried powdered plant material (13.7 kg.) was extracted by percolation at room temperature with about 74 liters of ethanol until the material was completely exhausted of alkaloids as evidenced by a negative Valser's test. The solvent was evaporated in vacuo at 40\(^\circ\) to give 2.24 kg. of residue. This was partitioned between chloroform and 2 percent aqueous citric acid in 100 g. quantities requiring 300 ml. of each phase.

\(^9\) Obtained from Alupharm Chemicals, New Orleans, Louisiana

\(^{10}\) Mallinckrodt Chemical Works, St. Louis, Missouri

\(^{11}\) Carl Zeiss, Oberkochen/Wuerttemberg, Germany
The citric acid phase was extracted three times with equal volumes of chloroform and then brought to pH 9 with concentrated ammonium hydroxide, and then extracted three times with equal volumes of chloroform. The combined chloroform extract was dried over anhydrous sodium sulfate and on evaporation of the solvent at reduced pressure gave 30.6 g. of crude tertiary alkaloids.

The ammonium hydroxide solution, after removal of the tertiary alkaloids, was acidified with 10% hydrochloric acid to give a gelatinous precipitate which was collected by filtration. The precipitate was treated with 600 ml. of hot n-amyl alcohol to give a yellow alcohol layer and a water layer. An excess of ether was added to the alcohol layer to give a yellow amorphous precipitate of caulosaponin.

The acidic filtrate was treated with a saturated 2% aqueous ammonium reineckate (w/v) while stirring. The precipitate of alkaloid reineckate was collected by suction filtration, sucked dry, and then washed with ether to give a yield of 285 grams. (See Figure 1 for flowsheet.)

E. Identification of Caulosaponin

The amorphous precipitate of caulosaponin was dissolved in hot absolute ethanol and decolorized with charcoal. On cooling, colorless globular crystals deposited (594 mg.) m.p. 257-259°. The melting point indicated that
Figure 1. Flowsheet for Separation of *C. thalictroides* Extract.
this compound might be caulosaponin. The literature values are m.p. 250-
255° (4) and 254–255° (6).

The isolated compound was soluble in dilute sodium hydroxide solu-
tion, and also gave a purple-red coloration, as does caulosaponin, in the
Liebermann–Burchardt test. An aqueous ethanolic solution of the compound
gave a persistent froth on shaking indicating it to be a saponin.

The saponin's infrared spectrum, determined in a potassium bromide
pellet, showed characteristic peaks at $\nu_{\text{max}}$ 3540, 3350, 1720, 1885, 1460,
and 1380 cm$^{-1}$.

1. Hydrolysis of Caulosaponin

To a solution of 200 mg. caulosaponin in 7 ml. 95% ethanol was
added 1.5 ml. 10% hydrochloric acid. The solution was refluxed for 5 hours
and upon cooling deposited $\xi$5 mg. of a crystalline product which was col-
lected by filtration.

This product was crystallized from absolute ethanol to give needles
of hederagenin (II), m.p. 333–335° (literature value m.p. 332–333° (6)). The
specific rotation was $[\alpha]_D^{29} + 80^\circ$ (c, 0.10% EtOH) and ultraviolet end ab-
sorption $\lambda$ EtOH 210 $\mu$ ($\varepsilon$ 4, 380). The infrared spectrum showed characteris-
tic bands at 3440, 3250, 1700, 1460 and 1380 cm$^{-1}$. McShefferty and Stenlake
(6) report a rotation of $[\alpha]_D^{29} + 78^\circ$ (c, 0.10% EtOH) and ultraviolet end absorp-
tion $\lambda$ EtOH 210 $\mu$ ($\varepsilon$ 2, 860).
2. Preparation of Hederagenin Acetate

To 0.5 ml. acetic anhydride was added 30 mg. of hederagenin. After refluxing for 1.5 hr. the solution was cooled and ice added followed by 10 ml. water. The aqueous layer was extracted with chloroform three times with equal volume and the extract dried over sodium sulfate and evaporated at reduced pressure to give 68 mg. of product. The residue was crystallized from aqueous methanol to give 20 mg. hederagenin acetate, m.p. 172-174°.) (6)

F. Isolation of Magnoflorine Chloride

The quaternary alkaloid reineckate (190 g.) was dissolved in 3 liters of acetone-water (1:1) and the solution passed through an ion exchange resin column containing 1700 ml. of Amberlite IRA-410 (Cl) resin to give 81 g. of crude quaternary chloride.

The entire crude chloride was dissolved in methanol and applied to a column (4.3 cm. I.D. x 54 cm.) of Woelm neutral alumina (800 g.) activity V suspended in methanol. Elution of the column with methanol was continued until the eluate gave a negative Dragendorff's test. Evaporation of the eluate gave 62.5 g. of crude magnoflorine chloride.

After crystallization from methanol, the magnoflorine chloride (IX) had m.p. 240-241° dec., [α]D^27 + 208° (c, 0.144 MeOH). The reported values (18) are m.p. 242-243° dec. and [α]D^31 + 213°. The isolated alkaloid and authentic magnoflorine chloride on silica gel G using a solvent system of n-
propanol–ammonium hydroxide–water (4:1:1) gave an identical \( R_f \) value of 0.62. The ultraviolet spectrum was characteristic of an aporphine, \( \lambda_{\text{Max}} \text{EtOH} \)

\begin{align*}
326 \text{m} &\quad (\epsilon \ 6,450) \\
279 \text{m} &\quad (\epsilon \ 15,100) \\
230 \text{m} &\quad (\epsilon \ 36,300).
\end{align*}

The infrared spectrum of the isolated substance and that of an authentic sample of magnoflorine chloride were superimposable.

1. Magnoflorine Perchlorate

Magnoflorine chloride (6 mg.) was dissolved in 5 ml. of glacial acetic acid on a steam bath. One drop of 70% perchloric acid was added to give a crystalline salt which was filtered and washed with ether. The material decomposed at 256\(^0\) (literature value (19) 257–258\(^0\) dec.).

G. Separation and Identification of the Tertiary Alkaloids

1. Partition Chromatography

Thin layer chromatography of the tertiary alkaloids on silica gel G with chloroform–methanol–ammonium hydroxide (200:20:1) indicated the presence of six tertiary alkaloids when detected with Dragendorff's reagent.

A test partition column according to the procedure of Brown and Kupchan (20) readily separated the tertiary alkaloids using a solvent system of Skellysolve B–ethylene dichloride–methanol–water (10:6:2.5:0.5). A column (3.7 cm. I.D. x 47 cm.) was prepared by mixing 182 ml. of stationary phase (upper phase) with 300 g. of Celite 545 and packing the material in 1 cm. segments with the aid of a ramrod. The mobile phase was passed through the
packed column until all the air had been removed (about 2 days). The holdup volume of the column was 395 ml.

The tertiary alkaloid mixture (1.32 g.) in 20 ml. of mobile phase was passed into the column. The flow rate was 30 ml. per hour. The effluent was collected in 20 ml. fractions after passage of the holdup volume and analyzed by thin layer chromatography (TLC). A summary of the separation obtained is given in TABLE 1.

Of the pooled fractions which were homogeneous by TLC, only fractions 2, 3 and 8 gave crystalline free bases or crystalline salts. All other fractions remained as oils and crystalline salt formation was unsuccessful. The non-crystallizable oils accounted for 9% of the crude tertiary alkaloid fraction.

2. Isolation of Anagyrine (VIII)

a. Anagyrine Perchlorate

Part of the pooled fraction 2 (69 mg.) was dissolved in 5 ml. methanol. To this was added 2 drops of 70% perchloric acid to give 45 mg. of rosettes which were filtered and washed successively with methanol and ether. After recrystallization from methanol, the rosettes (34 mg.) melted at 315°. Anagyrine perchlorate melts at 315° (21). The infrared spectrum of the perchlorate showed characteristic peaks of an α-pyridone (1650, 1570 and 1560 cm⁻¹) and the absence of any peak above 3000 cm⁻¹. These values are consistent with the values given by Okuda and co-workers (22). The ultraviolet
TABLE 1
Separation of Tertiary Alkaloid Mixture

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Tube No. ml.</th>
<th>TLC R_f</th>
<th>Pooled Wt. mg.</th>
<th>Crystalline Product, mg.</th>
<th>% yield</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12 - 14</td>
<td>0.74</td>
<td>35</td>
<td>...</td>
<td>...</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>16 - 19</td>
<td>0.67</td>
<td>144</td>
<td>109 b</td>
<td>0.012</td>
<td>Anagyrine</td>
</tr>
<tr>
<td>3</td>
<td>21 - 28</td>
<td>0.60</td>
<td>343</td>
<td>194</td>
<td>0.033</td>
<td>Methylcytisine</td>
</tr>
<tr>
<td>4</td>
<td>43 - 50</td>
<td>0.38</td>
<td>25</td>
<td>...</td>
<td>...</td>
<td>B</td>
</tr>
<tr>
<td>5</td>
<td>54 - 58</td>
<td>0.28</td>
<td>14</td>
<td>...</td>
<td>...</td>
<td>C</td>
</tr>
<tr>
<td>6</td>
<td>71 - 85</td>
<td>0.27</td>
<td>22</td>
<td>...</td>
<td>...</td>
<td>D</td>
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<tr>
<td>7</td>
<td>91 - 104</td>
<td>0.24</td>
<td>22</td>
<td>...</td>
<td>...</td>
<td>E</td>
</tr>
<tr>
<td>8</td>
<td>105 - 165</td>
<td>0.28</td>
<td>241</td>
<td>118</td>
<td>0.020</td>
<td>Baptifoline</td>
</tr>
</tbody>
</table>

a Based on dry plant material
b As perchlorate salt
spectrum of the perchlorate salt showed $\lambda_{\text{Max}}^{\text{EtOH}} = 308 \mu \text{ (e 7,460)}$ and $234 \mu \text{ (e 6,900)}$, which is characteristic of an $\alpha$-pyridone (22).

b. Anagyrine Picrate

Anagyrine picrate was prepared by heating 6 mg. of anagyrine and 6.1 mg. of picric acid (1:1 moles) in 1.0 ml. methanol for 10 minutes. After cooling, the yellow prisms of the picrate formed which melted at 247-249$^\circ$. The reported value (23) for anagyrine picrate is 249-251$^\circ$.

3. Isolation of Methylcytisine (I)

From pooled fraction number 3, the 343 mg. of crude material was crystallized from benzene-Skellysolve B to give 94 mg. of methylcytisine (I), m.p. 137$^\circ$ [\(\alpha\)]$_D^{30} = -224^0$ (c, 1.05 water); (literature value (4) m.p. 137$^\circ$ [\(\alpha\)]$_D^{20} = -221.6^0$); $\lambda_{\text{Max}}^{\text{EtOH}} = 309 \mu \text{ (e 7,560)}, 234 \text{ (e 6,860)}$. The infrared spectrum showed the absence of any peak above 3000 cm$^{-1}$ and the presence of peaks at 1650, 1570 and 1550 cm$^{-1}$.

a. Synthetic Methylcytisine

Cytisine (50 mg.) was dissolved in 0.13 ml. of 39% formic acid followed by the addition of 0.15 ml. of 37% formaldehyde (24). The mixture was heated for 3 hours at 95$^\circ$. After cooling, 12.0 ml. water was added and the mixture was extracted 3 times with 15 ml. of ether. The aqueous layer was basified with concentrated ammonium hydroxide and extracted 3 times with 25 ml. of ether. The combined ether layers were dried over sodium sulfate. The dried ether solution was evaporated to give 29 mg. of a residue
which was crystallized from ether to give synthetic methylcytisine, m.p. 137\(^\circ\).

The mixture melting point with the isolated methylcytisine showed no depression and the infrared and ultraviolet spectrum were identical, as well as a thin layer comparison of the two compounds.

b. Methylcytisine Perchlorate

Methylcytisine (5 mg.) was dissolved in 1.0 ml. of methanol and 1 drop of 70\% perchloric acid was added. After reduction of the volume, needles of crystals appeared which were filtered and washed with ether, m.p. 280-281\(^\circ\) (literature value (23) m.p. 282\(^\circ\)).

c. Methylcytisine Picrate

Methylcytisine (5 mg.) and picric acid (6.2 mg.) (1:1 mole ratio) were dissolved in a small volume of methanol and heated on a steam bath for a few minutes. On cooling, feathery needles appeared, m.p. 221-222\(^\circ\) (literature value (4) m.p. 228\(^\circ\)).

4. Isolation of Baptifoline (VIII)

The pooled fraction number 8 (241 mg.) was crystallized from benzene or acetone to give 118 mg. of baptifoline (VII) m.p. 210\(^\circ\) (literature value (21) m.p. 210\(^\circ\), \([\alpha]\_D^{27} - 133\) (c, 0.325 EtOH) (literature value (23) \([\alpha]\_D^{18} -147.7\) \(\text{EtOH}\), \(\lambda_{\text{Max}} 308 \mu\) (c 7,460), 234 \(\mu\) (6,900). The infrared spectrum showed a typical monomeric hydroxyl peak at 3600 cm\(^{-1}\) and an intermolecular hydrogen bonded peak at 3350 cm\(^{-1}\) which, on dilution of the sample, changed intensities (24). The infrared spectrum showed the usual \(\alpha\)-pyridone peaks at
1650, 1565, and 1550 cm$^{-1}$. The infrared spectrum of the isolated baptifoline in a Nujol mull was identical to a spectrum of authentic baptifoline*.

a. Baptifoline Perchlorate

Baptifoline (2.5 mg.) was dissolved in 1.0 ml. of methanol and 1 drop of 70% perchloric acid was added. On addition of ether, a crystalline material appeared. Recrystallization of this material from absolute ethanol gave rosettes of baptifoline perchlorate, m.p. 286-287$^\circ$ (literature value (21) m.p. 286-287$^\circ$).

b. Baptifoline Picrate

Baptifoline (3.0 mg.) and picric acid (2.93 mg.) (1:1) were dissolved in 0.4 ml. methanol and heated on a steam bath for a few minutes. After cooling, feathery needles of baptifoline picrate appeared which were collected. The salt sintered at 145$^\circ$ then melted, followed by resolidification and final m.p. 252$^\circ$ (literature value (23) m.p. 145$^\circ$ (sinters) then melts at 256$^\circ$).

*Kindly provided by Professor Shigenobu Okuda, University of Tokyo; Tokyo, Japan
DISCUSSION

The presence of methylcytisine in C. thalictroides was confirmed in 1913 by Power and Salway (4). With the advent of modern techniques of isolation and identification the tertiary alkaloids fraction was examined by thin layer chromatography and shown to contain eight alkaloids, three of which accounted for 91% of the fraction.

The three major tertiary alkaloids were shown to be anagyrine, methylcytisine and baptifoline.

An examination of the water-soluble fraction yielded caulosaponin which had been previously isolated and one quaternary alkaloid, magnoflorine chloride.

This work on C. thalictroides has been reported in 1967 (8).

A. Caulosaponin

This compound which had been isolated previously from C. thalictroides was characterized by its frothing in aqueous ethanol and solubility in dilute sodium hydroxide. Its identification was made by hydrolysis of caulosaponin to give hederagenin (II), whose m.p., infrared and ultraviolet spectrum were consistent with those reported in the literature (6). Acetylation of hederagenin gave the reported diacetate.
B. Magnoflorine Chloride (IX)

Magnoflorine was identified by comparison of the infrared and ultraviolet spectrum, optical rotation and relative mobility on TLC with an authentic sample. In addition, the perchlorate salt was prepared.

C. Anagyrine (VIII)

Its identification was made on the basis of the perchlorate and picrate salts whose melting points were consistent with those of the literature values (21, 23), as well as the characteristic infrared and ultraviolet spectrum.

D. Methylcystisine (I)

This alkaloid was identified on the basis of its melting point, ultraviolet and infrared spectrum and optical rotation which were consistent with those reported in the literature (4). In addition, synthetic methylcystisine was prepared which was identical to the natural product. Moreover, the perchlorate and picrate salts were prepared and their melting points were consistent with literature values (4, 23).

E. Baptifoline (VII)

Its identification was confirmed by superimposability of its infrared spectrum and that of a known sample. In addition, the melting points of the free base and the perchlorate and picrate salts were consistent with those
reported in the literature (21, 23). The ultraviolet spectrum and optical rotation were likewise consistent (23).
SUMMARY OF FINDINGS

1. The acidified aqueous extract after removal of the tertiary alkaloid yielded caulosaponin.

2. Adsorption chromatography of the quaternary alkaloid fraction yielded one alkaloid, magnoflorine chloride.

3. Partition column chromatography of the tertiary alkaloid fraction containing at least eight alkaloids yielded three crystalline tertiary alkaloids, methylcytisine, anagyrine and baptifoline.

4. The presence of anagyrine and baptifoline in C. thalictroides (L.) Michx. is the first example of isolation of these two alkaloids from the family Berberidaceae.
PART II

THE ISOLATION AND CHARACTERIZATION OF ALKALOIDS

AND NEUTRAL PRINCIPLES OF MAGNOLIA ACUMINATA L.
INTRODUCTION

A. **Botanical, Taxonomic, and Historical Description of Magnolia acuminata L.**

*Magnolia acuminata* L. or Cucumber tree is a large forest tree which grows to a height of 100 feet. The tree ranges from New York to Georgia to Illinois and Arkansas. The genus is named after Pierre Magnol (1638-1735), a French botanist, while the species name is derived from its acuminate leaves which taper to a slender point.

The common name, Cucumber tree, comes from the fact that the fruit, when young, resembles a cucumber (1). The acuminate green leaves are 6-10 inches long, while the greenish-yellow flowers are 2-3 inches long. The "cucumber" fruit grows to be 3-4 inches long, becoming pink or red (2).

*M. acuminata* L. belongs to the **Magnoliaceae** family. This family has ten genera of which *Elmerrillia*, *Liriodendron*, *Magnolia*, *Michelia* and *Talauma* have been examined chemically. The 80 or more species of these genera are found in the tropical, subtropical, and temperate zones of North America and Asia (2).

**Magnoliaceae** belongs to the large order Ranales which contains dicotyledonous herbs, shrubs and trees. Included in the order Ranales are
the families (3), Anonaceae, Berberidaceae, Himantandraceae, Lauraceae, Menispermaceae, and Ranunculaceae which have been found to contain some of the same constituents as Magnoliaceae.

According to the "Merck Index" (4) Magnolia acuminata had anti-periodic, tonic, and diaphoretic action and was used for malaria, rheumatism and indigestion.

B. Alkaloids of the family Magnoliaceae

The alkaloids of Magnoliaceae can be divided into five chemical types; aporphines (I), phenylethlamines (II), tetrahydroprotoberberines (III), benzyltetrahydroisoquinolines (IV) and bis-benzyltetrahydroisoquinolines which are dimers of (IV) joined by ether linkages.

Tomita and Nakano (5) examined the eight Magnolia species found in Japan for quaternary alkaloids and found the repeated occurrence of magnocurarine and salicifoline. Wall et al. (6) in a survey of Magnoliaceae for alkaloids found Liriodendron tulipifera and Magnolia acuminata to contain them while the other American Magnolias, M. fraseri, M. tripetala, and M. Virginiana contained none. Another examination of the Magnoliaceae alkaloids was found in an agricultural technical bulletin (7).

1. Elmerrillia species.--These species have been shown to contain alkaloids of undetermined structure (8).

2. Liriodendron tulipifera L. --The alkaloid liriodenine (V) was iso-
lated by Buchanan and Dickey (9) in 1960 and characterized by Taylor (10) in 1961 who also isolated 1, 2, 9, 10-tetramethoxy-7-oxodibenzo-(de, g)-quinoline (VI) and d-glaucine (VII).

3. **Magnolia acuminata** L. --An examination of the leaves by Kapadia et al., (11) led to the isolation of d-0-methylarmepavine (VIII) and the neutral ketone palmitone. Kapadia's examination of the stems (12) led to the isolation of one unknown and four known quaternary alkaloids. The quaternary alkaloids were choline (IX), magnocurarine (X), magnoflorine (XI), and salicifoline (XII).

4. **Magnolia coco** (Lour.) DC. --An examination (13) of this plant yielded liriodenine (oxoushinunine) (V) and the quaternary bases magnoflorine (XI) and salicifoline (XII).

5. **Magnolia denudata** Desr. --Tomita and Nakano (14) have found this plant to contain magnocurarine (X), magnoflorine (XI), and salicifoline (XII).

6. **Magnolia grandiflora** L. --An examination of the quaternary alkaloids from this plant by Nakano (15) led to the isolation and identification of magnoflorine (XI), salicifoline (XII) and canedicine (XIII). The tertiary alkaloid fraction (16) has been found to contain (-)anolobine (XIV), (-)anonaive (XV), liriodenine (V) and (-)N-Nornuciferine (XVI).

7. **Magnolia kachirachirai** Dandy.--Yang and coworkers have isolated (13, 17) D-(+)N-norarmepavine (XVII), glaucine (VII) and magnoflorine (XI).
8. **Magnolia kobus** DC. -- The quaternary alkaloid, salicifoline (XII) was isolated (18) followed by the later isolation (19) of magnoflorine (XI).

9. **Magnolia liliflora** Desr. -- Nakano's investigation of this plant (20) led to the isolation of the quaternary alkaloids, magnocurarine (X) and salicifoline (XII).

10. **Magnolia obovata** Thunb. -- Ito and Yoshida (21) have isolated the quaternary alkaloids, magnocurarine (X) and magnoflorine (XI) as well as the tertiary alkaloids liriodenine (V) anonaine (XV), and michelalbione (XVIII).

11. **Magnolia officinalis** or **Cortex magnoliae** -- The quaternary alkaloid, magnocurarine (X), was isolated from this plant (22).

12. **Magnolia parviflora** Sieb. & Zucc. -- Nakano and Uchiyana isolated (23) the quaternary alkaloids, magnocurarine (X) and magnoflorine (XI).

13. **Magnolia salicifolia** Maxim. -- The quaternary alkaloid magnocurarine (X) and salicifoline (XII) were isolated from this plant (24).

14. **Magnolia stellata** Maxim. -- The examination of this plant gave one quaternary alkaloid salicifoline (XII) (25).

15. **Michelia alba** DC. -- The isolation of the structurally related aoporphines, liriodenine (oxoushinsumine) (V), michelalbione (N-norshinsuaine) (XVIII), and shinsumine (XIX) with the quaternary alkaloid, salicifoline (XII) has been reported by Yang (26).

16. **Michelia champaca** L. -- An examination of this plant by Indian and Chinese workers (27, 28, 29) has led to the isolation of liriodenine (V),
ushinsunine (XIX) and the quaternary alkaloid, magnoflorine (XI).

17. **Michelia compressa**—The first examination of this plant by Ito (30) led to the isolation of magnoflorine (XI) and the tetrahydroprotobererines, tetrahydroberberine (XX) and tetrahydrojatrorrhizine (XXI) as well as the bis-benzyltetrahydroisoquinoline, oxyacanthine (XXII).

A later examination by Ito (31) led to the isolation of a quaternary alkaloid, michepressine (XXIII).

Yang and co-workers (32) examined the tertiary alkaloid fraction and found liriodenine (oxoushinsuine, micheline B) (V) and ushinsunine (micheline A) (XIX).

Other examinations (33, 34) of the heartwood yielded liriodenine and ushinsunine.

18. **Michelia figo**—An examination by Arthur et al. (35) has led to the isolation of the bis-benzyltetrahydroisoquinoline, magnolamine (XXIV).

19. **Michelia fuscata**—Prior investigations (36) of this plant revealed the presence of three bis-benzyltetrahydroisoquinolines, magnolamine (XXIV), magnoline (XXV) and tetrandine (XXVI). Later investigation (37) found the quaternary alkaloids, magnocurarine (X) and magnoflorine (XI).

20. **Talauma mexicana** G. Don.—**T. mexicana** has been shown (38, 39) to contain aztequine (XXVII) and an alkaloid of unknown structure, talaumine.
C. Sesquiterpenes of the family Magnoliaceae

Sesquiterpenes are widely distributed in the Compositae family but only a few isolated examples of sesquiterpenes have been found in Magnoliaceae.

The earliest report of the isolation of a sesquiterpene lactone was from Talauma mexicana (40). The plant contained costanolide (XXVIII) which was identified by comparison with an authentic sample.

Costanolide has been isolated from three plants in Compositae; Saussurea lappa (41), Artemisia balchanorum (42) and Cosmos sulphureus (43).

The most recent reported isolation of costanolide was from the Magnoliaceous plant Liriodendron tulipifera L. (44).

The other sesquiterpene lactone isolated from Magnoliaceae was from Michelia champaca (46). This compound was partholide (XXIX) which had been previously isolated from Chrysaethemum parthenium (family, Compositae) (47). The correct structure was determined by Govindachari et al., (46).

\[ \text{XXVIII} \]
\[ \text{XXIX} \]
D. Lignans of the family Magnoliaceae

Lignans are a class of natural products biosynthesized from cinnamic acid by oxidative coupling. The term lignan was first used by Haworth to describe the dimers of n-propylbenzene which are joined at the β-carbon of the aliphatic side chain (48).

The review by Hearon and MacGregor (49) lists the lignans by the following general types (where R is H or OH and the aromatic rings show oxygen substitution as hydroxyl, methoxyl or methylenedioxy groups).

Lignans of type (XXXV) and (XXXVI) have been studied recently by mass spectroscopy (49).

Klyne (50, 51) has investigated the optical rotatory dispersion of lignan type (XXXIII).

Lignans are widely distributed in plants with their occurrence in such families as Berberidaceae, Himantandraceae, Lauraceae, Pedaliaceae, Pinaceae, Piperaceae and Rutaceae.

The only previous report (52) of a lignan in Magnoliaceae occurs in Liriodendrin tulipfera L. (XXXVIII), which is liriodendrin.

However, an example of a n-propylbenzene dimer, magnolol (XXXIX) has been reported in Magnolia obovata and Magnolia officinalis by Sugii (53).
XXXVIII

XXXIX
E. **Objective of Research**

The purpose of this investigation was to isolate and identify the alkaloidal and neutral constituents from the root bark of *Magnolia acuminata* L. Since a sesquiterpene, costunolide, had been isolated from the Magnoliaceous plant *L. tulipifera* L., (44), it was hoped this plant might contain a sesquiterpene.
EXPERIMENTAL

A. Source of Plant Material

The root bark used in this investigation was obtained from a Magnolia acuminata L. tree at the Secrest Arboretum, Ohio Agricultural Research and Development Center, Wooster, Ohio.

B. Methodology--Chemical and Physical Analysis

The instrumentation and chromatography was the same as Part I. (See pp. 6-7 for details.)

C. Extraction and Initial Separation

The powdered root bark (1.0 kg.) was extracted by percolation at room temperature with 25 liters of U.S.P. ethanol. The solvent was evaporated in vacuo at 40° to give 190 g. of extract. The extract was partitioned between 900 ml. of 2% citric acid and 800 ml. of chloroform. The citric acid layer was extracted two additional times with 800 ml. of chloroform.

The chloroform solutions were dried over sodium sulfate and evaporated to give 78.5 g. of neutral constituents.

The neutrals were dissolved in 350 ml. 10% aqueous methanol and extracted with 3 portions of 350 ml. Skellysolve B. After drying over sodium
sulfate the Skellysolve B layer gave 31.0 g. of residue. The methanol layer gave 47.5 g. of residue.

In the workup of an additional 2 kg. of plant material, the partitioning between 10% aqueous methanol and Skellysolve B was excluded.

The citric acid solution containing the alkaloids was adjusted to pH 9 with concentrated ammonium hydroxide. The basification caused a precipitate to be formed, which was not separated but the entire mixture was extracted with 3-500 ml. portions of chloroform. The chloroform solution after drying over sodium sulfate gave 3.65 g. of a yellow amorphous tertiary alkaloid material.

The basic solution was acidified with 10% hydrochloric acid to pH 4. A saturated aqueous solution of ammonium reineckate (2% w/v) was added to give a precipitate of the quaternary alkaloid reineckates (28.4 g.). (See Figure 2 for Flowsheet.)

In another procedure, 1.0 kg. of plant was partitioned as before except that the basic solution after removal of the tertiary alkaloids was acidified to pH 5 with glacial acetic acid. To the acid solution was added 2 liters of Mayer's reagent (54) to give a precipitate of the quaternary alkaloid complex. The precipitate was suspended in water and stirred overnight with 300 ml. of Amberlite IR-410-A (chloride) ion exchange resin. After filtering the resin the solution was evaporated in vacuo at 40° to give 14.2 g. of quaternary chlorides.
Figure 2. Flowsheet for Separation of *M. acuminata* Extract.
D. Examination of Tertiary Alkaloid Fraction

A thin layer chromatographic examination of the crude alkaloid fraction showed it to contain one spot $R_f$ 0.47 on silica gel G using methanol concentrated ammonium hydroxide (99:1) as a solvent. The spot gave a positive color reaction with iodoplatinate, as well as with phosphomolybdate. However, in the case of this particular alkaloid, Dragendorff's reagent gave a fairly weak test. This one spot material was amorphous and darkened with time.

1. Adsorption Column Separation

A 3.5 cm. I.D. x 37 cm. column of silicic acid-celite (4:1) (120 g.) was poured in chloroform. The tertiary alkaloid fraction (2.90 g.) was dissolved in methanol:chloroform (1:1) and adsorbed onto 6 g. celite. After evaporation of the solvent, the residue remained as a brown powder. The powder was placed on top of the column and eluted with chloroform. The first 160 ml. of eluate was discarded. The column was placed on a fraction collector and 20 ml. effluent fractions collected. Two tubes were combined to give a total fraction of 40 ml. Final elution of the column gave no additional material. A summary of the data for the column is given in TABLE 2.

Alkaloid A ($R_f$ 0.62) failed to crystallize from methanol or ethanol. This compound was dark brown in color emphasizing the fact that it is an unstable compound.
<table>
<thead>
<tr>
<th>Eluate</th>
<th>Volume (ml.)</th>
<th>Fraction</th>
<th>Wt. Fraction (g.)</th>
<th>Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHCl₃</td>
<td>280</td>
<td>1 - 7</td>
<td>0.169</td>
<td>Non-alkaloidal</td>
</tr>
<tr>
<td>CHCl₃:MeOH(99:1)</td>
<td>1840</td>
<td>8 - 54</td>
<td>0.225</td>
<td>Alkaloid A</td>
</tr>
<tr>
<td>CHCl₃:MeOH(98:2)</td>
<td>3200</td>
<td>55 - 135</td>
<td>1.946</td>
<td>Anolobine</td>
</tr>
</tbody>
</table>

Anolobine (Rᶠ 0.47) also appeared to be unstable. Fractions 64 - 101, which were combined and crystallized from methanol, gave an amorphous material. Fractions 102 - 110 gave crystalline material (96 mg.), m.p. 260-261⁰ and fractions 111-135 gave crystalline material (248 mg.), m.p. 257-259⁰. The latter material was recrystallized from methanol twice to give slightly yellow needles, m.p. 265⁰. The compound has absorption bands in the infrared region at ν_max 3400, 3250, 3050, 2600, 1600, and 925 cm⁻¹.

Anolobine has been isolated from Magnolia grandiflora (16). Tomita and Kozuka (16) give the following data for anolobine: m.p. 240-241⁰ (dec), [α]D[^25] = 18° (c 0.57; CHCl₃), UV λ_max[^237, 282, 320 μm]. Another isolation of anolobine was from Asimina triloba (family, Anonaceae) by Manske (55), who reports a m.p. 262⁰, [α]D[^27] = 22.5° (c, 0.4; CHCl₃, MeOH). A reinvestigation of Asimina triloba (55) confirmed the presence of anolobine.
The ultraviolet absorption spectrum had \( \lambda_{\text{Shoulder}}^\text{MeOH} 320 \text{ nm} \) (c 5,100) \( \lambda_{\text{Max}}^\text{MeOH} 280 \text{ nm} \) (c 17,900) \( \lambda_{\text{Shoulder}}^\text{MeOH} 237 \text{ nm} \) (c 10,950) \( \lambda_{\text{Max}}^\text{MeOH} 214 \text{ nm} \) (c 24,800); containing 0.01 N KOH \( \lambda_{\text{Max}}^\text{MeOH} 313 \text{ nm} \) (c 22,700). The optical rotation was \([\alpha]_{D}^{27} = 12.3^\circ \) (c, 0.106, MeOH).

2. Labat's Test on Anolobine

Anolobine was suspected to contain a methylenedioxy group from the infrared spectrum (925 cm\(^{-1}\) peak) and NMR. The compound gave a positive Labat's test (57) using sulfuric and gallic acid.

3. Methylation of Anolobine

Anolobine was shown possibly to have a phenolic hydroxyl because of the bathochromic shift in the ultraviolet spectrum under alkaline conditions. Also, the infrared peaks at 3400, and 3250 cm\(^{-1}\) indicated the presence of a hydroxyl group.

An excess of diazomethane in ether prepared from 0.713 g. Diazald\(^{R}\) was added to a suspension of 100 mg. of anolobine in 10 ml. of CHCl\(_3\):MeOH (7:3). The flask was stoppered and allowed to remain overnight at 5\(^\circ\). The reaction mixture was evaporated to dryness and then taken up in chloroform. The chloroform solution (200 ml.) was washed with 2-30 ml. of portions of 5% NaOH and then with 3-25 ml. portions of water. The chloroform solution was dried over anhydrous sodium sulfate and evaporated to give an oil (118 mg.).

The oil was worked up in the following manner according to Manske (55). To the oil was added 20 ml. 5% hydrochloric acid. The insoluble materi-
ial was filtered (hydrochloride salt). The salt was dissolved in 30 ml. hot water (70°) and filtered again.

The filtrate was basified with concentrated ammonium hydroxide to give a precipitate which was not filtered. The aqueous suspension was extracted with ether. The ether layer was washed with water and dried over anhydrous sodium sulfate.

Evaporation of the ether gave an oil (64 mg.) which was submitted for NMR examination. The NMR spectrum showed an additional singlet at δ 3.76 (3H, OMe) indicated an O-methyl ether, most probably xyloplone.

4. Preparation of N, O-diacetyl Anolobine

Anolobine (130 mg.) was dissolved in 4.0 ml. pyridine and 2.0 ml. acetic anhydride. This mixture was set aside overnight and then poured into a mixture of ice and ether. The water was extracted with ether to give a total volume of 200 ml. The ether was extracted with 5% NaOH (3-30 ml. portions) and then washed with water. The ether layer was dried over anhydrous sodium sulfate and evaporated to give a crystalline residue. To this material was added toluene which was evaporated in vacuo to remove the residual pyridine. The weight of the residue was 193 mg. Crystallization of this residue from absolute ethanol gave 96 mg. crystals, m.p. 213°.

The NMR shows singlets at δ 2.18 (3H, NCOCH₃). The methylene-dioxy group appears as an AB quartet at δ 5.88 (1H, J = 1.5 Hz) and 5.01 (1H, J = 1.5 Hz). The H₃ and H₈ protons appear as singlets at δ 8.51 (1H).
and δ 7.35 (1H), respectively. The H₁₀ proton is a multiplet at δ 7.00 and the H₁₁ appears as a doublet at δ 8.08.

Anolobine diacetate as prepared by Tomita and Kozuka (16) gave the following data: m.p. 213-214°; NMR singlets at δ 2.20 (3H, NCOCH₃) and δ 2.30 (3H, OCOCH₃), and an AB quartet at δ 5.95 (1H) and δ 6.07 (1H) (OCH₂O).

E. Examination of the Quaternary Alkaloid Fraction

The quaternary alkaloid reineckate (30.0 g.) was dissolved in 700 ml. of acetone-water (1:1) and passed through a 3.8 cm. I.D. x 46 cm. column of 500 ml. of Amberlite IRA-410 (C1) resin. The first 200 ml. of eluate was discarded and 1500 ml. of eluate was collected. It was reduced by evaporation to a volume of 200 ml. and absolute ethanol was added to give 1.99 g. of a precipitate which was an inorganic salt. The filtrate was reduced to a black oily material (24.02 g.) of quaternary alkaloid chloride.

The crude quaternary alkaloid chloride was chromatographed on silica gel G with a solvent system of n-propanol-ammonium hydroxide-water (4:1:1) to give six Dragendorff positive spots. The Rf values were 0.51, 0.48, 0.45, 0.42, 0.38, and 0.27. The spot with Rf 0.45 gave a blue fluorescence when exposed to ultraviolet light while the spot with Rf 0.38 gave a dark blue fluorescence.

1. Adsorption Chromatography of Quaternary Alkaloids

An adsorption column (3.7 cm. I.D. x 42 cm.) of 200 g. silicic acid-
celite (4:1) was poured in chloroform. The quaternary chlorides (2.10 g.) were dissolved in methanol and 4.0 g. celite 545 added to it. The suspension was evaporated to give a powder which was slurried in chloroform and applied to the top of the column. Twenty milliliter fractions were collected. A summary of the column data is given in TABLE 3.

**TABLE 3**

**Separation of Quaternary Alkaloid from *M. acuminata***

<table>
<thead>
<tr>
<th>Eluate</th>
<th>Volume (ml.)</th>
<th>Fraction</th>
<th>Wt. (mg.)</th>
<th>Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>550</td>
<td>1 - 54</td>
<td>391</td>
<td>Non-alkaloidal</td>
</tr>
<tr>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;-MeOH(99:1)</td>
<td>550</td>
<td>55 - 85</td>
<td>542</td>
<td>Alkaloidal oil</td>
</tr>
<tr>
<td>CHCl&lt;sub&gt;2&lt;/sub&gt;-MeOH(95:5)</td>
<td>360</td>
<td>86 - 90</td>
<td>593</td>
<td>N-Methyllindcarpaine methochloride</td>
</tr>
<tr>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;-MeOH(9:1)</td>
<td>440</td>
<td>91 - 116</td>
<td>391</td>
<td>Alkaloid Mixture</td>
</tr>
</tbody>
</table>

Fraction 87, R<sub>f</sub> 0.38, was crystallized from absolute ethanol to give white needles (32 mg.), m.p. 242-244°. Fractions 88-90 were crystallized twice from absolute ethanol containing potassium iodide to give 105 mg. of product, m.p. 249° (uncorrected). N-Methyllindcarpine methiodide had broad peaks at 3400 and 3160 cm<sup>-1</sup> in the infrared, as well as characteristic peaks at ν<sub>max</sub> 3000, 2960, 2920, 2840 and 1590 cm<sup>-1</sup> (see Figure 3). The optical
Figure 3. Infrared Spectrum of N-Methylindcarpine methiodide in KBr.
rotation was \([\alpha]_D^{27} + 213^\circ\) (c 0.60, MeOH).


The ultraviolet spectrum had peaks at \(\lambda_{\text{Max}}^{\text{EtOH}}\) 305 \(\mu\) (\(\varepsilon\) 5730), 275 \(\mu\) (\(\varepsilon\) 10,450), 222 \(\mu\) (\(\varepsilon\) 41,000); In 0.01 N KOH \(\lambda_{\text{Max}}^{\text{EtOH}}\) 330 \(\mu\) (\(\varepsilon\) 7,930), \(\lambda_{\text{Shoulder}}^{\text{EtOH}}\) 312 \(\mu\) (\(\varepsilon\) 7,730), \(\lambda_{\text{Max}}^{\text{EtOH}}\) 280 \(\mu\) (\(\varepsilon\) 7,500), \(\lambda_{\text{Shoulder}}^{\text{EtOH}}\) 272 \(\mu\) (\(\varepsilon\) 7,080). The bathochromic shift in base indicated the presence of a phenolic hydroxyl group.

The NMR spectrum shows singlets at \(\delta\) 2.95 (2H, NMe), 3.38 (3H, NMe), 3.81 (3H, OMe), 3.83 (3H, OMe) and 6.92 (3H, aryl H) (see Figure 4). The 0,0-dimethylether as the iodide of the alkaloid proved to be identical with 0,0-dimethylmagnoflorine iodide (58). Therefore, the isolated quaternary alkaloid and magnoflorine have the same basic skeleton.

Magnoflorine (XI) has an aromatic hydrogen at position 8. In order to establish the absence or presence of this hydrogen in N-Methyllindocarpine methiodide a Gibb's test was carried out (59). A positive Gibb's test is indicated by a blue or blue-green color, with ultraviolet absorption in the visible region at about 600 \(\mu\).

2. Gibb's Test

N-Methyllindocarpine methiodide (1.5 reg.) and 1.0 ml. pyridine were mixed. To this suspension was added 5.0 ml. of a freshly prepared solution of Gibb's reagent in pyridine \((25 \text{ mg. per 15 ml.})\) and the mixture was diluted
Figure 4. NMR Spectrum of N-Methylindocarpine methiodide in DMSO-$d_6$. 
with pH 9.2 boric acid-sodium hydroxide buffer (60). Immediately there was a blue-green color indicating a positive reaction. Magnoflorine chloride was run under the same conditions to give the same color. Likewise, the ultraviolet spectrum of the Gibb's complex of magnoflorine gave a λ max at 582 μm with approximately the same ε values.

3. Quastel Test

The Quastel test (61) is a test for catechol systems, giving a reddish-brown coloration with the reagent.

To a 2.0 ml. of an aqueous solution of N-Methylindocarbamine methiodide (3 mg.) was added 0.5 ml. of glacial acetic acid and 1.0 ml. of concentrated (14%) solution of ammonium molybdate. No color was produced indicating the absence of two adjacent phenolic groups. Under the same conditions catechol gave an immediate reddish-brown coloration.

F. Examination of the Neutral Fraction of M. acuminata

The Skellysolve B fraction (15.38 g.) was chromatographed on 600 g. of Woelm neutral alumina (activity I). The column was poured in benzene to give the dimensions of 4.2 cm. I.D. x 50 cm. A summary of the eluting solvent is presented in TABLE 4.
TABLE 4

Eluting Solvents for Column of Neutral Fraction of *M. acuminata*

<table>
<thead>
<tr>
<th>Eluate</th>
<th>Volume (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>1640</td>
</tr>
<tr>
<td>Benzene-chloroform (9:1)</td>
<td>420</td>
</tr>
<tr>
<td>Benzene-chloroform (9:2)</td>
<td>600</td>
</tr>
<tr>
<td>Benzene-chloroform (1:1)</td>
<td>600</td>
</tr>
<tr>
<td>Chloroform</td>
<td>400</td>
</tr>
<tr>
<td>Chloroform-methanol (1:1)</td>
<td>900</td>
</tr>
<tr>
<td>Methanol</td>
<td>1800</td>
</tr>
</tbody>
</table>

The first 560 ml. of benzene was discarded. The benzene, benzene-chloroform, and chloroform layers were combined to give 0.422 g. material. Elution with chloroform-methanol (1:1) gave 6.31 g. of an orange oil. The methanol wash gave 0.781 g.

1. Adsorption Column Chromatography of Purified Skellysolve B Fraction

The orange oil (6.31 g.) from the alumina column was chromatographed on 300 g. of silicic acid-celite (4:1) in a column 3.8 cm. I.D. x 54 cm. The column was poured as a slurry in chloroform-benzene (9:1) and the column was continuously eluted with the same solvent to give 15 ml. frac-
tions which were collected and evaporated. TABLE 5 is a summary of the column data.

**TABLE 5**

Separation of Skellysolve B Fraction from *M. acuminata*

<table>
<thead>
<tr>
<th>Fraction Number</th>
<th>Material</th>
<th>Weight (mg.)</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 28</td>
<td>Oil</td>
<td>550</td>
<td>-</td>
</tr>
<tr>
<td>29 - 40</td>
<td>Acuminatin</td>
<td>678</td>
<td>0.48(^1)</td>
</tr>
<tr>
<td>41 - 50</td>
<td>Mixture</td>
<td>179</td>
<td>-</td>
</tr>
<tr>
<td>51 - 87</td>
<td>β-Sitosterol</td>
<td>834</td>
<td>0.21(^1)</td>
</tr>
<tr>
<td>88 - 101</td>
<td>Calopiptin</td>
<td>335</td>
<td>0.27(^1)</td>
</tr>
<tr>
<td>102 - 105</td>
<td>Mixture</td>
<td>198</td>
<td>-</td>
</tr>
<tr>
<td>106 - 111</td>
<td>Galgravin</td>
<td>354</td>
<td>0.32(^2)</td>
</tr>
<tr>
<td>112 - 118</td>
<td>Mixture</td>
<td>416</td>
<td>-</td>
</tr>
<tr>
<td>119 - 123</td>
<td>Veraguensin</td>
<td>324</td>
<td>0.32(^2)</td>
</tr>
<tr>
<td>124 - 150</td>
<td>Mixture</td>
<td>1138</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^1\) Thin layer chromatography on silica gel G with chloroform as solvent.

\(^2\) Thin layer chromatography on silica gel G with chloroform-ethyl acetate (40:1) as solvent.
The methanol wash gave 0.200 g. of residue. After thin layer chromatography the plates were sprayed with ether-sulfuric acid (4:1). Acmi
matin, calopiptin, galgravin, and veraguensin gave reddish-purple spots while 
β-sitosterol gave a bluish-red spot.

2. Investigation of β-sitosterol

Fractions 51–87 were crystallized from 95% ethanol to give shiny platelets (253 mg.), m.p. 139–140°, which gave in the Lieberman-Burchard test a transitory violet followed by a green color characteristic of a 3-
hydroxy-Δ5-sterol. The infrared spectrum showed hydroxyl absorptions at 
νmax 3600 and 3420 cm⁻¹ and the absence of aromatic peaks. The compound was suspected of being β-sitosterol from its melting point. A mixture melting point with an authentic sample showed no depression and the infrared spectrum of an authentic sample was superimposable with the isolated sub-
stance.

a. β-sitosterol acetate

The acetate was prepared by the overnight reaction at room tempera-
ture of the sterol in pyridine with acetic anhydride and had a m.p. 131°. A mixture m.p. with an authentic sample showed no depression.

3. Investigation of Calopiptin

Fractions 88–101 were crystallized from isopropyl ether and then from hexane to give rosettes, m.p. 94.5°. The infrared spectrum has peaks at νmax 3000, 2955, 2925, 2865, 2875, 2840, and 2770 cm⁻¹ indicating the
compound has methoxy and methylenedioxy groups. The ultraviolet spectrum has peaks at $\lambda_{\text{Max}}^\text{MeOH}$ 280-284 nm ($\varepsilon 6,640$), 233 nm ($\varepsilon 14,500$). The optical rotation was $[\alpha]^26_D + 45.8^\circ$ (c, 0.66, CHCl$_3$). The data indicates that the isolated compound is identical to calopiptin, a lignan isolated from Piptocalyx moorei (family, Trimeniaceae). The physical constants reported by Riggs and Stevens (62) for calopiptin are m.p. 95.5$^\circ$, $[\alpha]^20_D + 28^\circ$ (c, 2.09, CHCl$_3$) $\lambda_{\text{Max}}^\text{EtOH}$ 281-285 nm ($\varepsilon 6,700$), 234 nm ($\varepsilon 13,200$), infrared absorption $\nu_{\text{max}}$ at 1612 m, 1596 m, 1520 vs, 1490 vs, 1444 vs, 1417 m, 1397 m, 1345 m, 1325 m, 1283 m, 1250 vs, 1234 vs, 1190 ms, 1162 s, 1127 vs, 1100 m, 1027 vs, 1010 s, 959 m, 930 s, 868 m, 860 m, 808 s $\text{cm}^{-1}$.

The NMR spectrum (Figure 5) confirms the structure showing a methylenedioxy group and two aromatic methoxy groups and two secondary methyl groups. The peaks in the NMR spectrum are at $\delta$ 0.67 (d, 3H, $J$ = 6.5 Hz, CHCH$_3$), 1.95 (d, 3H, $J$ = 6 Hz, CHCH$_3$), 1.5-2.4 (m, 2H, 2 CHCH$_3$), 3.83 (s, 3H, ArOMe), 2.90 (s, 3H, ArOMe), 4.32 (d, 1H, ArCHOR), 5.05 (d, 1H, ArCHOR), 5.79 (s, 2H, OCH$_2$O) and 6.7-7.1 (m, 6H, aryl-H). The spectrum corresponds to the one (62) reported in the literature for calopiptin. The reported values are $\delta$ 0.68 (d, 3H, CHCH$_3$), 1.07 (d, 3H, CHCH$_3$), 1.7 - 2.3 (m, 2H, 2 CHCH$_3$), 3.85 (s, 6H, ArOMe), 4.37 (d, 1H, ArCHOR), 5.11 (d, 1H, ArCHOR), 5.33 (s, 2H, OCH$_2$O) and 6.55-7.02 (m, 6H, ArH). Structure (XL) was assigned to calopiptin on the basis of the NMR spectrum.
Figure 5. NMR Spectrum of Calopiitin in CDCl₃.
An examination of the NMR spectrum of veraguensin shows it to be structurally similar to calopiptin except that calopiptin has one 3,4-methyleneedioxy group instead of the 3,4-dimethoxy groups. Veraguensin has a molecular rotation of +14,850° and calopiptin's value was +16,550°. These values compare favorably and, therefore, the stereochemistry is probably the same as veraguensin (63). The absolute stereochemistry of calopiptin was shown later (64) to be that of structure XLI.

4. Investigation of Galgravin

Galgravin and veraguensin were found to have identical behavior in TLC. The fractions 106-123 were all crystalline. Gas chromatography of these fractions on a 4 ft. column containing 3.8% silicone rubber UC W 98 on Diatoport 80-100 mesh (supplied by F. and M. Scientific Company) using a
Model 402 F and M Gas Chromatograph showed these fractions to be a mixture of two compounds.

Fractions 106-111 were combined and crystallized from hexane to a constant m.p. 121°. The optical rotation was $\left[\alpha\right]_{D}^{22} = 0^\circ$ (c, 1.30, CHCl$_3$). The infrared spectrum was similar to that of calopiptin with the absence of the peak at $\nu_{\text{max}}$ 2780 cm$^{-1}$ which is characteristic of the methylenedioxy group. The infrared spectrum shows peaks at $\nu_{\text{max}}$ 3000, 2955, 2940, 2905, 2870, 2840, 1620, and 1600 cm$^{-1}$. The compound appears to be a lignan containing methoxy groups. The ultraviolet spectra has peaks at $\lambda_{\text{Max}}^{\text{MeOH}}$ 278 $\mu$m ($\varepsilon$ 6, 600), 233 $\mu$m ($\varepsilon$ 20, 300).

A plane of symmetry in the molecule is shown by its lack of optical rotation and its simple NMR spectrum. (Figure 6). The methyl groups appear as a doublet (6H, $J = 6.8$ Hz) at $\delta$ 1.03 typical of secondary methyl on a carbon bearing other carbon atoms. The methine protons on the carbons bearing the methyl groups appear as a multiplet $\delta$ 2.1 - 2.6 (2H). The benzyl protons appear as a doublet at $\delta$ 4.58 (2H, $J = 6.5$ Hz) and the aryl protons as a multiplet at $\delta$ 6.8 - 7.2 (6H). The aromatic methoxyls are at $\delta$ 3.88 (12H).

The presented data corresponds to galgravin, a lignan isolated from Himantandra belgraveana (family, Himantandraceae). Galgravin (LIII) was shown by Hughes and Ritchie (65) to have a m.p. 121°, $\left[\alpha\right]_{D}^{20} = 0^\circ$. 
Figure 6. NMR Spectrum of Galgravin in CDCl$_3$. 
5. Investigation of Veraguensin

Fraction 119-123 was crystallized from ether to give prisms m.p. 128-129°, \([\alpha]_D^{25} + 41.7° (c, 0.96 \text{ MeOH}). The infrared spectrum is identical with that of galgravin except that veraguensin shows an extra peak at 1110 cm\(^{-1}\).

This would indicate that the two compounds are diastereoisomers. The ultraviolet spectrum shows peaks at \(\lambda_{\text{max}}^{\text{MeOH}}\) 278 \(\mu\) (\(c 6,130\)), 233 \(\mu\) (\(c 18,800\)). The data coincides with that of veraguensin, a lignan isolated from Octoa veraguensis (family, Lauraceae) (63).

Veraguensin has a molecular weight of 372 by mass spectrometry (\(C_{32}H_{28}O_4\)). The compound was submitted for elemental analysis.

Anal. Calcd for \(C_{32}H_{28}O_4\): C, 70.94; H, 7.58; 4 OMe, 33.21.

Found: C, 71.25; H, 7.56; 4 OMe, 33.66.

The NMR spectrum (Figure 7) is distinctly different from galgravin. The methyl group that is cis to the veratryl group is shielded and found at \(\delta 0.66 (d, 3H)\). The other methyl group is deshielded being found at \(\delta 1.08 (d, 3H)\). The benzylic protons are found at \(\delta 4.48 (1H, d)\) and \(\delta 5.20 (1H, d)\).

The isolated lignan was compared with an authentic sample of veraguensin.* The infrared spectra were superimposable and the mixture melting point showed no depression.

* I thank Professor Carl Djerassi for this sample.
Figure 7. NMR Spectrum of Veraguensin in CDCl₃.
6. Alumina Column Purification of the Neutral Fraction

From another 1.0 kg. of plant, 77.5 g. of neutrals were obtained. The neutrals were subjected directly to column chromatography on alumina.

A column (4.2 cm. I.D. x 56 cm.) of Wessel neutral alumina (activity I) 700 g. was packed in chloroform. The neutrals (77.5 g.) were dissolved in 250 ml. chloroform and applied to the column. The first 250 ml. were discarded and the next 900 ml. of chloroform was collected. Evaporation of the band gave 54.032 g. which on thin layer chromatography showed the presence of the lignans as evidenced by their color reaction with the sulfuric acid-ether spray.

The next 800 ml. of CHCl₃ eluted a yellow band which on evaporation gave a red-orange oil (6.334 g.). This fraction contained no lignans as evidenced by TLC.

Further elution with 3500 ml. of CHCl₃ gave a yellow oil (3.57 g.). The column was then washed with 2 ℥ of methanol to give 8.41 g. of an oil.

7. Silicic Acid-Celite Column for Purification of the Neutrals

The alumina purified neutral fraction containing the lignans was chromatographed on silicic acid-celite (4:1) 1.0 kg. in a column 6.5 cm. I.D. x 64 cm. The adsorbent was poured in chloroform-benzene (9:1) and the column was eluted with the same solvent.

The purified neutrals containing the lignans (25.18 g.) were dissolved
in 125 ml. of chloroform–benzene (3:1) and applied to the column at a flow rate of 50 ml. per hour. The first 1500 ml. of eluate was discarded and the 40 ml. of effluent fractions then collected and evaporated in tared 125 ml. erlenmeyer flasks. A summary of the data for the column is given in TABLE 6.

### TABLE 6

**Separation of Purified Neutral Fraction from *M. acuminata***

<table>
<thead>
<tr>
<th>Fraction Number</th>
<th>Material</th>
<th>Weight (g.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 20</td>
<td>oil mixture</td>
<td>3.960</td>
</tr>
<tr>
<td>21 - 22</td>
<td>oil</td>
<td>0.236</td>
</tr>
<tr>
<td>23 - 29</td>
<td>acuminatin</td>
<td>1.573</td>
</tr>
<tr>
<td>30 - 40</td>
<td>costunolide</td>
<td>0.633</td>
</tr>
<tr>
<td>41 - 46</td>
<td>mixture</td>
<td>0.380</td>
</tr>
<tr>
<td>47 - 80</td>
<td>calopiptin</td>
<td>1.457</td>
</tr>
<tr>
<td>81 - 89</td>
<td>mixture</td>
<td>0.881</td>
</tr>
<tr>
<td>90 - 100</td>
<td>galgravin</td>
<td>1.969</td>
</tr>
<tr>
<td>100 - 113</td>
<td>mixture</td>
<td>2.227</td>
</tr>
<tr>
<td>114 - 120</td>
<td>veraguensin</td>
<td>1.290</td>
</tr>
<tr>
<td>121 - 128</td>
<td>mixture</td>
<td>1.708</td>
</tr>
<tr>
<td>MeOH wash</td>
<td>mixture</td>
<td>8.00</td>
</tr>
</tbody>
</table>
All of the previously isolated compounds were identified by the $R_f$ value on TLC and their purplish-red coloration with sulfuric acid:ether spray. All of the fractions were crystallized as before and fractions 101-113 were fractionally crystallized from acetone:hexane to give pure galgravin.

Fractions 30-40 when sprayed with sulfuric acid:ether (1:4) give a green color indicating that the material was probably not lignan in nature.

8. Investigation of Costunolide

Fractions 30-40 (633 mg.) were crystallized from isopropyl ether to give 87 mg. of rods, m.p. 106-107°. The infrared spectrum had characteristic bands at $\nu_{\text{max}}$ 3000 or 1600 cm$^{-1}$ indicating that this compound is not aromatic. The peak at $\nu_{\text{max}}$ 1760 cm$^{-1}$ suggests a $\gamma$-lactone and that at 1670 cm$^{-1}$ can be attributed to a double bond.

The compound is optically active $[\alpha]_D^{26} + 124^\circ$ (c, 0.44, CHCl$_3$). The NMR spectrum shows the presence of an exocyclic methylene which is indicated by two doublets at $\delta$ 5.60 and 6.24 ($J = 4$ Hz) and two singlets at $\delta$ 1.41 and 1.69 each integrating for 3 protons. These are methyl groups on a double bond. From this data and the fact that costunolide (XXVIII) had been previously isolated from two plants in the family Magnoliaceae, it was suspected to be costunolide.

A mixture melting point taken with an authentic sample of costunolide showed no depression. The infrared and NMR spectra of the isolated compound and costunolide were found to be superimposable. Thin layer chromatography
on silica gel G using chloroform as a solvent showed both to have a $R_f$ 0.49.

9. Investigation of Acuminatin (XLI)

After several months in the freezer, acuminatin (fraction 29-40, TABLE 5) crystallized from isopropyl ether to give colorless needles, m.p. 77.5°. The infrared spectrum showed $\nu_{\text{max}}$ at 3005, 2960, 2940, 2910, 2870, 2840, 1601, 1525, 1505, 1270 and 965 cm$^{-1}$ (see Figure 8). The ultraviolet spectrum has the following characteristics: $\lambda_{\text{MeOH}}^{\text{Shoulder}}$ 312 $\mu$m ($c$ 2,680); $\lambda_{\text{MeOH}}^{\text{Max}}$ 275 $\mu$m ($c$ 19,300), 219 $\mu$m ($c$ 35,100); $\lambda_{\text{MeOH}}^{\text{Min}}$ 247 $\mu$m ($c$ 8,780), 213 $\mu$m ($c$ 34,200). The molecular formula was confirmed by high resolution spectroscopy. Calcd. for $C_{21}H_{24}O_4$: 340.1674. Found: 340.1678 (Figure 9).

The optical rotation was $\left[ \alpha \right]_D^{27} + 43.3^\circ$ (c, 0.22, MeOH). The NMR spectrum (Figure 10) of acuminatin had the following peaks: $\delta$ 6.7 - 7.0 (m, 5H, aryl H), centered at 6.2 (A and B of ABX$_3$, 2H, olefinic H), 5.12 (d, 1H, J = 9.5 Hz), 3.82 (s, 3H, OMe), 3.87 (s, 6H, 2OMe), 3.42 (two overlapping quartets, 1H), 1.85 (d, 3H, J = 5.5 Hz) and 1.37 (d, 3H, J = 6.5 Hz).

Anal. Calcd for $C_{21}H_{24}O_4$: C, 74.99; H, 7.11. Found: C, 73.94; H, 7.22.

A decoupling experiment was run on acuminatin. Irradiation of the methyl group at $\delta$ 1.85 caused the pattern at $\delta$ 6.2 to change. Irradiation of the doublet at $\delta$ 5.12 caused the multiplet at $\delta$ 3.42 to collapse to a quartet while irradiation at $\delta$ 3.42 caused the peak at $\delta$ 5.12 and $\delta$ 1.37 to collapse to
Figure 8. Infrared Spectrum of Acuminatin in CHCl₃.
Figure 9. Mass Spectrum of Acuminatin.
Figure 10. NMR Spectrum of Acuminatin in CDCl$_3$.
singlets. Irradiation at δ 1.37 caused the peak at δ 3.42 to collapse to doublet 
(J = 9.5 Hz).

The secondary methyl groups are deshielded. One may be assigned
as an epoxide methyl (δ 1.37) and the other as an olefinic methyl.

The peak at δ 5.12 could be the methine proton on an epoxide adja-
cent to the aromatic ring split to a doublet by the other methine proton on the
epoxide. The two overlapping quartets at δ 3.42 is from the epoxide methine
proton which is split by the secondary methyl group and the other epoxide
methine proton.

a. Oxidation of Acuminatin with Potassium
Permanganate and Acetone

\[
\text{XLII} \quad \rightarrow \quad \text{XLIII}
\]

Acuminatin (200 mg.) was dissolved in 30 ml. of acetone and 800 mg.
of potassium permanganate was added over a half hour to the refluxing solu-
tion. The mixture was refluxed another half hour and the volume was re-
duced to 10 ml., then 6 ml. water was added and the mixture was refluxed for 5 additional minutes.

An additional 40 ml. of water were added and the manganese dioxide was removed by filtration to give a cloudy yellow solution which, on evaporation of the acetone, still gave a cloudy solution that was at pH 8. A few drops of 0.10 N potassium hydroxide were added to bring the solution to pH 9 and the still cloudy solution was extracted with chloroform (3 x 20 ml.).

The aqueous solution was acidified with concentrated hydrochloric acid and the aqueous layer was extracted with 3 x 25 ml. portions of chloroform. The chloroform layer was washed once with water and dried over anhydrous sodium sulfate. Evaporation of the chloroform gave 187 mg. of a froth.

Thin layer chromatography of the acid (XLIII) on silica gel G using chloroform-acetic acid (93:7) showed one spot at Rf 0.73 which was visualized with bromophenol solution (66). The infrared spectrum in chloroform of the acid showed a peak at ν_max 3500 and a broad peak from 3320 to 3000 cm⁻¹ typical of a carboxylic acid hydroxyl group plus peaks at ν_max 2600 and 1685 cm⁻¹. The NMR spectrum showed a peak at 8 8.80 (broad singlet) which disappeared on deuteration (D₂O exchange) indicating that the oxidation was indeed an acid.
b. Methylation of the Acid from Oxidation of Acuminatin

The acid XLIII (50 mg.) was dissolved in 3 ml. of methanol and 7.0 ml. of ether. An excess of diazomethane prepared from *Diazald R was added and the reaction was left overnight at 0°. Evaporation of the solvent gave an oil which was dissolved in chloroform and passed through a 400 mg. column of silicic acid-celite (4:1) to give 32 mg. of an oil. The infrared spectrum showed the absence of a hydroxyl peak and the presence of carbonyl peak at \( \nu_{\text{max}} = 1708 \text{ cm}^{-1} \).

The NMR spectrum of this material clearly indicated that one of the C-methyl groups had been removed, since the olefinic methyl group of acuminatin at \( \delta = 1.85 \) (doublet, 3H) had disappeared while the epoxide methyl group at \( \delta = 1.33 \) (doublet, 3H) still remained. The spectrum shows these singlets at \( \delta = 3.85, 3.90, \) and 3.92 (12H) corresponding to 4-OMe groups, 3 aromatic OMe groups and the COOMe group. The NMR shows peaks at \( \delta = 7.56 \) (d, \( J = 1 \text{ Hz}, 2H \)), 6.93 (m, 3H), 5.22 (d, \( J = 9 \text{ Hz}, 1H \)) and 3.50 (quintet, 1H). The molecular formula is \( \text{C}_{20}\text{H}_{22}\text{O}_6 \) as shown by high resolution mass spectroscopy. Calcd. for \( \text{C}_{20}\text{H}_{22}\text{O}_6 \): 358.1416. Found: 358.1411.

* N-methyl-N-nitroso-p-toluenesulfonamide
c. Oxidation of Acuminatin with Potassium Permanganate and Sodium Periodate

\[\text{XLII} \xrightarrow{\text{MeO}} \text{XLIV} + \text{XLV}\]

Acuminatin (186 mg.) (XLII) and anhydrous sodium carbonate (41.5 mg.) were dissolved in 67 ml. of t-butanol and 111 ml. of water. To this was added 44.5 ml. of an oxidizing solution prepared according to von Rudloff (67). The reaction mixture was stirred for forty-eight hours at room temperature. Then solid sodium bisulphite was added until the reaction went colorless. Solid sodium carbonate was added until the reaction went basic and then the water and t-butanol were evaporated in vacuo. Toluene was added to azetrop off the remainder of water and t-butanol mixture.

\[1\text{Sodium peridate (10.43 g.) and potassium permanganate (0.198 g.) dissolved in 500 ml. of water.}\]
To the solid residue were added 10 ml. of water. The basic aqueous solution was extracted with 3-20 ml. of chloroform. The chloroform layer was dried over sodium sulfate and evaporated in vacuo to give 77 mg. of an oil which gave a positive 2,4-dinitrophenylhydrazine reaction.

This oil was chromatographed on 5 g. of silicic acid in chloroform. The first 28 ml. of eluate were evaporated to give 3.5 mg. of a mixture. The next 45 ml. eluate gave 67 mg. of an oil that appeared homogeneous on TLC but whose NMR spectrum revealed the presence of t-butanol.

This purified oil was rechromatographed on 5 g. of silicic acid in benzene. The first 50 ml. of eluate gave 14 mg. of a material that gave a negative 2,4-dinitrophenylhydrazine test. The column was then eluted with 45 ml. chloroform to give 3 mg. of a material that likewise gave a negative test. The next 22 ml. of chloroform eluate gave 31 mg. of an oil (XLIV) that appeared homogeneous, Rf 0.40, on TLC using benzene-chloroform (1:1) and gave a positive 2,4-dinitrophenylhydrazine test, but would not crystallize.

The infrared spectrum of this material gave a $\nu_{\text{max}}$ at 1682 cm$^{-1}$ characteristic of an aromatic aldehyde. The mass spectrum whose most intense peak was the molecular ion peak corresponding to 328. This agrees with C$_{19}$H$_{20}$O$_{5}$ whose molecular weight is 328. The ultraviolet spectrum had peaks at $\lambda_{\text{Max}}^{95\% \text{ EtOH}}$ 304 nm ($\epsilon$ 15,100), 288 nm ($\epsilon$ 15,300), 235 nm ($\epsilon$ 24,400); $\lambda_{\text{Min}}^{95\% \text{ EtOH}}$ 294 nm ($\epsilon$ 14,600), 254 nm ($\epsilon$ 3,000), 225 nm ($\epsilon$ 20,000). The optical rotation was $[\alpha]_D^{26} + 89.2^\circ$ (c, 2.2, MeOH). The NMR spectrum had the
following peaks: 6 1.46 (d, 3H, $J = 7.0$ Hz, C-Me), 3.60 (m, 1H), 3.89 (s, 3H, OCH$_3$), 3.98 (s, 6H, 2, OCH$_3$), 5.28 (d, 1H, $J = 9.0$ Hz), 6.95 (m, 3H), 7.38 (m, 2H), and 9.09 (s, 1H, ArCHO).

d. Isolation and Methylation of 2,2',3'-Trimethoxy-5,5'-Carboxy Biphenyl (XLV)

Acuminatin (594 mg.) was oxidized as above (see 9c) with von Rudloff's mixture. The basic aqueous solution after extraction with chloroform to remove the aldehyde was acidified to pH 2 with concentrated hydrochloric acid. The aqueous layer was extracted with 3-75 ml. portions of chloroform. The chloroform layer was dried over sodium sulfate and evaporated to give 64 mg. of an oil. This oil was chromatographed on 1 g. of silicic acid in chloroform to give 58 mg. of a purified material that was still a mixture as evidenced by TLC.

This oil was dissolved in 3 ml. of methanol and treated with an excess of diazomethane in ether at room temperature for 2 hr. Immediately there was nitrogen evolution. The mixture was evaporated in vacuo at 40° to give 60 mg. of an oil.

This oil was chromatographed on 10 g. of silica gel G in chloroform on a column 1 x 23 cm. The column was eluted with chloroform. The first 44 ml. gave a mixture (29 mg.), but the next 24 ml. gave an oil (29 mg.) that appeared homogeneous on three different TLC systems. They were benzene (R$_f$ 0.09), benzene-chloroform (1:1) (R$_f$ 0.48), and chloroform (R$_f$ 0.82). The oil crystallized from ether to give 12 mg. of 2,2',3'-trimethoxy-5,5'-dicarbo-
methoxy biphenyl (XLVI) m.p. 107-108°. This material proved identical to the synthetic derivative as obtained from the Ullman condensation (see page 93). Both had the same relative mobility in three different TLC systems. The NMR and infrared spectra were superimposable and the mixture m.p. showed no depression.

![Chemical structure](image)

**XLVI**

G. **Syntheses of Epoxides and Biphenyls**

1. Anethole trans-epoxide

![Chemical structure](image)

Anethole trans-dibromide (68), 700 mg., was dissolved in 15 ml. of
acetone and 15 ml. of 5% sodium carbonate were added. The mixture was refluxed on a steam bath for one hour and twenty-five minutes and then evaporated in vacuo at 40° to remove the acetone. The aqueous layer was extracted with three fifteen-ml. portions of benzene. The benzene layer was washed with water till neutral and dried over anhydrous sodium sulfate. The benzene was evaporated in vacuo at 40° to give an oil (343 mg.) b.p. 142°, lit. b.p. 1132° (69). The infrared spectrum showed the absence of a hydroxyl peak and $\nu_{\text{max}}$ at 1250 and 840 cm$^{-1}$, epoxide. The NMR was compatible with the structure: NMR peaks at $\delta$ 3.0 (1H, m) methine hydrogen adjacent to methyl group; $\delta$ 3.52 (1H, d, $J = 2$ Hz) methine hydrogen adjacent to aryl ring; $\delta$ 1.42 (3H, d, $J = 6$ Hz) methyl group. The compound has U.V. extremes at $\lambda_{\text{Max}}^{95\% \text{EtOH}}$ 273 $\mu$ ($\epsilon 1,760$) and 228 $\mu$ ($\epsilon 12,900$); $\lambda_{\text{Min}}^{95\% \text{EtOH}}$ 250 $\mu$ ($\epsilon 1,050$) and 213 $\mu$ ($\epsilon 5,350$); $\lambda_{\text{Shoulder}}^{95\% \text{EtOH}}$ 233 $\mu$ ($\epsilon 1,510$).

2. Isoeugenol methyl ether trans-epoxide

![Chemical structure diagram]

Isoeugenol methyl ether dibromide (70), 700 mg. was dissolved in 15 ml. of acetone and 15 ml. of 5% sodium carbonate solution was added. The
mixture was refluxed for one hour and twenty-five minutes. The mixture was evaporated in vacuo to remove the acetone and then extracted with three fifteen-ml. portions of benzene. The benzene layer was washed with water till neutral and dried over anhydrous sodium sulfate to give an oil (446 mg.). The I.R. shows the absence of a hydroxyl peak and $\nu_{\text{max}}$ at 1265 and 840 cm$^{-1}$ (epoxide). The NMR is consistent with the structure. NMR peaks at $\delta$ 1.45 (3H, d, $J = 5$ Hz) C-methyl group; $\delta$ 3.05 (1H, m) methine hydrogen adjacent to methyl group; $\delta$ 3.55 (1H, d, $J = 2$ Hz) methine hydrogen adjacent to aryl ring. The compounds have UV extremes at $\lambda_{\text{Max}}^{95\%\text{ EtOH}}$ 282 nm ($\varepsilon$ 3,010) and 235 nm ($\varepsilon$ 10,400); $\lambda_{\text{Min}}^{95\%\text{ EtOH}}$ 237 nm ($\varepsilon$ 2,780).

3. Isoeugenol trans-epoxide benzoate

\begin{center}
\begin{align*}
\text{CH}_3 & \\
\text{H} & \\
\text{O} & \\
\text{OMe} & \\
\text{OCOC}_6\text{H}_5 & \\
\text{CH}_3 & \\
\text{H} & \\
\text{O} & \\
\text{OMe} & \\
\text{OCOC}_6\text{H}_5 & \\
\end{align*}
\end{center}

Trans-isoaugenol benzoate (71), 1.61 g., was dissolved in thirty ml. chloroform. The mixture was cooled to 0° and meta-chloroperbenzoic acid, 1.23 g., dissolved in 30 ml. of chloroform was added dropwise to the stirring, cooled reaction. The addition took thirty-five min. and the cooled reaction was stirred an additional twenty-five min. The reaction mixture was
placed in the refrigerator at 5° for 24 hr. The reaction was extracted with three sixty-mL portions of 5% sodium bicarbonate and then water till neutral. The chloroform was dried over anhydrous sodium sulfate and evaporated in vacuo to give an oil which crystallized in the freezer. This material was recrystallized from benzene-petroleum ether (30-60°) (1:1) to give 857 mg., m.p. 78-80°. An analytical sample had m.p. 79-80°. The IR had ν_max at 1738 cm⁻¹ (carbonyl) and 1255 and 842 cm⁻¹, (epoxide). The UV had peaks at λ_max 95% EtOH 282 μ (ε 5,200), 276 μ (ε 5,320), 230 μ (ε 25,400) and λ_min 95% EtOH 278 μ (ε 5,130), 257 μ (ε 2,630), 213 μ (ε 14,900). The side chain had the following peaks in the NMR: δ 3.59 (1H, d, J = 2.0 Hz), 3.03 (1H, m) and 1.47 (3H, d, J = 5.0 Hz).


4. Isoeugenol cis-epoxide benzoate

\[ \text{cis-isoeugenol benzoate (71), 1.61 g., was dissolved in 30 mL.} \]
1.23 g., dissolved in 30 ml. of chloroform was added dropwise to the stirring cooled reaction. The addition took 35 min. and the cooled reaction was stirred an additional 25 min. The reaction mixture was placed in the refrigerator at 5° for 31 hr. The chloroform was extracted with three 125-ml. portions of 5% sodium bicarbonate and then water till neutral. The chloroform was dried over anhydrous sodium sulfate and evaporated in vacuo to give an oil, 1.30 g. The oil was chromographed on 9 g. of Woelm neutral alumina (Act. V) in benzene. The first 30 ml. of benzene eluate on evaporation gave an oil 1.12 g. which crystallized from benzene-petroleum ether (30-60°) to give 267 mg., m.p. 80-82°.

An analytical sample had m.p. 83-84°. The IR had ν_max at 1738 cm⁻¹ (carbonyl) and 1260 cm⁻¹ and 830 cm⁻¹ (epoxide). The UV had peaks at λ_max 282 μ (ε 4,350), 275 μ (ε 4,620), 228 μ (ε 20,800) and λ_min 280 μ (ε 4,260), 257 μ (ε 2,660), 213 μ (ε 14,500). The side chain had the following peaks in the NMR: δ 4.09 (1H, d, J = 4.8 Hz), 3.40 (1H, m), and 1.15 (3H, d, J=5.5 Hz).

Anal. Calcd for C₁₇H₁₆O₄: C, 71.82; H, 5.67. Found: C, 71.92; H, 5.89.

5. 3-Iodo-4-Methoxybenzoic acid

The aldehyde, 3-iiedo-4-methoxybenzaldehyde, 180 g., (72) was added to 2.5 l. of 10% sodium bicarbonate that was heated on a steam bath. To the heated, stirring mixture was added dropwise 1.5 l. of a previously heated aqueous solution containing 164.8 g. of potassium permanganate. The time of addition was 1 hr. and the stirring mixture was heated further on the steam bath for an additional 2 hr. 15 min. The hot aqueous solution was
filtered and the filtrate acidified with 150 ml. of cold concentrated sulfuric acid. The precipitated organic acid was filtered and washed well with water. After drying at 100° the yield was 138 g., m.p. 237-238°. Literature value, m.p. 234-235° (73). The IR had \( \nu_{\text{max}} \) at 3200-300 cm\(^{-1}\), (hydroxyl) and 1661 cm\(^{-1}\), (carboxylic acid). The UV had peaks at 95% EtOH 293 m\(\mu\) (\(\varepsilon 1,640\)), 256 m\(\mu\) (\(\varepsilon 12,100\)), 224 m\(\mu\) (\(\varepsilon 26,000\)); \(\lambda_{\text{Min}}\) 95% EtOH 290 m\(\mu\) (\(\varepsilon 1,530\)), 241 m\(\mu\) (\(\varepsilon 7,860\)); \(\lambda_{\text{Shoulder}}\) 284 m\(\mu\) (\(\varepsilon 2,250\)).

Anal. Calcd for C\(_8\)H\(_7\)O\(_3\): C, 34.56; H, 2.54. Found: C, 34.46; H, 2.74.

6. 3-Iodo-4-Methoxy Benzoic acid, Methyl Ester

To 744 ml. of cold methanol was added dropwise 56 ml. of acetyl chloride (24), followed by 87.0 g. of 3-iodo-4-methoxy benzoic acid. The esterification was carried out under reflux for 18 hr. and the now clear solution was evaporated in vacuo to remove 350 ml. of solvent. Then 100 ml. of Skelly B were added and the mixture crystallized to give 87.0 g. of product, m.p. 93-94°. An analytical sample was further crystallized from Skelly B, m.p. 94-95°. Literature value (73) m.p. 94-95°. The IR spectrum had \( \nu_{\text{max}} \) at 1717 cm\(^{-1}\), (ester). The UV had peaks at 95% EtOH 293 m\(\mu\) (\(\varepsilon 1,940\)), 258 m\(\mu\) (\(\varepsilon 14,300\)), 228 m\(\mu\) (\(\varepsilon 27,500\)); \(\lambda_{\text{Min}}\) 95% EtOH 290 m\(\mu\) (\(\varepsilon 1,700\), 242 m\(\mu\) (\(\varepsilon 7,780\)); \(\lambda_{\text{Shoulder}}\) 284 m\(\mu\) (\(\varepsilon 2,680\)). The following values for the NMR were found: 5 3.88 (s, 3H, OMe), 3.92 (s, 3H, OMe), 6.83 (d, 1H, J = 9 Hz), 8.03 (q, 1H, J = 2.0 Hz) and 8.05 (d, 1H, J = 2.0 Hz).

7. Symmetrical and Unsymmetrical Biphenyls

The 5-iodoveratraldehyde (75), 84.7 g., and 3-iodo-4-methoxy methyl benzoate, 84.7 g., were dissolved in 700 ml. of dimethylformamide in a 3-necked 3 l. flask fitted with a condenser and stirrer. To the solution was added copper bronze (44 fine), 140 g. and the mixture was heated under reflux with stirring for 12 hr. Then an additional 140 g. of copper bronze were added and the continuously stirred mixture was refluxed an additional
12 hr. The reaction was cooled to room temperature and the copper salts were filtered and then washed with chloroform. The organic filtrate was evaporated in vacuo at 62° to give 105.1 g. of a dark brown oil. To the oil was added 500 ml. of ether which gave 22.5 g. of 2,2',-dimethoxy-5,5'-dicarbomethoxy biphenyl, m.p. 153-157°. Several crystallizations from ether gave pure material, m.p. 173-174°. The IR had ν max at 1715 cm⁻¹, ester carbonyl. The UV had peaks at λ Max 95% EtOH 257 μ (ε 28,500), 237 μ (ε 29,700); λ Min 95% EtOH 248 μ (ε 25,500). The following values for the NMR were found:
δ 3.82 (s, 6H), 3.89 (s, 6H), 7.0 (d, 2H, J = 8.4 Hz) and 7.92–8.20 (m, 4H).


The 500 ml. of ether was reduced in volume to 200 ml. which gave impure 2,2',3,3'-tetramethoxy-5,5'-diformylbiphenyl, m.p. 122-124°.
Several crystallizations from ether gave pure material, m.p. 137-138°. Literature value (76) m.p. 137-138°. The IR had ν max at 1690 cm⁻¹, (aldehyde). The UV had peaks at λ Max 95% EtOH 310 μ (ε 13,300), 273 μ (ε 20,100), 227 μ (ε 38,600); λ Min 95% EtOH 298 μ (ε 12,100), 257 μ (ε 16,500). The following values for the NMR were found: δ 3.78 (s, 6H), 4.01 (s, 6H), 7.48 (ABq, 4H, J = 2 Hz), and 9.88 (s, 2H).


The 200 ml. of ether were then evaporated in vacuo to give 75 g. of
an oil. This oil was taken up in 175 ml. of benzene which was then extracted with 3-300 ml. portions of 1.35 M sodium bisulfite.

The aqueous bisulfite was basified with 6.5 M sodium hydroxide solution and then extracted with 3-500 ml. portions of chloroform. After discarding the aqueous layer, the chloroform layer was dried over anhydrous sodium sulfate and then evaporated to give 22.8 g. of an oil. The oil appeared to be homogeneous from the NMR spectrums, and crystallized from ether to give 12.2 g. of veratraldehyde, m.p. 44-45°. A mixture m.p. was taken with an authentic sample of veratraldehyde showed no depression. Also, the IR spectrum of the isolated substance was identical to that of an authentic sample of veratraldehyde.

The 175 ml. of benzene was evaporated in vacuo to give 47.5 g. of an oil. To this oil was added a solution made from 100 ml. of saturated aqueous sodium bisulfite solution and 70 ml. of 95% ethanol. A precipitate of 2,2'-dimethoxy-5,5'-dicarbomethoxybiphenyl (m.p. 167-169°) appeared.

The aqueous ethanolic sodium bisulfite solution was evaporated in vacuo to remove the ethanol. The volume was brought up to 400 ml. with water and then extracted with ether. Evaporation of the ether gave an oil which appeared to be a mixture of 2,2'-dimethoxy-5,5'-dicarbomethoxybiphenyl and 2,2',3'-trimethoxy-5-carbomethoxy-5'-formyl biphenyl. The mixture crystallized from Et₂O to give pure 2,2',3'-trimethoxy-5-carbomethoxy-5'-formyl biphenyl (5.34 g.), m.p. 91-92°. The IR spectrum had ν_max at 1725
(ester) and 1695 cm$^{-1}$ (aldehyde). The UV had peaks at $\lambda_{\text{Max}}^{95\% \text{ EtOH}}$ 258 nm ($\varepsilon$ 20, 200), 230 nm ($\varepsilon$ 30, 300); $\lambda_{\text{Min}}^{95\% \text{ EtOH}}$ 252 nm ($\varepsilon$ 19, 800). The following values for the NMR were found: $\delta$ 3.78 (s, 3H), 3.84 (s, 3H), 3.89 (s, 3H), 7.02 (d, 1H, $J = 8.4$ Hz), 7.46 (q, 2H, $J = 1.8$ Hz), 7.92-8.24 (m, 2H), and 9.91 (s, 1H).

Anal. Calcd for C$_{18}$H$_{18}$O$_6$: C, 65.44; H, 5.49. Found: C, 65.42; H, 5.60.

The 400 ml. aqueous layer that had been extracted with ether was acidified to pH 1 with hydrochloric acid. The acidified aqueous layer was then extracted with 2-500 ml. of chloroform. After discarding the aqueous layer, the chloroform was dried over anhydrous sodium sulfate and evaporated to give 13.9 g. of an oil. The oil appeared homogeneous in the NMR and was identical to the NMR of 2,2',3'-trimethoxy-5-carbomethoxy-5'-formyl biphenyl. The oil was dissolved in ether and Skellysolve B followed by a seed crystal of the pure biphenyl. The material crystallized to give 9.90 g., m.p. 83-85$^\circ$. After crystallization from ether pure 2,2';3'-trimethoxy-5-carbomethoxy-5'-formyl biphenyl m.p. 91-92$^\circ$ was obtained.

8. Oxidation of 2,2',3'-trimethoxy-5'-formyl-5-carbomethoxybiphenyl
Solid potassium permanganate (6.0 g.) were added to a magnetically stirred mixture of the aldehyde (1.50 g.) and 75 ml. of 5% sodium carbonate solution at room temperature. The mixture was continuously stirred at room temperature for 21 hours after which time enough solid sodium bisulfite was added to destroy the excess permanganate. Then additional solid sodium bisulfite was added followed by 5 ml. of concentrated hydrochloric acid. All of the manganese dioxide dissolved and a white precipitate of the acid formed. The organic acid was extracted with 3-150 ml. of dichloromethane. The organic layer was dried over anhydrous sodium sulfate and evaporated in vacuo to give a solid material, weight 1.30 g., m.p. 274-277° which is probably a mixture of the mono- and diacid.

9. Methylation of 2, 2', 3'-trimethoxy-5' -carboxy-5'-carbomethoxybiphenyl
The crude acid (1.30 g.) obtained from the oxidation was dissolved in 23 ml. of methanol containing 1.8 ml. of acetyl chloride (74). The mixture was refluxed for 21 hr. and then the solvent was removed in vacuo. The solid residue was crystallized from methanol–ether to give 619 mg. of 2,2',3'-trimethoxy-5,5'-carbomethoxybiphenyl, m.p. 104-105°. An analytical sample m.p. 109-110° was obtained after further crystallization from ether. The IR has a ν_max at 1710 cm⁻¹. The UV has peaks at λ_{Max}^{95\%\text{ EtOH}} 258 \text{ m} (ε 14,600), 225 \text{ m} (ε 19,500); λ_{Min}^{95\%\text{ EtOH}} 248 \text{ m} (ε 14,100); λ_{Shoulder}^{95\%\text{ EtOH}} 298 \text{ m} (ε 2,540). The NMR spectrum had the following values: singlets at δ 3.68 (3H), 3.82 (3H), 3.88 (6H), and 3.95 (3H); δ 7.00 (d, 1H, J = 8.4 Hz), 7.62 (q, 2H, J = 1.8 Hz) and 7.88-8.20 (m, 2H).

Anal. Calcd for C_{19}H_{20}O_{7}: C, 63.33; H, 5.59. Found: C, 63.37; H, 5.65.
DISCUSSION

An examination of the tertiary alkaloid fraction revealed the presence of one major alkaloid anolobine. The quaternary alkaloid fraction was shown to contain at least six alkaloids. The major one was N-Methylindocarpine methiodide. This alkaloid was shown to be identical with an alkaloid isolated from *Menispermum canadense* L. (family, Menispermaeae). N-Methylindocarpine methiodide is isomeric with magnoflorine.

Chromatography of the neutral fraction yielded six crystalline substances. Four of these compounds are lignans. Three are known compounds while the fourth is a new substance which was assigned the name of acuminatin. Costunolide, a sesquiterpene lactone, and β-sitosterol were also identified.

A. *Anolobine (XIV)*

The ultraviolet spectra was characteristic of an aporphine (77) and alkali caused a bathochromic shift indicating the presence of a phenol. The alkaloid was identified by its m.p., optical rotation, and ultraviolet and NMR spectrum. In addition the acetate and methyl ether were prepared which were all consistent with the literature values (16, 55, 56), and the infrared spectrum
is identical to that given by Holubek and Strouk (78). This data indicates that the alkaloid is anolobine (XIV).

B. **N-Methylindocarpine Methiodide (LI)**

N-Methylindocarpine Methiodide isolated from *M. acuminata* was compared with a quaternary alkaloid isolated by Dr. Joseph Knapp from *Menispermum canadense* L. and found to be identical by ultraviolet and infrared spectroscopy as well as TLC and mixture m.p. Since they were isolated within two days of each other a collaborative effort was undertaken to identify the alkaloid.

The ultraviolet spectra of this compound was characteristic of an aporphine (77) and alkali caused a bathochromic shift in alkali indicating that the compound was also phenolic. The infrared spectrum of the isolated alka-
loid was similar to that of magnoflorine indicating that these compounds might be related. The NMR spectrum showed 2 NMe and 2 OMe groups and 3 aryl protons indicating that the compound was related to magnoflorine.

To confirm the basic skeleton and oxygenation pattern the methyl ether was prepared. This proved to be identical to a sample of 0.0-dimethyl-magnoflorine (compared as the iodide salt) as evidenced by the superimposability of the infrared spectra, the same relative mobility on TLC and the mixture decomposition points. In addition the diacetate of N-methylindcarpine was prepared indicating that there were two phenolic groups.

Therefore, the alkaloid has one of the five following structures:
The first two structures (XLVII) and (XLVIII) involve a catechol system. Since the isolated alkaloid gave a negative Quastel test for catechols, these structures can be ruled out.

Only one of the remaining structures (LI) contains an unsubstituted position para to a phenolic group. The isolated alkaloid was submitted for a Gibb's test which was positive indicating that only structure (LI) was consistent with all the data.

The stereochemistry of the asymmetric center was determined by ORD and CD (58).

Lindcarpine, the noraporphine of the isolated quaternary alkaloid, was isolated from Lindera pipericarpa (family, Lauraceae) and characterized as 2,11-dihydroxy-1,10-dimethoxynoraporphine by Kiang and Sim (79).

The 2,11-dihydroxy-1,10-dimethoxyaporphine was isolated from Phoebe clemensii (family, Lauraceae) (80) but it was not named.
C. β-Sitosterol (LII)

Crystallization of fractions 51-87 (see TABLE 5) gave β-sitosterol (LII). Comparison of the isolated material with an authentic sample of β-sitosterol showed them to be identical by infrared spectroscopy, mixture m.p. and TLC behavior. The acetate was prepared and a mixture m.p. with authentic β-sitosterol acetate showed no depression.

\[ \text{LII} \]

D. Calopiptin (XLI)

Crystallization of fractions 88-101 (see TABLE 5) gave calopiptin (XLI). The infrared, ultraviolet and NMR spectra, m.p. and optical rotation of the isolated compound corresponded to a lignan, calopiptin, isolated from \textit{P. moorei} (62).
E. *Galgravin* (LIII)

Crystallization of fractions 106-111 (see TABLE 5) gave *galgravin* (LIII). The characteristic NMR spectrum indicated that the compound belongs to the class of tetrahydrofuran lignans. The infrared, ultraviolet, and NMR spectra, m.p. and optical rotation of the isolated compound corresponds to a lignan, *galgravin*, isolated from *H. belgraveana* (65).
F. **Veraguensin (LIV)**

Crystallization of fractions 119-123 gave veraguensin (LIV). The infrared spectrum of the isolated compound and galgravin are almost identical in all respects and the ultraviolet spectrum of each was the same. The elementary analysis and mass spectrum showed the isolated compound and galgravin to have the same molecular formula. The data corresponds to a lignan, veraguensin, isolated from *O. veraguensis* (63).

Comparison of the isolated substance with an authentic sample of veraguensin showed no depression in the mixture m.p. determination and the infrared spectra were superimposable.

![Chemical Structure](image)

**LIV**

G. **Costunolide (XXVIII)**

Crystallization of fractions 30-40 (see TABLE 6) gave costunolide. The infrared and NMR spectra indicated that the compound was aliphatic and contained an \( \alpha,\beta \)-unsaturated-\( \gamma \)-lactone.
A comparison with an authentic sample of costunolide showed no depression of the mixture m.p. The infrared and NMR spectra were found to be superimposable and TLC showed identical behavior of the isolated compound and authentic costunolide.

H. Acuminatin (XLII)

The infrared spectrum of acuminatin, calopiptin, galgravin and veraguensin were similar indicating all of these compounds were lignans. The infrared spectrum of acuminatin showed no peaks for a hydroxyl group or carbonyl group but did show that the compound was aromatic and contained aryl methoxyl groups.

However, the ultraviolet spectra of the isolated lignan was quite different than the other three lignans and in addition the ε value was higher, indicating the presence of an additional chromophore.

The elemental analysis showed the compound to have the molecular formula C_{21}H_{24}O_{4} which was confirmed by high resolution mass spectroscopy.
The mass spectrum of galgravin was completely different than that of acuminatin (49). The molecular ion peak of galgravin had an intensity of 8% while the most intense peak was due to the fragmentation of the parent ion to give a stabilized carbonium ion m/e 206. In addition a peak at m/e 151, intensity 13%, was also found corresponding to the dimethoxytropylion ion (49).

In the case of acuminatin the most intense peak was the parent ion peak which is characteristic of biphenyls (81) and thus acuminatin was not an \( \alpha, \alpha' \) diaryl tetrahydrofuran like the other three isolated liganans. There was little fragmentation in the mass spectrum but a peak at m/e 325 corresponding to \( \text{C}_{20}\text{H}_{21}\text{O}_4 \) was formed. This peak indicates the loss of a methyl group.

The molecular formula \( \text{C}_{21}\text{H}_{24}\text{O}_4 \) indicates ten double bond equivalents, eight of which are satisfied by the aromatic rings. Since the NMR spectrum of the ABX\(_3\) pattern centered at about \( \delta 6.2 \) is identical to that of the olefinic protons of trans-isodeugenol (82), one of the double bond equivalents is an olefinic bond. The other double bond equivalent is satisfied by an epoxide ring since the NMR spectra clearly indicates the presence of three aryl methoxyls, the other oxygen must be an ether oxygen. NMR spectra of anethole and trans-isodeugenol methyl ether gave identically the same ABX\(_3\) pattern for the two olefinic protons and doublets for the methyl group on a double bond. Rotten-dorf (82) reports a chemical shift of \( \delta 1.84 \) (d, \( J = 5.0 \) Hz) for the methyl on the double bond for the trans-isodeugenol wherein the methyl group for cis-isodeugenol appears as a double doublet. The olefinic proton pattern is com-
pletely different for the cis-isoeugenol thereby indicating the presence of the trans-propenyl group on the biphenyl.

The presence of a biphenyl is also indicated from the NMR spectrum showing five aryl protons. Since there are three aryl methoxy groups and two side chains, the aryl rings must be joined as a biphenyl.

The peak at δ 5.12 (d, J = 9.5 Hz) corresponds to the methine proton on the epoxide adjacent to the aromatic ring. Several epoxides were prepared synthetically. The J value of 2.0 Hz was found for the trans and a J value of 4.8 Hz was found for the cis. The large J value (9.5 Hz) found for the natural product indicates that the epoxide is cis. From Karplus's equation as the dihedral angle between adjacent protons approaches 0° the J value increases. The abnormally high value of 9.5 Hz can be explained by the fact that one is not dealing with a simple system. In addition, the largest J value for the synthetic epoxides was for the cis epoxide. Therefore, in light of this evidence the epoxide is most probably cis.

The peak at δ 1.37 (d, 3H, J = 6.5 Hz) corresponds to a methyl group on a carbon bearing the epoxide oxygen and a methine proton.

Acuminatin was treated under a variety of conditions which gave complex mixtures. Example conditions were potassium dichromate and glacial acetic acid at room temperature, concentrated nitric acid and glacial acetic acid on a steam bath, dilute sulfuric acid on a steam bath and a Birch reduction.
The monoacid from the potassium permanganate and acetone oxidation was then treated with an excess of potassium permanganate and sodium carbonate on a steam bath but the compound was destroyed.

The epoxide aldehyde was treated with dilute sulfuric acid, potassium permanganate and t-butanol but the compound was destroyed as it was when heating in a sealed tube with 6 N hydrochloric acid.

Treatment of acuminatin with potassium permanganate and sodium periodate gave an acid (XLV) which on methylation was identical by infrared and NMR spectroscopy, TLC behavior and undepressed mixture m.p. to 2,2',3'-trimethoxy-5,5'-dicarbomethoxydiphenyl (XLVI) prepared synthetically.

![Chemical Structure](image)

XLVI

This derivative proves the biphenyl linkage as well as the position of the methoxy groups and side chains. Other biphenyls were possible but this was the most likely since it was biogenetically attractive being joined by ortho, ortho phenol coupling process.

The position of the side chains was ascertained from the ever presence
of a peak at \textit{m/e} 151 corresponding to \(C_9H_{11}O_2\) or the dimethoxytropyl ion in the natural product and its derivative.

Oxidation of acuminatin with potassium permanganate in acetone gave an acid in which the epoxide was still intact and the propenyl side chain had been oxidized. The methyl ester of this acid gave a peak at \textit{m/e} 151 of 16.7\% intensity as well as the M-15 peak and parent ion peak being the most intense peak.

The mass spectrum of acuminatin gave a peak at \textit{m/e} 151 of 11.0\% intensity.

The other compound isolated from the von Rudloff oxidation was the biphenyl aldehyde in which the epoxide was still intact and the propenyl side chain had been oxidized to the aromatic aldehyde. The mass spectrum showed the most intense peak as that of the molecular ion, a M-15 peak and a peak corresponding to \textit{m/e} 151 (intensity 25\%).

In light of the consistent mass spectra, acuminatin (XII) fragments according to the following scheme where \(R\) is \textit{trans}-propenyl, or carbomethoxy or formyl:
Thus the two methoxys can only be on the ring bearing the epoxide side chain since by varying R the peak at m/e 151 still appeared in all three of the mass spectra.

Therefore, in light of the NMR and mass spectral data as well as the unequivocal proof of the structure of the diester (XLVI) derived from acuminatin, only structure XLII is compatible with acuminatin.
SUMMARY OF FINDINGS

1. The tertiary alkaloid fraction yielded the known alkaloid ancolobine.

2. The quaternary alkaloid fraction yielded an unknown alkaloid, N-methylindocarpine methiodide. This alkaloid also found in Menispernum canadense, was characterized as N,N'-dimethyl-2,11-dihydroxy-1,10-dimethoxyaporphine iodide.

3. Four lignans were found in the neutral fraction. Three lignans, calopiptin, galgravia, and veraguensin were known but isolated for the first time from this plant. The fourth is an unknown compound and a proposed structure is given for acuminatin.

4. The sesquiterpene lactone, costunolide, was isolated from the neutral fraction.

5. The ubiquitous sterol, \( \beta \)-sitosterol was also isolated from the neutral fraction.
BIBLIOGRAPHY

Part I

1. Lloyd, J. U., Drugs and Medicines of North America, 2, 141 (1887).
3. Ebert, A. E., ibid., 36, 203 (1864).


BIBLIOGRAPHY

Part II


32. Yang, S., Huang, W., Lin, L., Yeh, P., Chemistry (Taipei), 1961, 144; C. A., 56, 1489c (1962).


42. Herout, V. and Sorm, F., ibid., 1959, 1067.


45. Private communication from F. S. El-Feray.


53. Sugii, Y., J. Pharm. Soc. Japan, 50, 183 (1930); C. A., 24, 3505


68. Beilstein Organische Chemie, VII, 506.

69. ibid., XVII, 115.

70. ibid., VI, 921.

76. Beilstein Organische Chemie, VIII, 542.