THESIS

THE CONSTITUENTS OF CASTILLEJA RHEXIFOLIA,

MAHONIA REPENS AND ONCIDIUM CEBOLLETA

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER
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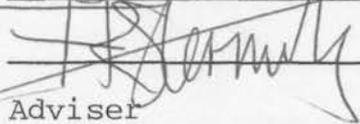FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

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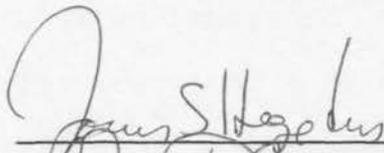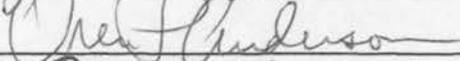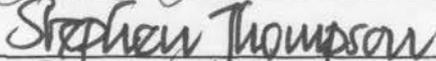
ABSTRACT OF THESIS

THE CONSTITUENTS OF CASTILLEJA RHEXIFOLIA,

MAHONIA REPENS AND ONCIDIUM CEBOLLETA

A total of 54 species of plants from 21 different plant families were collected throughout the subalpine region of the Colorado Rocky Mountains. These plants were tested for antitumor activity, alkaloid content and grasshopper anti-feedant activity. Although none of the plants showed significant antitumor activity, 13 showed the presence of alkaloids and eight showed activity in the grasshopper antifeedant screen.

One of the plants that showed the presence of alkaloids was Castilleja rhexifolia Rydb. Investigation of C. rhexi-folia extracts led to the isolation of senecionine, a pre-viously reported hepatoxic, pyrrolizidine alkaloid. This is the first report of a pyrrolizidine alkaloid in the Scrophulariaceae, and the first alkaloid isolated from Castilleja.

The constituents of Mahonia repens (Lindl.) G. Don, a plant used by the Ramah Navajo of the Southwest in folk medicine, were also investigated. The leaves of M. repens contained four known aporphine alkaloids: isocorydine, corydine, thaliporphine and glaucine. The stems and roots of M. repens contained one known aporphine alkaloid (magno-florine), four known bisbenzylisoquinoline alkaloids (obaberine, thalrugosine, oxyacanthine and obamegine), four known protoberberine alkaloids (palmatine, berberine,

jatrorrhizine and columbamine),the known lignan, syringa-
resinol, and the sugar, sucrose. The isolation of these
secondary metabolites allows a chemotaxonomic comparison of
M. repens to the other Mahonia sp. studied, and also to the
closely related genus, Berberis.

As part of a continuing study to clarify the spectral
data of 1,2,10,11-dihydroxydimethoxyaporphines, N-methyl-
isocorytuberine was synthesized by the Pomeranz-Fritsch
reaction and Pschorr cyclization. Spectral and thin layer
chromatography data for N-methylisocorytuberine were then
compared to two other reported isomers, magnoflorine and
N,N-dimethyllindcarpine.

The orchid, Oncidium cebolleta (Jacq.) Sw., is used by
the Tarahumara Indians of Mexico as an important substitute
for peyote. The chloroform extract of O. cebolleta was
found to contain five previously unreported phenanthrenes
and 9,10-dihydrophenanthrenes. Four of these compounds
were identified as 2,3-dihydroxy-4,6,7 - trimethoxyphenan-
threne, 2,3-dihydroxy-4,6,7-trimethoxy-9,10-dihydrophenan-
threne, 2,7-dihydroxy-3,4-dimethoxyphenanthrene and 2,8-
dihydroxy-4,7-dimethoxyphenanthrene. Although it is not
known if these compounds are responsible for the hallucino-
genic activity of O. cebolleta, their structural similarity to
tetrahydrocannabinol makes this conceivable.

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TABLE OF CONTENTS

CHAPTER I

BIOLOGICAL SCREENING OF ROCKY MOUNTAIN PLANTS

Page

CHAPTER II

THE ISOLATION OF SENECIONINE
FROM CASTILLEJA RHEXIFOLIA RYDB.

CHAPTER III

THE CONSITTUENTS OF MAHONIA REPENS

CHAPTER IV

THE SYNTHESIS OF N-METHYLISOCORYTUBERINE

CHAPTER V

THE CONSTITUENTS OF ONCIDIUM CEBOLLETA

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

BIOLOGICAL SCREENING OF ROCKY MOUNTAIN PLANTS

I. Introduction

In 1977 approximately 386,700 people in the United States died of some form of cancer. Cancer deaths accounted for 20.4% of all the deaths in that year.[1] The percentage of deaths attributed to cancer in the United States has been steadily rising, and as a result, extensive research is focused on the causes and the cures of cancer.

Plants and plant extracts have a long history of use in folk medicine as cancer remedies. Hartwell[2] has compiled a long list of plants reported to be used for this purpose. The National Cancer Institute has had an extensive testing program of plant, animal, fermentation and synthetic products for a number of years. The philosophy of this approach to finding new drugs for the treatment of cancer has been summarized by Kupchan.[3]

> One aspect of the approach of our program differs
> significantly from the classical, and most widely
> practiced, approach to the biological study of
> plant constituents. In the classical phyto-
> chemical approach, those components are studied
> which are most easily separated from a plant
> extract and most easily crystallized. In our
> program, however, the fractionation and isolation
> studies are guided at every stage by biological
> assays. The systematic fractionation, guided by
> biological assays, has made possible the isolation
> of minor constituents which would most probably
> have been missed in the classical approach.

Plants may be selected for biological screening by three main methods: random collection of plants and screening of their extracts, screening of plants used as folk remedies and screening of plants closely related to those with activity shown in previous screens. All three methods were used for the selection of the plants used in this study. Very little work has been done on subalpine plants of the Rocky Mountain region, so the majority of the plants were collected from this area. Many of these same subalpine plants have been used by the Ramah Navaho[4] of the Southwest as folk remedies, and therefore provide an additional reason for their testing. Finally, Eriogonum campanulatum Nutt. showed some antitumor activity, so several other Eriogonum sp. were collected and tested.

The two primary tests for plant extracts are the KB Cell Culture Screen and the P388 Screen. The KB Cell Culture Screen is an in vitro test using cells derived originally from a human epidermoid carcinoma of the mouth. Results are recorded as ED 50%, or the dose that inhibits 50% of the control growth three days after addition. This test is based on cytotoxicity and values of ED $\leq$ 30 μg/ml are a criterion of activity. To pass a second activity criterion level, an average activity (first and second test) of ED $\leq$ 20 μg/ml is necessary. The P388 Screen is an in vivo test using a lymphocytic leukemia induced in mice. The results are expressed as a T/C value - the mean animal weight of the test group divided by the mean animal

weight of the control group. An initial T/C $\geq$ 125% is considered necessary to demonstrate antitumor activity and a reproduced T/C $\geq$ 125% is necessary for further study.[5]

Many of the plant extracts were also tested for the presence of alkaloids and for the ability to inhibit grasshopper feeding. Alkaloids constitute a class of secondary metabolites well known for their physiological activity. Testing the plants for alkaloids might determine which plants have a possibility of possessing interesting biological activity. Although this method cannot guarantee biological activity, it is a simple test that could be productive.

Synthetic insecticides are currently the primary method of insect control, but there are drawbacks to these insecticides. Some are toxic not only to insect pests, but also to beneficial insects, fish and vertebrates through concentration in the food chain. One answer to this problem is to find and use naturally occuring substances, such as pheromones or antifeedants, to protect desired plants from specific insect pests. This may prove to be a far better and less damaging answer to the problem of insect control. This field is currently under intense investigation.

II. Results and Discussion

A total of 54 plants from 21 different families were collected, extracted and tested for antitumor activity.

Of these plants, <u>Eriogonum</u> <u>campanulatum</u> Nutt. was the only plant extract that had sufficient activity in either the KB Cell or the P388 test to pass the National Cancer Institute's primary screen. <u>E</u>. <u>campanulatum</u> did pass the initial KB Cell test, but it failed to pass the second stage screen.

Although none of the plants possessed significant anti-tumor activity, some showed toxicity in the P388 test. Seven plants showed toxicity at levels of 100 mg/kg or less: <u>Polemonium</u> <u>pulcherrimum</u> <u>ssp</u>. <u>delicatum</u> (Rydb.) Brand; <u>Primula</u> <u>parryi</u> A. Gray; <u>Epilobium</u> <u>hornemanni</u> Reichb.; <u>Eriogonum</u> <u>campanulatum</u> Nutt.; <u>Dodecatheon</u> <u>radicatum</u> Greene; <u>Eriogonum</u> <u>alatum</u> Torr.; <u>Zygadenus</u> <u>paniculatus</u> (Nutt.) S. Wats. <u>E</u>. <u>campanulatum</u> was the most toxic of these plants, having toxicity at the 12.5 mg/kg level. The toxic constituents of these plants are unknown, with the exception of <u>Zygadenus</u> <u>sp</u>., which possess toxic steroidal alkaloids. Further investigation is necessary to determine the toxic principles of these plants. <u>E</u>. <u>campanulatum</u> is currently being investigated by another member of the research group.

Alkaloid testing was carried out on 34 of the plants collected. Of those plants tested, 13 showed at least one alkaloid spot on tlc. Three of the plants - <u>Castilleja</u> <u>rhexifolia</u> Rydb., <u>Trollius</u> <u>laxus</u> Salisb. <u>var</u>. <u>albiflorus</u> A. Gray and <u>Thermopsis</u> <u>montana</u> Nutt. ex. T. & G. - contained fairly large quantities of alkaloid. A literature search

revealed that the alkaloids of Castilleja sp. had never been investigated, so that study was undertaken and is reported in Chapter 2.

Of the 18 plant extracts tested for grasshopper antifeedant activity, eight showed some activity. Five of the extracts - Polemonium pulcherrimum ssp. delicatum (Rydb.) Brand, Trollius laxus Salisb. var. albiflorus A. Gray, Phacelia sericea (Graham) Gray, Arabis holbeii Hornem. and Epilobium hornemanni Reichb. - showed antifeedant activity at a statistically significant ($P \leq 0.01$) level. Three of the plants - Erythronium grandiflorum Persh., Ranunculus alismaefolius Geyar ex. Benth. and Senecio dimorphophyllus Greene - showed a feeding stimulant activity at $P \leq 0.01$. Further research is needed to determine what plant constituents are responsible for the activity of these extracts and if they have a possibility of further use in the control of insects.

III. Experimental

A. Preliminary Treatment of Plant Material

The plants used in these studies were collected in subalpine regions of Northern Colorado. Voucher specimens, identified by Dr. Dieter H. Wilken (Department of Botany), were placed in the Colorado State University Herbarium. All plant materials were allowed to thoroughly air dry before they were finely ground. The plants were then extracted and tested for antitumor activity, for alkaloidal

content and some were tested for insect antifeedant
activity.

B. Extraction and Testing Procedure for Plants Collected in 1976

10 g of ground plant material were allowed to stand
for 24 hr in 100 ml of 50% aqueous ethanol. The solution
was filtered and the filtrate concentrated in vacuo below
50°C. 10 ml of water was added, the solution concentrated
to a paste and the paste was allowed to air dry. The dried
material was sent to National Cancer Institute contractors
for antitumor testing. The results are given in Appendix
1. Alkaloid testing was carried out by the acid-base pro-
cedure shown in Figure 1.

The unused portion of the extract, returned by the
National Cancer Institute, was then used by Renee Renaud
and Dr. John L. Capinera (Department of Zoology and
Entymology) for grasshopper antifeedant studies.

C. Extraction and Testing Procedure for Plants Collected After 1976

The procedure for plants tested after 1976 is shown
in Figure 2. Antitumor testing was carried out by the
National Cancer Institute contractors and the results are
given in Appendix 2.

```
                    ┌─────────────────────────────┐
                    │   10 g ground plant material │
                    └─────────────────────────────┘
                                  │
                        extract with 100 ml
                       (1:1) benzene:n-butanol

        ┌─────────────┐                    ┌──────────────────────┐
        │   soluble   │                    │  insoluble material  │
        └─────────────┘                    └──────────────────────┘
                │                                      │
        extract with 2.0 N H₂SO₄                   discard

    ┌───────────────┐                      ┌──────────────────────┐
    │ organic phase │                      │  aqueous acid phase  │
    └───────────────┘                      └──────────────────────┘
            │                                        │
         discard                            extract with CHCl₃

    ┌─────────────────────┐                ┌────────────────────┐
    │ aqueous acid phase  │                │   CHCl₃ solution   │
    └─────────────────────┘                └────────────────────┘
            │                                        │
                                                  discard
```

(1) Make basic to pH 7 with
    NaOH pellets, and then to
    pH 8.6 with NaHCO₃

(2) extract with CHCl₃

```
    ┌──────────────────────────┐          ┌────────────────────┐
    │  basic aqueous solution  │          │   CHCl₃ solution   │
    └──────────────────────────┘          └────────────────────┘
            │                                        │
         discard
```

(1) concentrate and then
    spot on tlc
(2) develop tlc in (3:2)
    benzene:methanol
(3) spray with iodoplatinic
    acid (blue-purple spot
    indicates alkaloid
    positive)

Figure 1   Alkaloid Testing of 1976 Plants

```
                    ┌─────────────────────────┐
                    │   300 g plant material  │
                    └─────────────────────────┘
                                 │
              extract with hexane in Soxhlet for 8 hr
                                 │
            ┌────────────────────┴────────────────────┐
    ┌───────────────────┐                    ┌──────────────────┐
    │   plant material  │                    │     soluble      │
    └───────────────────┘                    └──────────────────┘
    extract with 95% EtOH in                 test for alkaloids by tlc
    Soxhlet for 8 hr
              │
      ┌───────┴──────────────────────────┐
 ┌──────────────────┐            ┌──────────────────┐
 │  plant material  │            │  EtOH solution   │
 └──────────────────┘            └──────────────────┘
          │                               │
       discard                  (1) concentrate to 100 ml
                                (2) test for alkaloid by
                                    tlc
                                        │
                    ┌───────────────────┴───────────────────┐
        ┌──────────────────────┐            ┌──────────────────────┐
        │  25 ml of solution   │            │  75 ml of solution   │
        └──────────────────────┘            └──────────────────────┘
                 │                                     │
    concentrate to paste,               (1) concentrate to paste
    Sample A sent to NCI                (2) partition between CHCl₃:
                                            H₂O (1:1) 200 ml of each
                                                   │
                            ┌──────────────────────┴──────────────────────┐
                  ┌──────────────────────┐            ┌──────────────────────┐
                  │    CHCl₃ soluble     │            │    H₂O soluble       │
                  └──────────────────────┘            └──────────────────────┘
                           │                                     │
                concentrate to solid,                         discard
                Sample B sent to NCI
```

In the flowchart, the chemical labels read: partition between $CHCl_3$: H_2O (1:1) 200 ml of each; $CHCl_3$ soluble; H_2O soluble.
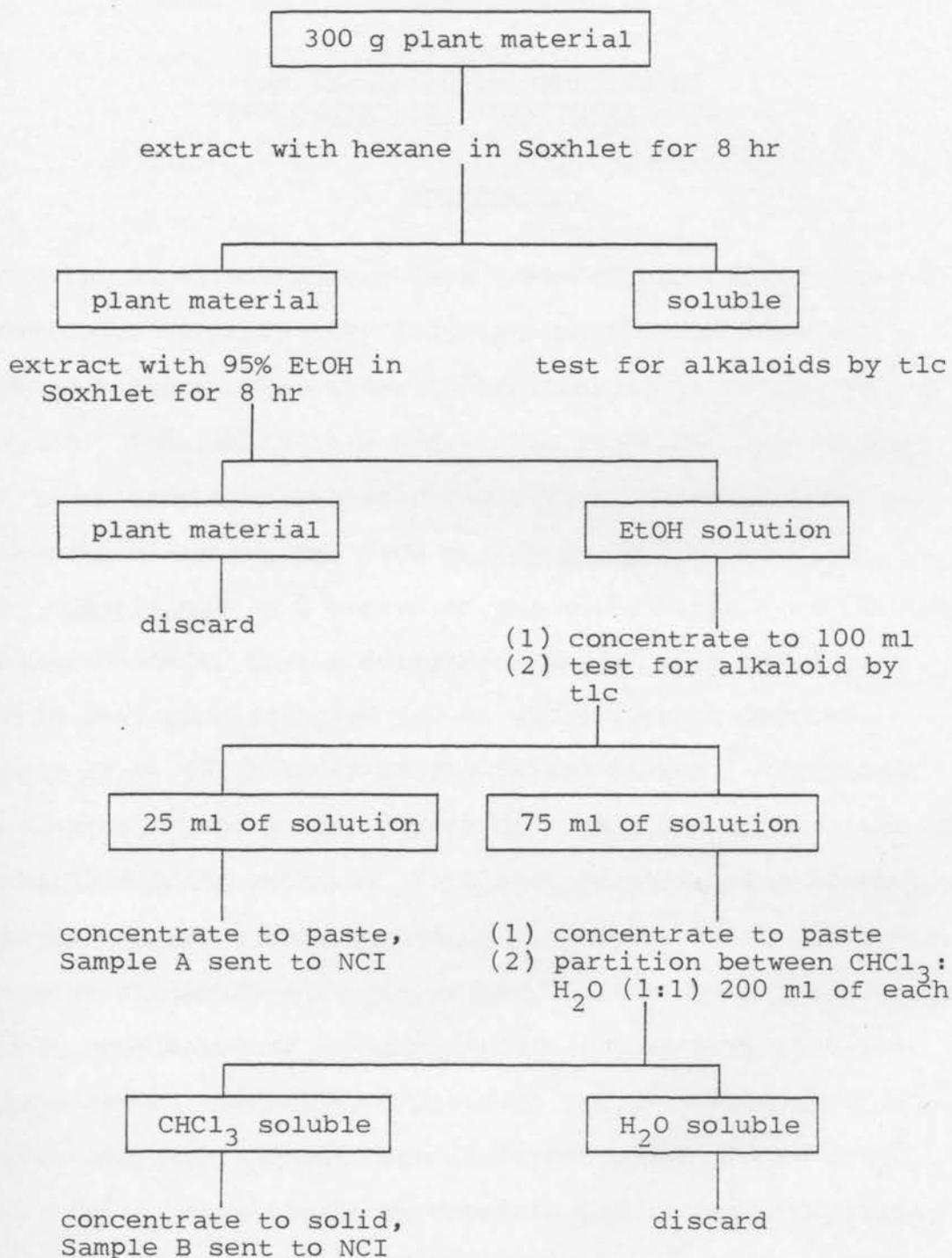
Figure 2 Plant Extraction and Testing Procedure After 1976

CHAPTER II

THE ISOLATION OF SENECIONINE
FROM CASTILLEJA RHEXIFOLIA RYDB.

I. Introduction

The Scrophulariaceae is a cosmopolitan family con-
sisting of approximately 3000 species divided into 220
genera. About 40 of these genera are native to the United
States. The family is economically important because many
of the plants are used as ornamentals. Important cardiac
glycosides are derived from Digitalis sp.[6]

Castilleja is a member of the hemiparasitic subfamily,
Rhinanthoideae, of the Scrophulariaceae. The genus is a
large one, consisting of 200 to 250 different species,
about 35 of which occur in the United States.[7] Taxonomic
treatment of the genus is very difficult due to the lack of
clearly defined species. This lack of definition between
species is due mainly to the hybridization which can occur
between distantly related species.[8] Chromosome counts have
shown many cases of polyploid plants, with many reported
instances of natural hybridization and artificial hybridi-
zation between species with different polyploid levels.[9]

While screening Rocky Mountain plants for biological
activity (Chapter I), Castilleja rhexifolia Rydb. was found
to contain a large amount of alkaloidal material. Since
alkaloids had previously never been reported in the

<u>Castilleja</u> genus, the isolation and structural elucidation of this component were undertaken.

II. <u>General Experimental</u>[10]

^{1}H-NMR shifts are reported using the δ scale with tetramethylsilane (TMS) as the internal standard except in the case of deuterium oxide, where sodium-2,2-dimethyl-2-silapentane sulfonate (DSS) was used as the internal standard. 60 MHz spectra were taken on either a Varian T-60 or a Varian EM-360 instrument. 100 MHz spectra were recorded on either a Jeol MH-100 or a Jeol FX-100, and 360 MHz spectra were recorded on a Nicolet NT-360 instrument. ^{13}C-NMR were recorded on a Jeol FX-100 instrument.

Infrared (IR) spectra were taken on a Beckman Acculab 3 or a Beckman IR 4240 as KBr pellets, a chloroform solution or a thin film on a NaCl plate.

Ultraviolet-Visible (UV-Vis) spectra were recorded on a Varian Techtron 635 in ethanol solution. Several drops of 1 N potassium hydroxide were added to determine base shifts, while concentrated hydrochloric acid was added to determine acid shifts.

Mass spectra (MS) were obtained on an AEI MS-12, an AEI MS 902 or a V.G. Micromass 16F. High resolution mass spectra were done on a AEI MS 902.

Optical rotations were determined on a Perkin-Elmer Model 241 Polarimeter using the Na 589 Line.

All melting points were obtained on a Laboratory Devices Mel-Temp and are uncorrected.

Thin layer chromatography (tlc) and preparative layer chormatography (plc) were performed on precoated silica gel 60 F-254 plates or on aluminum oxide (Al_2O_3) F-254 (Type T) plates, both manufactured by E. Merck of Darmstadt, Germany. Alkaloids were visualized by spraying the developed plates with iodoplatinic acid spray.[11]

Two standard tlc solvent systems were used. They were "S_4" (15:3:1 - methanol:water:ammonium hydroxide) and "formamide" (7:7:4:1 - chloroform:methanol:formamide: water).

III. <u>Results</u>

A. <u>Plant Material and Preliminary Extraction</u>

Stems, leaves and flowers of <u>C</u>. <u>rhexifolia</u> Rydb. were collected on June 22, 1976, northwest of Cameron Pass Campground, Larimer County, Colorado (Colorado State University Accession Number 57109). <u>C</u>. <u>rhexifolia</u>, commonly called Indian paintbrush, is a perennial growing 20-60 cm tall with conspicuous red bracts enclosing the small flowers.

263 g of finely ground plant material were successively extracted with 2.5 l of Skellysolve-F, chloroform and then methanol. The extracts were concentrated under reduced pressure and a tlc was developed using (3:2) benzene: methanol on Si gel. The tlc plate was sprayed with

iodoplatinic acid to check for alkaloidal material. Only
the methanol fraction showed sizeable quantities of alka-
loid, so this was the only fraction investigated.

B. Isolation of Senecionine[12]

The residue from the methanol extract was dissolved in
200 ml of 2 $\underline{N}$ sulfuric acid and extracted with chloroform.
The aqueous phase was adjusted to pH 8.6 with sodium bi-
carbonate and again extracted with chloroform. This
chloroform extract was dried with sodium sulfate and con-
centrated to give 0.114 g of crude alkaloidal material.
Tlc on Si gel using (2:1) acetone:water showed two alkaloid
positive spots at R_f 0.34 and R_f 0.08. A Si gel plc
plate of the crude material was run with water:acetone
(1:2), and the R_f 0.20-0.30 region eluted. Crystallization
provided 15 mg (0.006% yield) of an alkaloid whose IR
spectrum showed two carbonyl stretches at 1710 and 1740
cm^{-1}. A mass spectrum showed a molecular weight of 335.
The IR, MS, ^{1}H-NMR, UV and tlc were identical to those of
senecionine (Figure 3), a pyrrolizidine alkaloid previously
isolated from $\underline{Caltha}$ $\underline{leptosepala}$.[13] Flowers of $\underline{C}$. $\underline{rhexi}$-
$\underline{folia}$ gave a very strong alkaloid test on tlc, but insuf-
ficient material was available for isolation. A semi-
quantitative test suggested that the flowers had a higher
alkaloid content than did the whole plant.

Three other species of $\underline{Castilleja}$ - $\underline{C}$. $\underline{flava}$ Watson,
$\underline{C}$. $\underline{sulphurea}$ Rydb. and $\underline{C}$. $\underline{chromosa}$ A. Nels. - were also
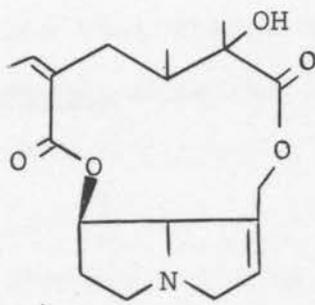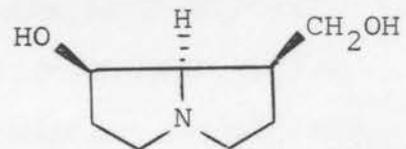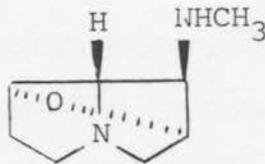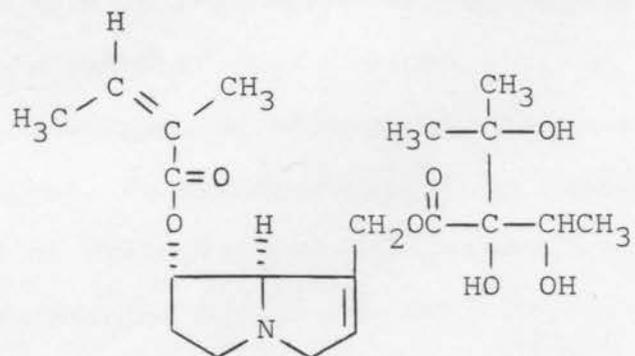
Senecionine
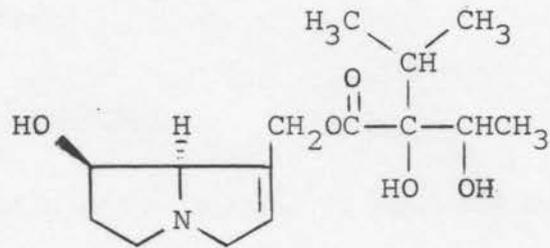
Indicine
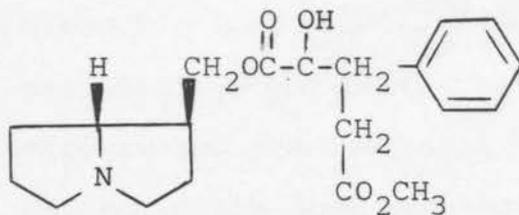
Phalaenopsine

Platynecine

Loline

Lasiocarpine

Figure 3 Pyrrolizidine Alkaloids

tested for alkaloids. Of the three, C. chromosa was the only one that showed alkaloid by tlc. The alkaloids in C. chromosa were not identified.

IV. Discussion

The isolation of senecionine is the first example of a pyrrolizidine alkaloid in the Scrophulariaceae and the first alkaloid isolated from the genus Castilleja. Studies of Castilleja have shown that they are facultative para- sites. They are not host specific and are able to grow without a host plant.[14] Because of the lack of host specificity, the high concentration of alkaloid in the flowers and the lack of a direct connection between the phloem of the host and the Castilleja plant,[15] it is likely that C. rhexifolia - not the host plant - produces the alka- loid. This question is under investigation at the present time.

Pyrrolizidine alkaloids have been found in many plant families, including the Apocynaceae, Boraginaceae, Celastraceae, Compositae, Graminae, Legumionsae, Orchidaceae, Ranunculaceae, Rhizophoraceae, Santalaceae, and Sapotaceae. There are five main types of pyrrolizidine alkaloids (Figure 3). One type, represented by loline, is a 1- aminopyrrolizidine derivative. The other four types are either simple derivatives or diastereomers of 1-hydroxy- methylpyrrolizidine. Platynecine is a simple aminoalcohol derivative; indicine and lasiocarpine are representative of

the aliphatic monocarboxylic acid derivatives and phalaenopsine is typical of the aminoalcohol esters with either an aryl or an alkylaryl side chain. The fifth group, represented by senecionine, is macrocyclic diester derivatives.

Pyrrolizidine alkaloids are well known for their physiological activity. Plants containing pyrrolizidine alkaloids and some of the purified alkaloids (lasiocarpine, for example) have been shown to be carcinogenic[16, 17, 18] and toxic to animals and humans.[19] The toxic alkaloids are all esters of 1-hydroxy-1,2-dihydropyrrolizidine containing branched chain acids. These alkaloids are chronic toxins, causing liver and lung lesions along with other symptoms. Hepatotoxic pyrrolizidine alkaloids have been found to be transferred into milk by cows[20] and into honey by bees.[21] Pyrrolizidine alkaloids are somewhat soluble in water (e.g., senecionine, 35 mg/l cold water) and were apparently responsible for human deaths when Senecio flowers were incorporated into herbal teas.[22] Since senecionine is one of the chronic hepatotoxins, in areas where C. rhexifolia is abundant the possibility of transfer into milk and honey must be considered. Since two of the four Castilleja sp. examined in this study were shown to contain alkaloids, their incorporation into herbal teas would not be wise.

CHAPTER III

THE CONSTITUENTS OF MAHONIA REPENS

I. Introduction

The Berberidaceae is a small family, with nine genera
and approximately 590 species native to the northern
hemisphere and South America. The Berberidaceae are shrubs
or perennial herbs having either simple or pinnately com-
pound, alternate leaves. Many of these plants are used as
ornamentals.[6]

The two largest genera in the Berberidaceae are
Berberis, containing about 450 species, and Mahonia, con-
taining about 70 species. These two genera are taxonomical-
ly divided on the basis of several characteristics. In
Berberis, the leaves are always simple, whereas in Mahonia,
the leaves are always imparipinnate. This is the only
reliable distinction, but others do exist. Mahonia sp.
never have thorns on the stems and are always evergreen,
while Berberis sp. usually have thorns on the stems and
may be either evergreen or deciduous.[23]

Many Berberis sp. have been intensively studied due to
their wide distribution and common use in folk medicine.
There is wide use of these plants in India for the treat-
ment of gastric duodenal ulcers, in chronic diarrhea and
piles, in jaundice, and for the treatment of rheumatic
conditions.[24] The alkaloids of many Berberis sp. have
been thoroughly investigated. Those alkaloids isolated

have all been of the isoquinoline type. Seven major structural types have been found: protoberberines, aporphines, bisbenzylisoquinolines, phthalido-isoquinolines, protopines, proaporphines and aporphine-benzylisoquinoline dimers.

Although they are very closely related to Berberis, Mahonia sp. have not been investigated as thoroughly. The alkaloidal constituents of 18 different species of Mahonia have been reported (Table 1). Ten different alkaloids have been isolated from these species (Figure 4).

The genus Mahonia is divided into two groups - the Orientales and the Occidentales. The Orientales grow in the Himalayas from Kashmir and Punjab to Bhutan, in Tibet, China, India, the Phillipines, and Formosa, and one species grows on the California coast. The Occidentales grow in Northwest and Central America. Of the 18 species studied, 15 belong to the Orientales group, while only three - M. swaseyii, M. trifoliolata and M. aquifolium - belong to the Occidentales group. Each group is further divided into sections; the Occidentales are divided into three sections: Aquifoliateae, Paniculatae and Horridae. M. swaseyii and M. trifoliolata belong to the section Horridae, while M. aquifolium belongs to the Aquifoliatae. Some of the sections are further divided into subsections. Aquifoliatae is divided into the Schiedeanea and the Euaquifoliatae; M. aquifolium is in the subsection Euaquifoliatae.[23]

	1	2	3	4	5	6	7	8	9	10
M. acanthifolia G. Don[29,31]	x	x	x						x	
M. aquifolium (Pursh.) Nutt.[39]	x	x		x	x	x		x	x	
M. borealis Takeda[32,36]	x	x	x						x	
M. fortunei (Lindl.) Fedde[35]	x	x	x				x	x	x	
M. griffithii Tadeda[30]	x	x	x					x	x	
M. japonica (Thundb.) DC.[34]	x	x	x					x		x
M. leschenaultii (Wall) Takeda[33]	x	x					x		x	
M. lomariifolia Takeda[38]	x	x	x				x	x		x
M. manipurensis Takeda[32,33]	x	x	x						x	
M. morrisonensis Takeda[38]	x	x	x				x	x		x
M. napaulensis DC.[29]	x	x								
M. nepalensis Fedde[28]	x	x								
M. philippinensis Takeda[25]	x	x	x					x		x
M. sikkimensis Takeda[33]	x	x							x	
M. simonsii Takeda[32,36]	x	x	x						x	
M. swaseyi (Buckley) Fedde[26,27]	x							x		
M. thunbergii DC.[37]		x					x	x	?	
M. trifoliolata (Moric.) Fedde[26]	x									

1) Berberine   6) Isoboldine
2) Jatrorrhizine   7) Magnoflorine
3) Palmatine   8) Berbamine
4) Isocorydine   9) Oxyacanthine
5) Corydine   10) Isotetrandine
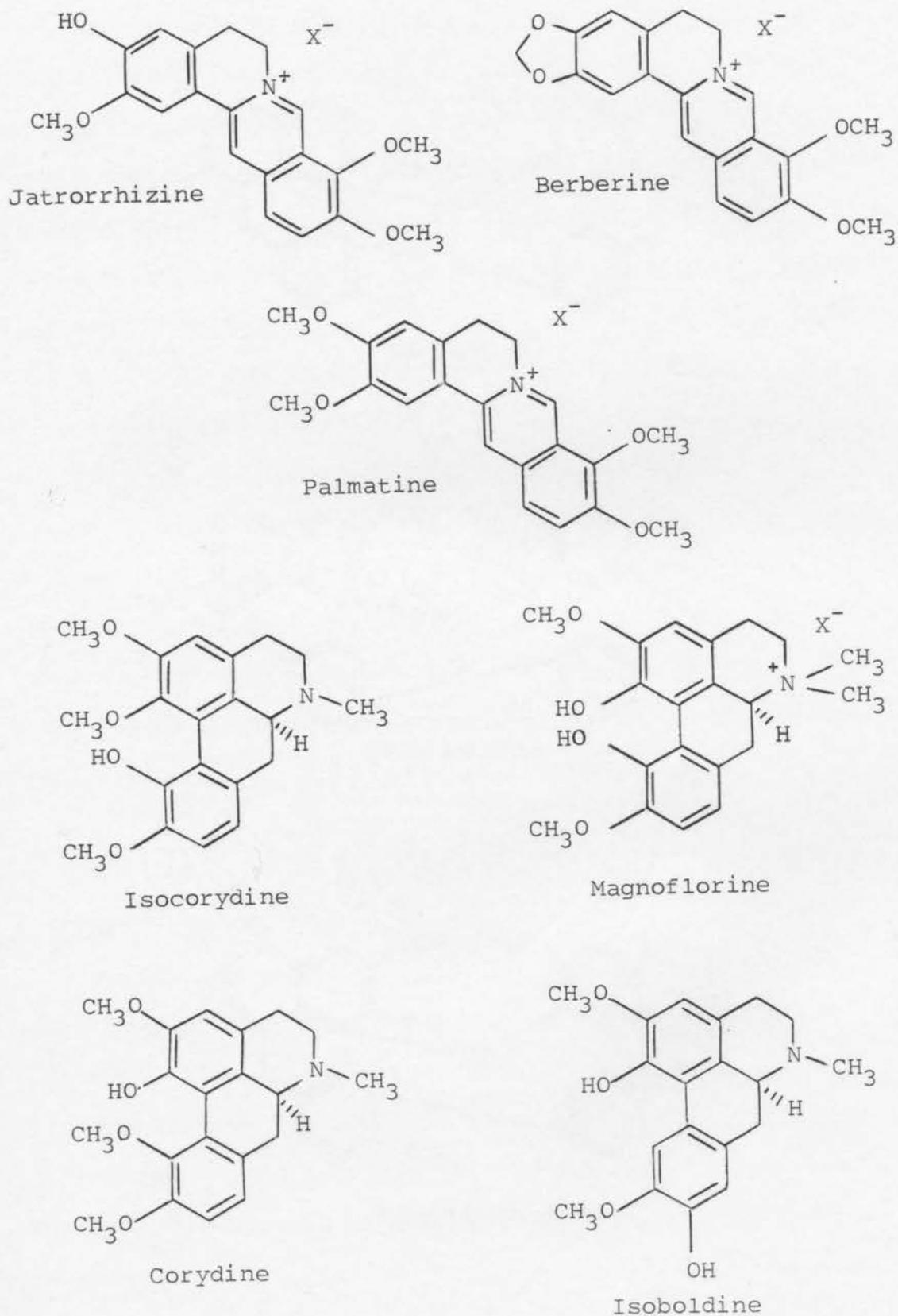
Table 1 Mahonia Species Studied

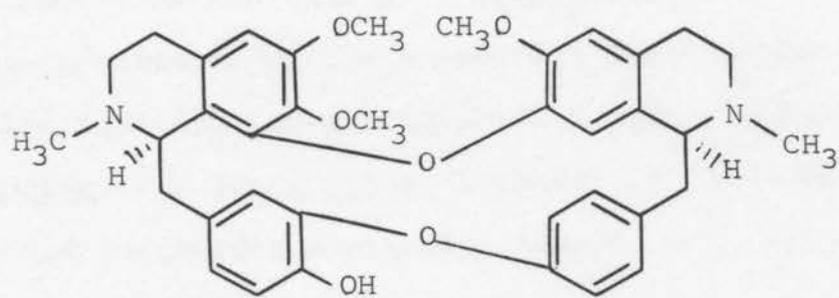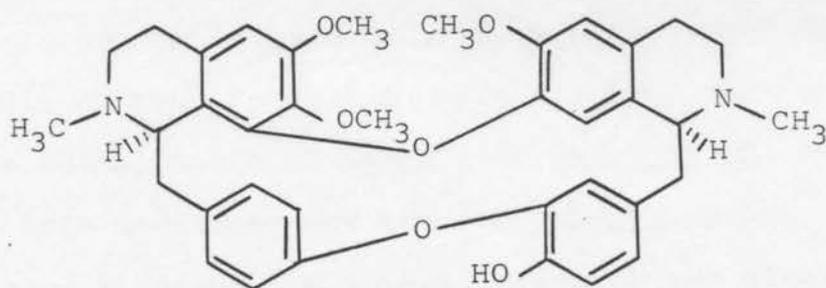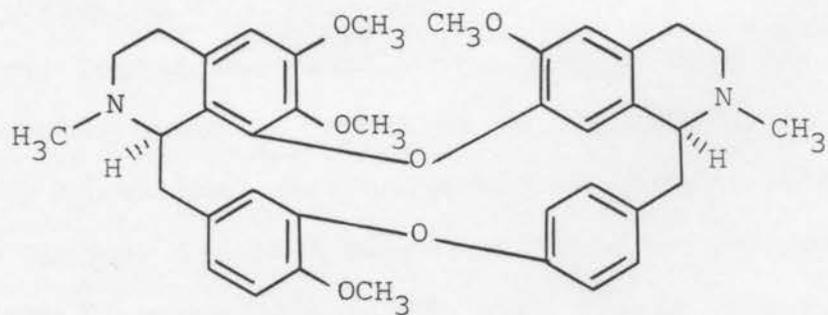Figure 4   Alkaloids Previously Isolated from Mahonia

Berbamine

Oxyacanthine

Isotetrandine

Two species of <u>Mahonia</u> are native to Colorado: <u>M</u>.
<u>repens</u> (Lindl.) G. Don. and <u>M</u>. <u>fremontii</u> (Torr.) Fedde.
<u>M</u>. <u>repens</u> is a member of the subsection Euaquifoliatae of
the section Aquifoliatae and as such, is closely related to
<u>M</u>. <u>aquifolium</u>. <u>M</u>. <u>fremontii</u> is a member of the section
Horridae and is therefore closely related to <u>M</u>. <u>trifolio-</u>
<u>lata</u> and <u>M</u>. <u>swaseyii</u>.

<u>M</u>. <u>repens</u> was reported to be used by the Ramah Navajo
in several ways.[4] The whole plant was used as an emetic; a
decoction of the roots was made to treat constipation, and
a cold infusion or lotion was used to treat scorpion bite.
As a result of the use made of this plant by the Navajo,
the close relationship of <u>Mahonia</u> to <u>Berberis</u> <u>sp</u>. used in
India as folk medicines and the few <u>Mahonia</u>, group
Occidentales studied, the investigation of the alkaloidal
constituents of <u>M</u>. <u>repens</u> was undertaken.

II. <u>Results</u>

A. <u>Plant Material</u>

Stems, leaves and roots of <u>M</u>. <u>repens</u>, known as Oregon
grape (voucher specimen deposited in the Colorado State
University Herbarium) were collected in August, 1978, on
Colorado Highway 14, 36.3 miles northwest of the junction
of Colorado Highways 14 and 287. The plants were growing
on the southeast side of the road, scattered throughout
the forest.

M. repens is a small, creeping ground cover with stems
rarely more than 25 cm tall. It is found throughout the
mountainous regions of Montana and British Columbia, south
to New Mexico and California.[40] The wood and inner bark
of the roots and stems is a bright yellow color, which also
appears bright yellow under long wave UV radiation. The
stems, leaves and roots were separated prior to grinding
to allow analysis of the alkaloidal constituents of each
plant part.

B. Extraction of the Leaves and Isolation of the Aporphine Alkaloids

925 g of finely ground M. repens leaves were succes-
sively extracted in a Soxhlet apparatus for 24 hr each
with 2.5 1 of hexane and ethanol, Evaporation of the hexane
extract in vacuo provided 36 g of material. An acid-base
extraction (Figure 1) was performed on the extract, yield-
ing only a small amount of alkaloid positive material by
tlc.

Evaporation of the ethanol extract yielded 329 g of a
viscous, green oil. Chloroform extraction of the pH 9
aqueous phase from an acid-base extraction provided 8.23 g
of alkaloidal material. Tlc showed the presence of the
same alkaloid in the hexane extract, along with at least
four others.

6 g of the material isolated from the acid-base extrac-
tion of the leaves was adsorbed to 12 g of basic alumina
(Baker). This material was then placed on top of a 4.8 by

12.7 cm column of basic alumina. The column was eluted first with 600 ml of toluene:methanol (15:1), and five fractions were collected. The solvent was then changed to methanol (150 ml) and an additional fraction collected. Tlc of the fractions showed that 1 and 2 contained primarily one alkaloid; 3 through 5 contained a mixture of that alkaloid and three others, and fraction 6 contained two polar alkaloids.

Fractions 1 and 2 were combined to give one gram of material. The UV (EtOH) (Figure 5) having absorptions at λ max 221, 266, 302, with a bathochromic shift to 343 upon addition of base, proved the alkaloid to be a 10,11-substituted aporphine alkaloid with at least one phenolic substituent. An NMR (CDCl$_3$) (Figure 6) showed the presence of three methoxy substituents with peaks at 3.66, 3.83, 3.86 and a phenol at 8.82. It also showed the presence of an N-methyl at 2.46 and three aromatic protons at 6.65 (1H, H-3) and 6.80 (2H, H-9 and H-8). A MS (M+ 341, 326, 310, 295, 280, 266) established the molecular weight of 341. The sample was crystallized from ethanol to give material having mp 183-185°C and $\angle\alpha\angle_D$ = +204 (c = 0.026, CHCl$_3$). The NMR,[41] UV,[42] MS,[43] mp[44] and optical rotation[45] are consistent with the aporphine alkaloid isocorydine (Figure 7).

The mother liquour from fractions 1 and 2 was combined with fractions 3, 4 and 5. This material was injected onto a 2.5 by 95 cm MPLC (Medium Pressure Liquid Chormatography) column and eluted with chloroform:methanol
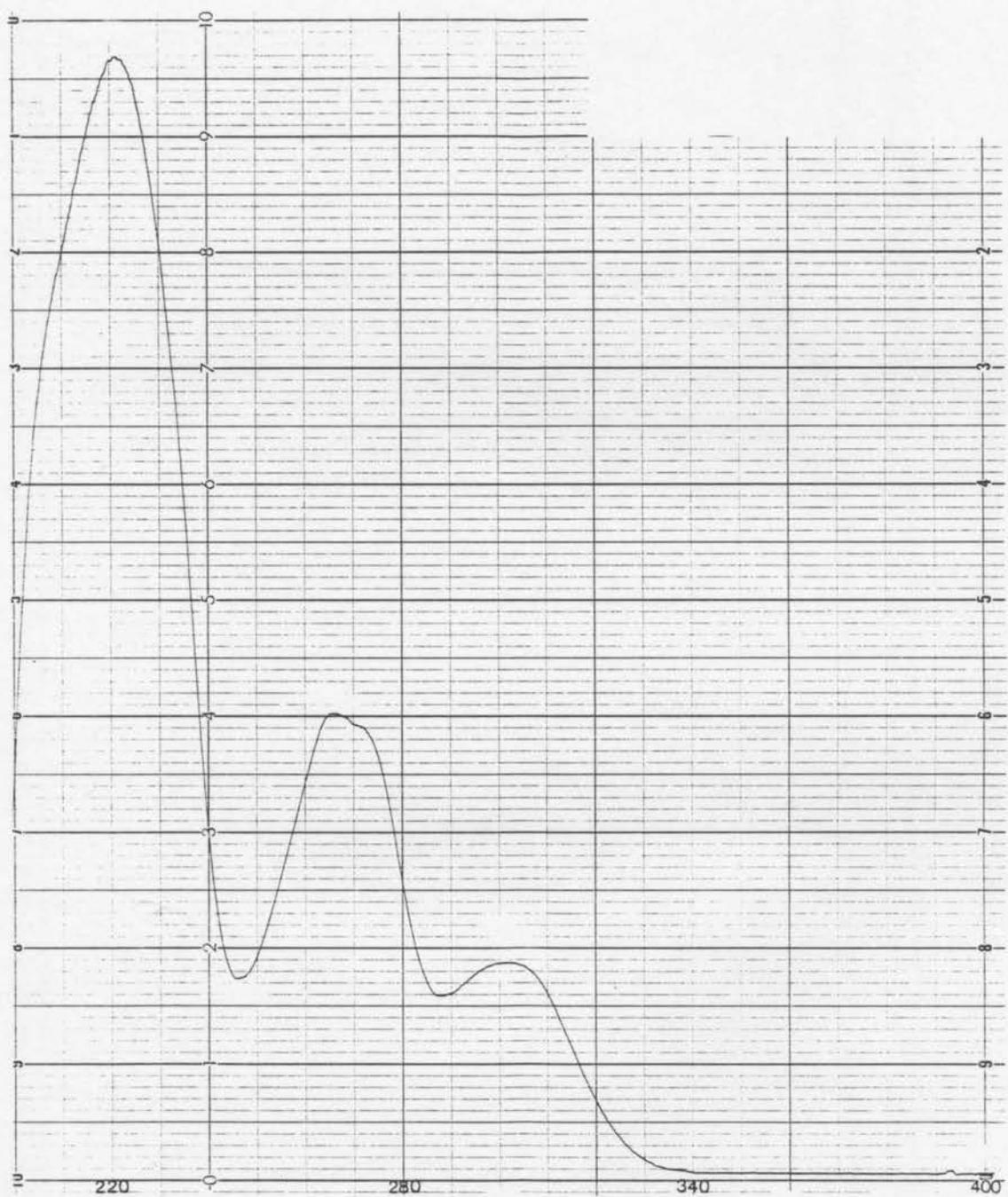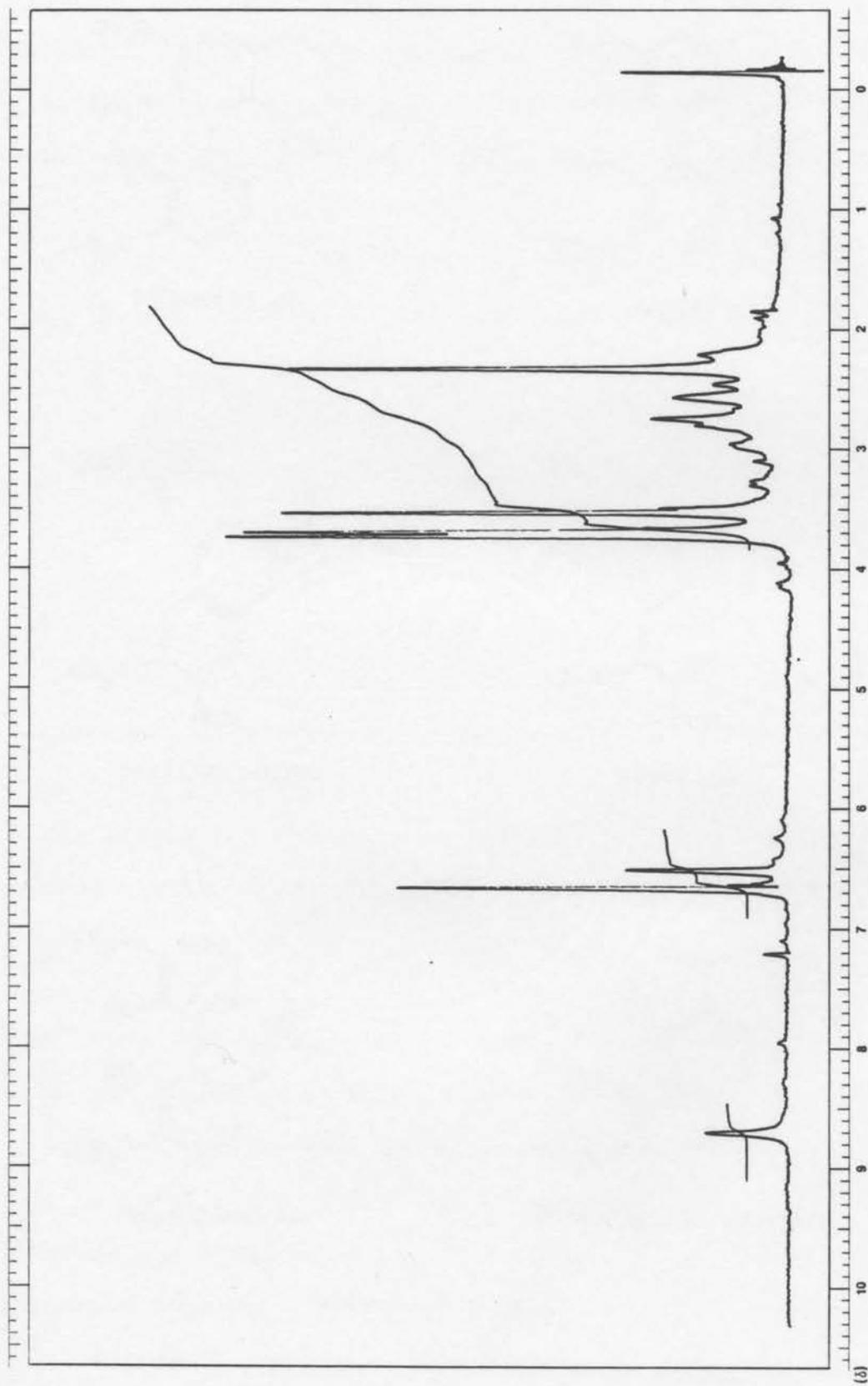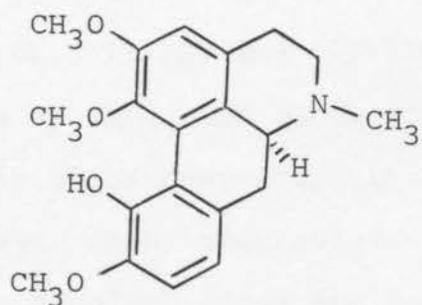
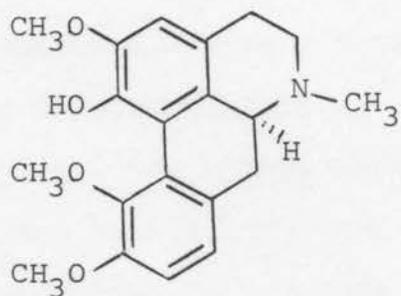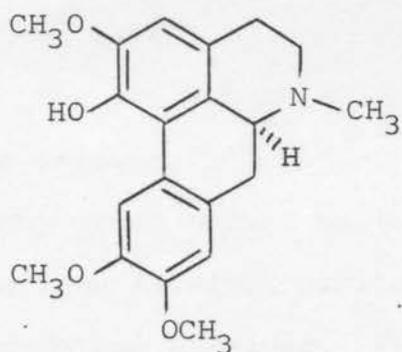Figure 5 UV (EtOH) of Isocorydine

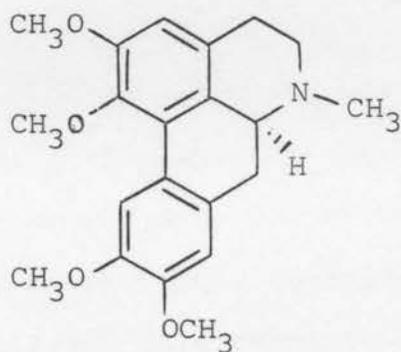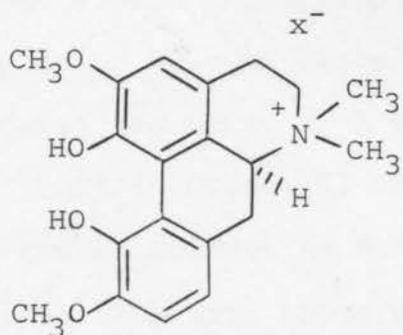Figure 6 ^{1}H-NMR (CDCl$_3$) of Isocorydine
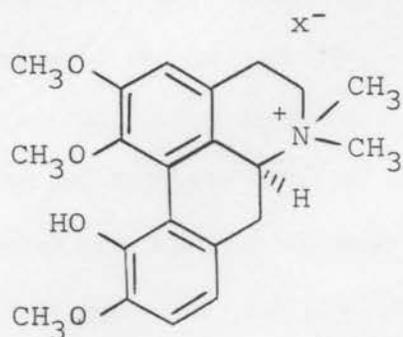
Isocorydine

Corydine

Thaliporphine

Glaucine

Magnoflorine

N-methylisocorydine

Figure 7 Aporphine Alkaloids of <u>M</u>. <u>repens</u> Leaves

(99:1). 15 ml fractions were collected. Fractions 66-97,
98-108, 111-160 and 170-230 each contained a single alka-
loid by tlc. The spots were easily distinguishable by
their color reaction. 16 to 24 hr after the plates were
sprayed with iodoplatinic acid, it was noticed that the
four alkaloid spots had developed different colors when the
back of the plate was viewed. The spot corresponding to
fractions 66-97 showed a bright green color, 98-108 showed
yellow, 111-160 showed grey-green, and 170-230 showed an
orange color.

The two alkaloids which gave rise to greenish colors
were investigated first. Fractions 66-97, showing the
bright green color, were found to contain more isocorydine.
Fractions 111-160, giving the grey-green color, contained
a different alkaloid. Fractions 111-160 were combined and
evaporated to give 0.5 g. A UV (EtOH) (Figure 8) had adsorp-
tions at λ max 224, 266, 273 and 305. Addition of base
caused a bathochromic shift to λ max 347 nm. This UV is
similar to that of isocorydine, showing again the 10,11-sub-
stituted aporphine with at least one phenolic hydroxy. An
NMR (CDCl$_3$) (Figure 9) showed three methoxys: one at 3.73
and two coincident at 3.93. An N-methyl resonance was pre-
sent at 2.53 with three aromatic protons: a one proton sing-
let at 6.70, a one proton doublet at 6.85 (J = 8 Hz) and
another one proton doublet at 7.12 (J = 8 Hz). The phenolic
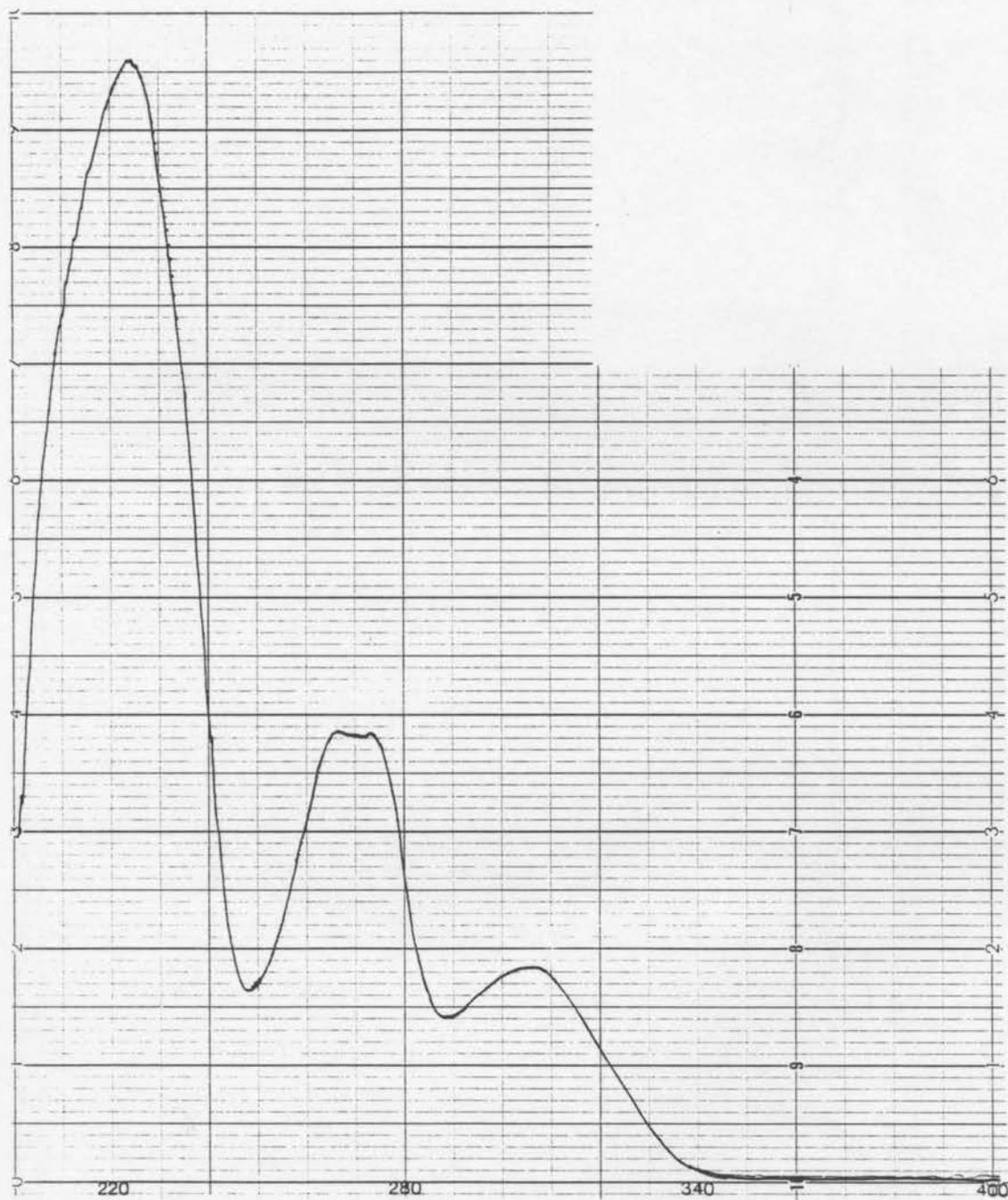hydrogen appeared as a broad singlet at 8.72. The UV[46]
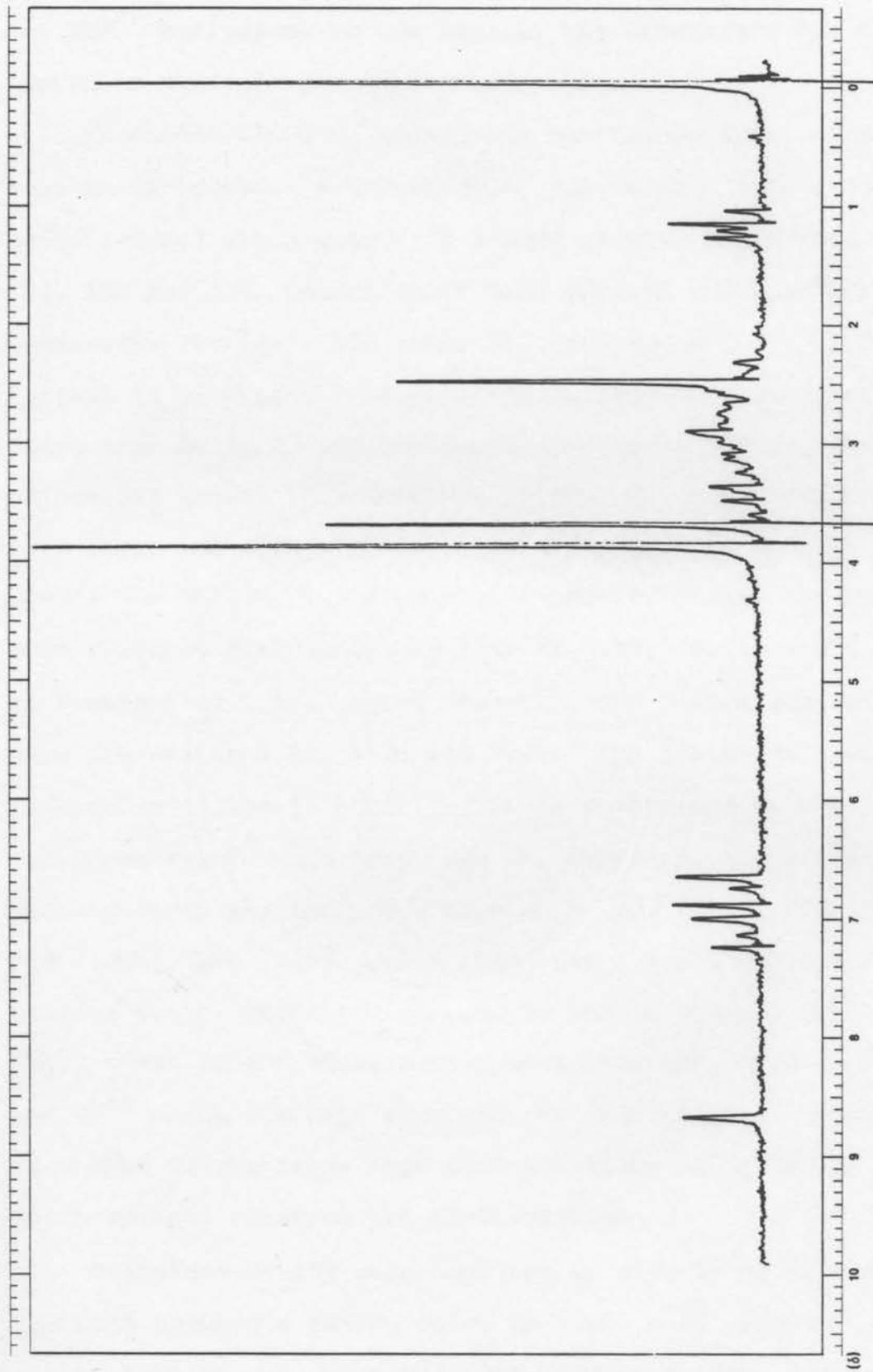
Figure 8   UV (EtOH) of Corydine

Figure 9 ^{1}H-NMR (CDCl$_3$) of Corydine

and NMR[47] correspond to the data in the literature for the aporphine alkaloid corydine (Figure 7).

Fractions 170-230, containing the orange spot, were next investigated. Evaporation of the solvent left a dark solid (20 mg) which gave a UV (EtOH) (Figure 10) with λ max 224, 280 and 304. Addition of base shifted the spectrum to λ max 222, 268 (sh), 280 (sh), 310 (sh) and 343. This UV pattern is different from those of corydine and isocorydine, being that of an 11-unsubstituted aporphine. These aporphines are generally 9,10-substituted. The bathochromic base shift indicated the presence of a phenolic hydroxy group. The NMR $(CDCl_3)$(Figure 11) spectrum showed the presence of three methoxy groups (one at 3.85, two at 3.93) and an N-methyl at 2.52. Three aromatic, one proton singlets were present at 6.51, 6.76 and 8.06. The proton at 8.06 is indicative of the 11 position; it is deshielded by the aporphine system much more than the other positions are. A mass spectrum was obtained, showing M^+ 341 (26%), 339 (87%), 324 (100%), 266 (28%), 169.5 (48%) and 139.5 (34%). A melting point, 185-186°C (decomp.), and an optical rotation, $\angle\alpha\angle_D$ = +55 (c = 0.0046, EtOH), were obtained. The NMR[48] and UV[48] match the data recorded for thaliporphine (Figure 7). This is the first report of a melting point and an exact optical rotation for thaliporphine.

Fractions 98-108 were combined to give 50 mg of the alkaloid showing a yellow color on tlc. A UV (EtOH) (Figure 12) showed λ max 221, 265, 280 (sh), 298, 311 (sh).
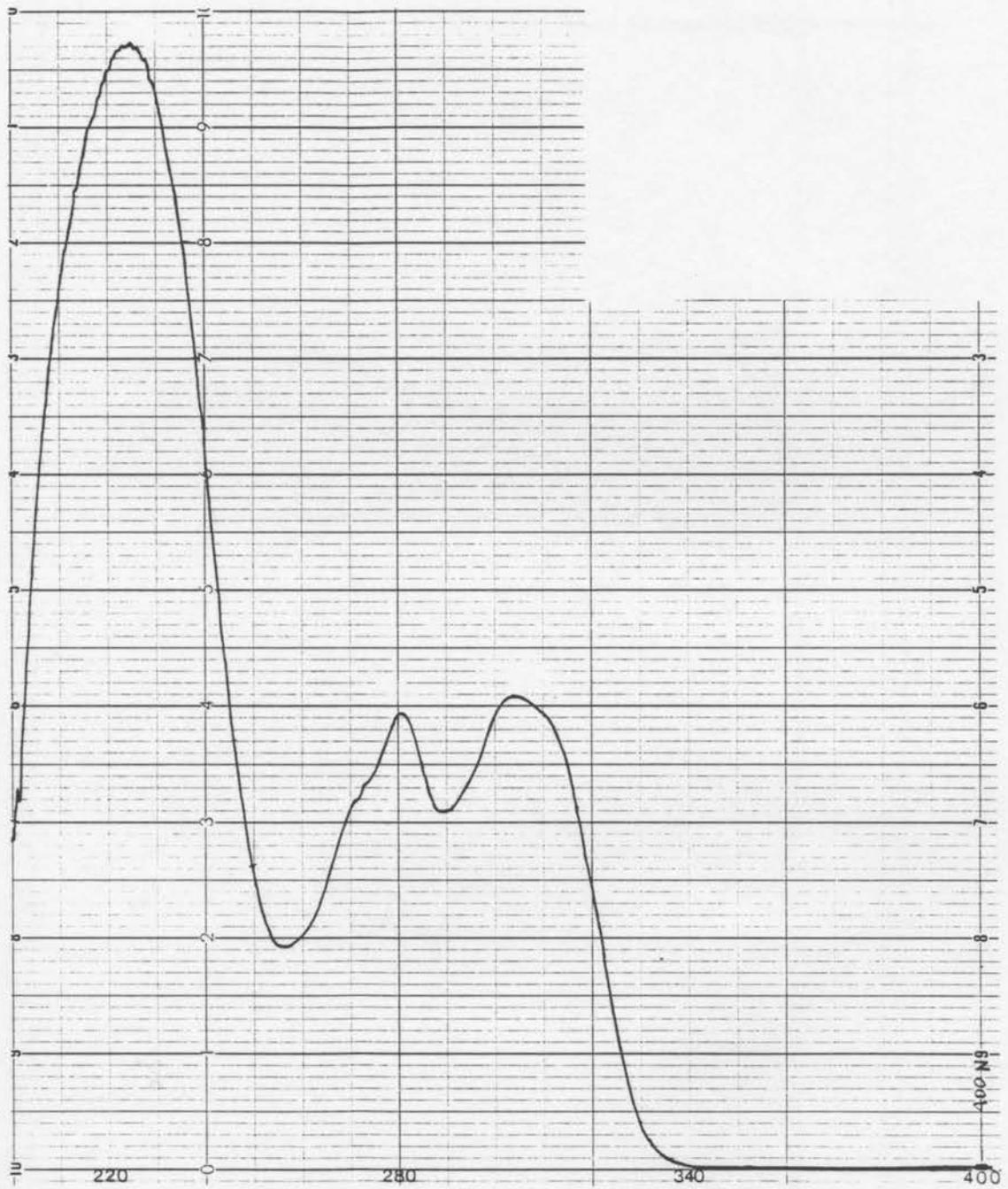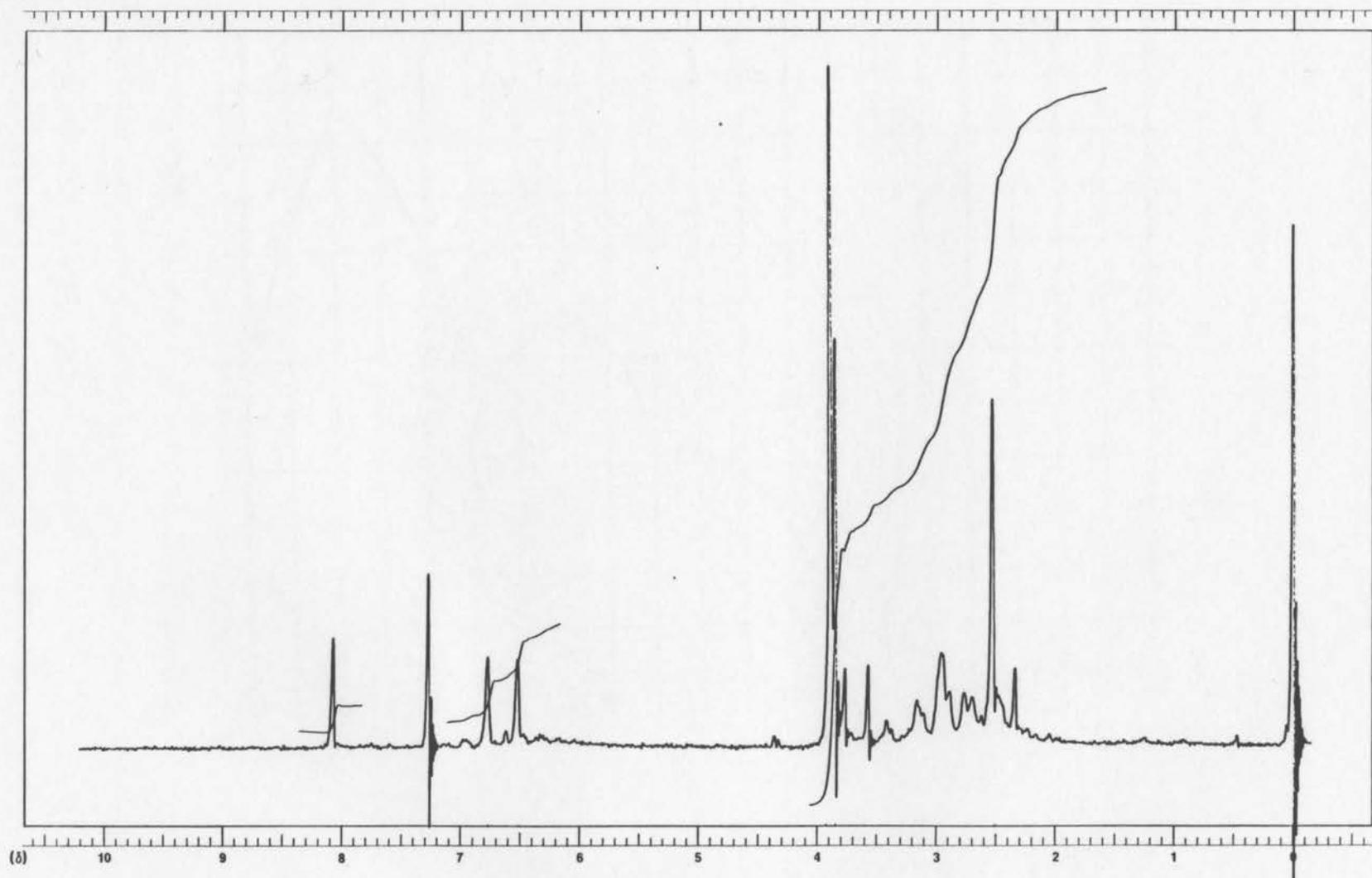
Figure 10 UV (EtOH) of Thaliporphine

Figure 11 ^{1}H-NMR (CDCl$_3$) of Thaliporphine
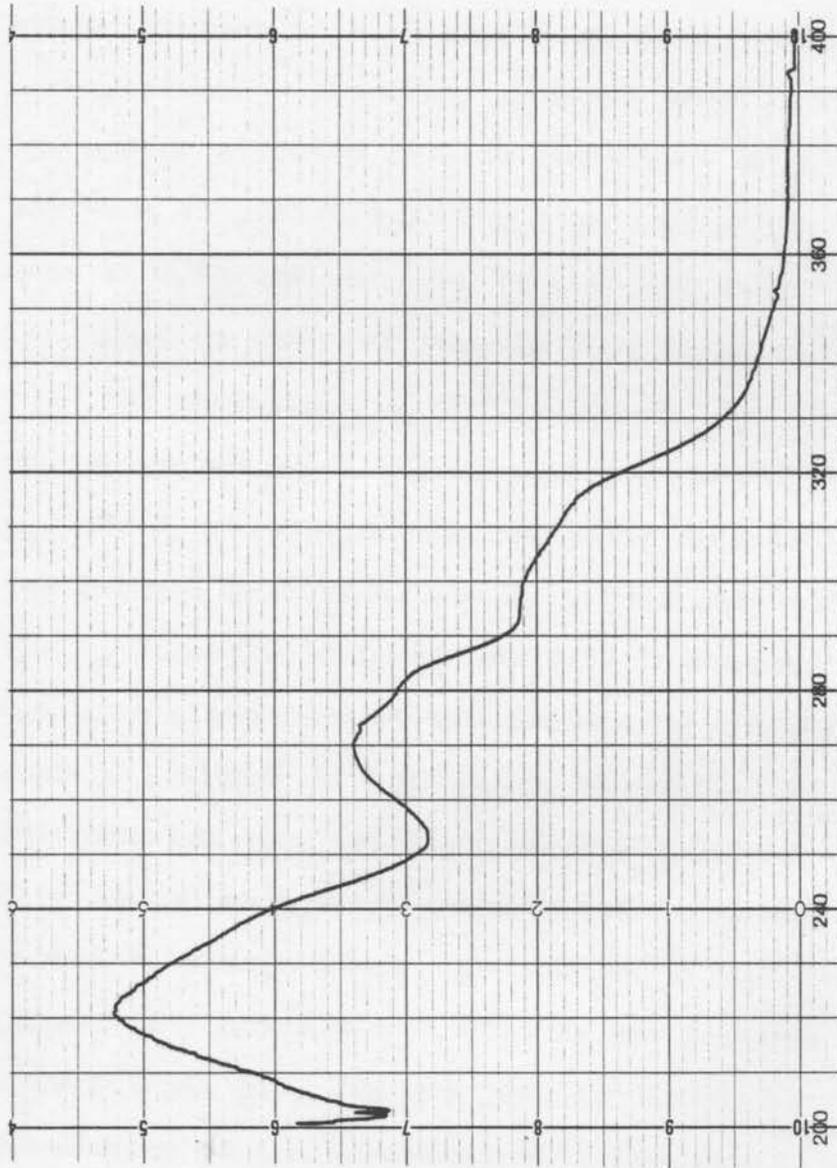
Figure 12   UV (EtOH) of Glaucine

Addition of base did not shift any peaks, proving the lack of a phenolic hydroxy group. The UV was similar to that of the 11-unsubstituted aporphines such as thaliporphine. In the NMR ($CDCl_3$) (Figure 13), the presence of a one proton singlet at 8.13 confirmed that the 11-position was unsubstituted. The other aromatic resonances occurred as one proton singlets at 6.80 and 6.60. An N-methyl signal was seen at 2.53 along with 4 methoxy peaks: two coincident at 3.93 and one each at 3.86 and 3.66. The NMR was identical to that for the alkaloid glaucine[49] (Figure 7).

Tlc investigation of fraction 6 from the alumina column showed two alkaloid positive spots. In the solvent S_4, the R_f values for the two spots were 0.30 and 0.0. In the solvent formamide, the R_f values were 0.57 and 0.34. The R_f values 0.30 in S_4 and 0.57 in formamide were identical with a standard of the quaternary aporphine magnoflorine (Figure 7), while the values 0.0 in S_4 and 0.34 in formamide were identical with a standard of the quaternary aporphine N-methylisocorydine. A small amount of the higher R_f alkaloid was separated on a cellulose column using 0.1 N hydrochloric acid as the eluent. A UV (EtOH) showed λ max 226, 270 and 304. Addition of base caused a broadening of the spectrum, with the only discernable peak at 245. This UV and tlc data is consistent with that of magnoflorine, but further data and complete identification of these two alkaloids was precluded by the absence of enough pure material to record an NMR or mass spectrum.
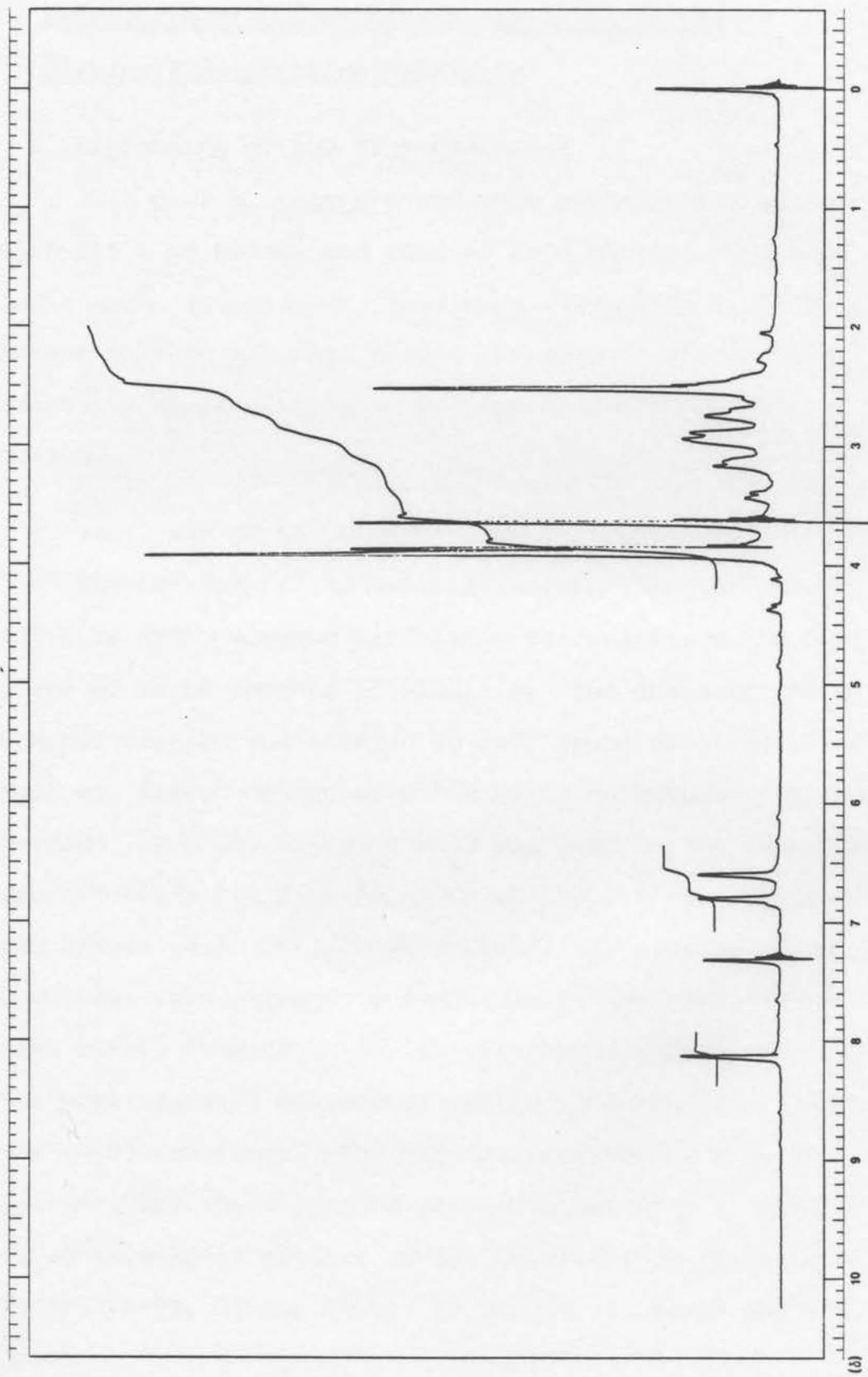
Figure 13 ^{1}H-NMR (CDCl$_3$) of Glaucine

C. Extraction of the Stems and Isolation of the
   Bisbenzylisoquinoline Alkaloids

1. Extraction of the Plant Material

1013 g of M. repens stems were successively extracted
with 2.5 l of hexane and ethanol in a Soxhlet apparatus for
24 hr each. Evaporation, acid-base extraction and tlc of the
hexane soluble material showed the absence of alkaloidal
material, so investigation of this fraction was not
pursued.

2. Isolation of the Ethanol Soluble Components

Evaporation of the ethanol soluble material provided
137 g of dark, viscous material. Tlc confirmed the pre-
sence of large amounts of alkaloid. Two grams of the crude
ethanol extract was adhered to four grams of Si gel, and
this was placed on top of a 2.4 by 37 cm column of Si gel.
Methanol:chloroform (1% to 60%) was used as the eluent and
approximately 175 fractions, 25 ml each, were collected.
Tlc showed very little separation of the alkaloids, so the
fractions were allowed to evaporate in the hood. Several
days later, fractions 113-116 (18% MeOH) contained crystals.
The crystals were collected, washed with methanol, and an
NMR (D_2O) was taken. The NMR corresponded to that of
sucrose, and the structure was confirmed by a ^{13}C-NMR
(D_2O) showing 12 signals at 62.25, 63.86, 70.10, 71.14,
73.59, 74.93, 75.10, 76.51, 78.89, 83.33, 94.65 and 106.16
ppm.

45 g (about one-third) of the ethanol extract was put through a modified acid-base extraction (Figure 14). The modified acid-base extraction provided three alkaloid fractions: 0.72 g of chloroform soluble material, 0.16 g of the ether soluble non-phenolics and 0.54 g of the ether soluble phenolic material.

The 0.16 g of non-phenolic material was placed on a 1 by 19 cm column of neutral alumina (Merck). The column was eluted with chloroform and then with increasing amounts of methanol in chloroform. Fractions 9-11 (25 mg, $CHCl_3$ eluent) contained one alkaloid positive spot on tlc at R_f 0.59 in "Solvent F" (17:2:1 - ethyl acetate: methanol: ammonium hydroxide). An NMR ($CDCl_3$) (Figure 15) showed the presence of four methoxy peaks at 3.90, 3.80 3.63 and 3.18. Two separate N-methyl resonances were also apparent at 2.65 and 2.55. Aromatic protons were present in the region 6.3 to 7.6. A MS showed a molecular weight of 622 (28%) along with other prominent peaks at 607 (10%), 591 (4%), 515 (5%), 396 (11%), 395 (29%), 381 (22%), 311 (48%), 282 (31%), 209 (33%), 198 (42%), 194 (100%) and 175 (25%). The NMR and MS were indicative of a bisbenzylisoquinoline type alkaloid. The mass spectrum was particularly impor- tant in the elucidation of the structure of these alkaloids. The principal fragmentation patterns for some simple bisbenzylisoquinolines have been worked out.[50] Analysis of the mass spectrum (Figure 16)[50a] along with the NMR[51] showed this alkaloid to be obaberine (Figure 17).
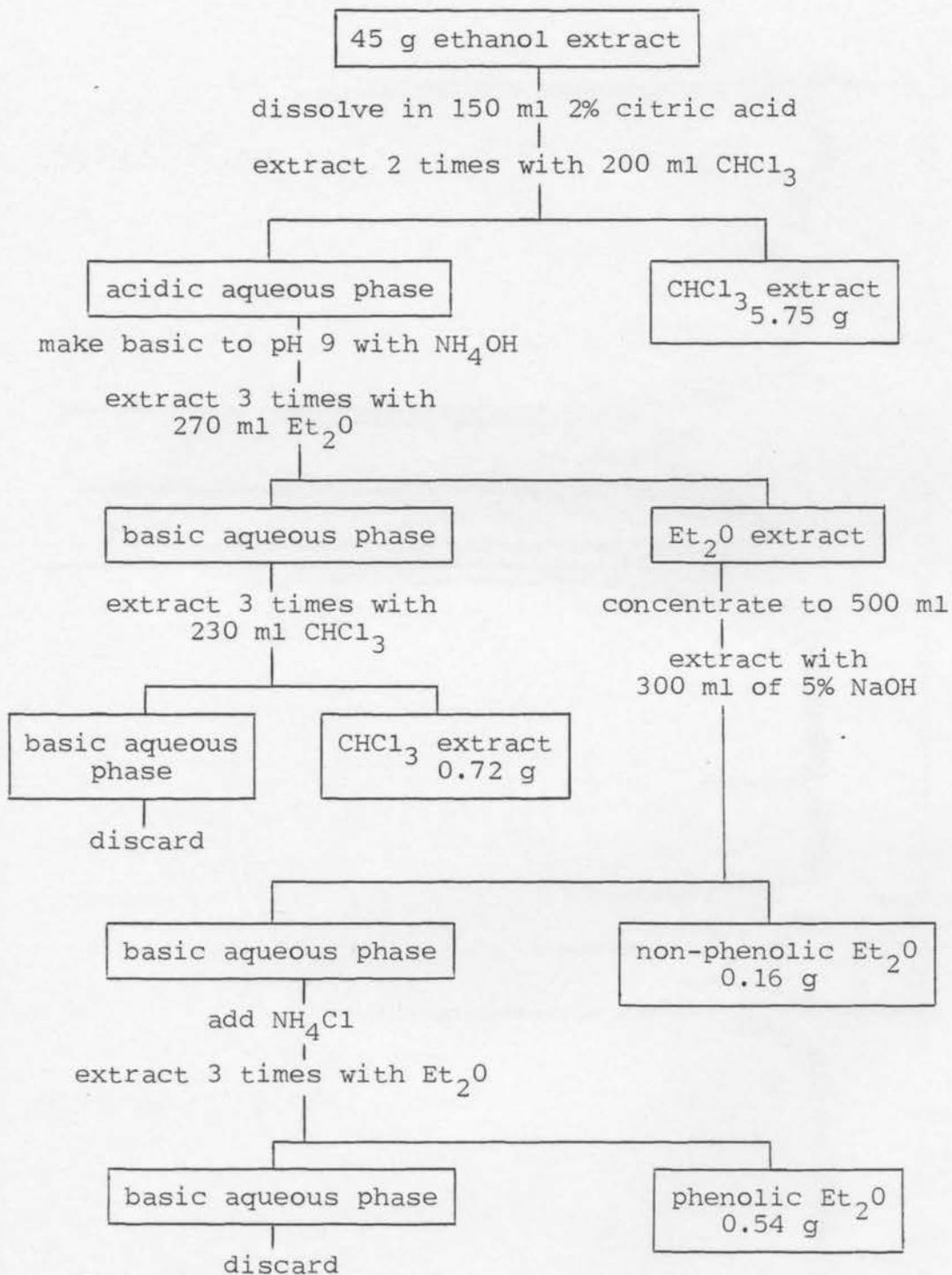
Figure 14   Modified Acid-Base Extraction
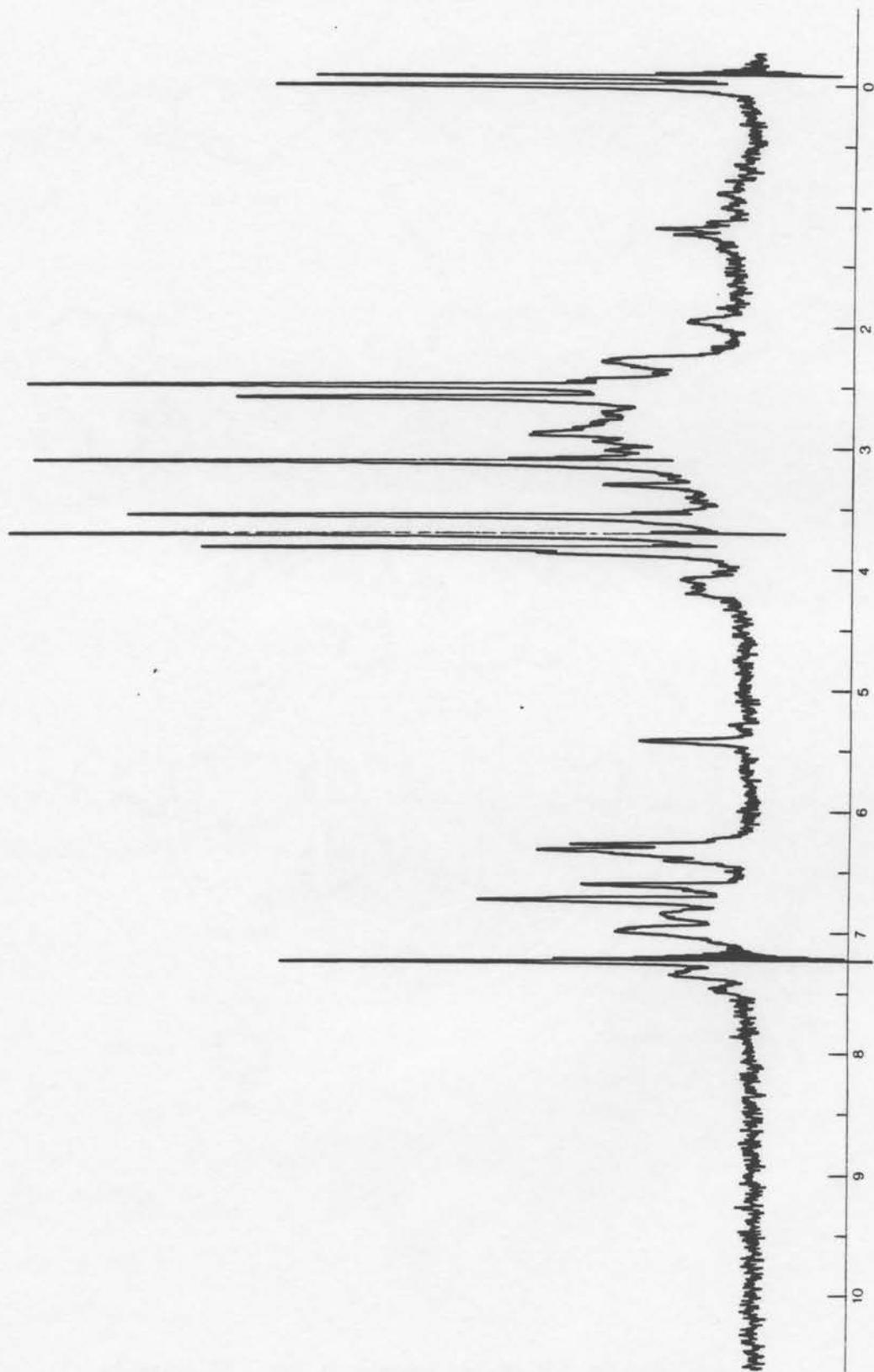
Figure 15 ^{1}H-NMR (CDC1$_3$) of Obaberine
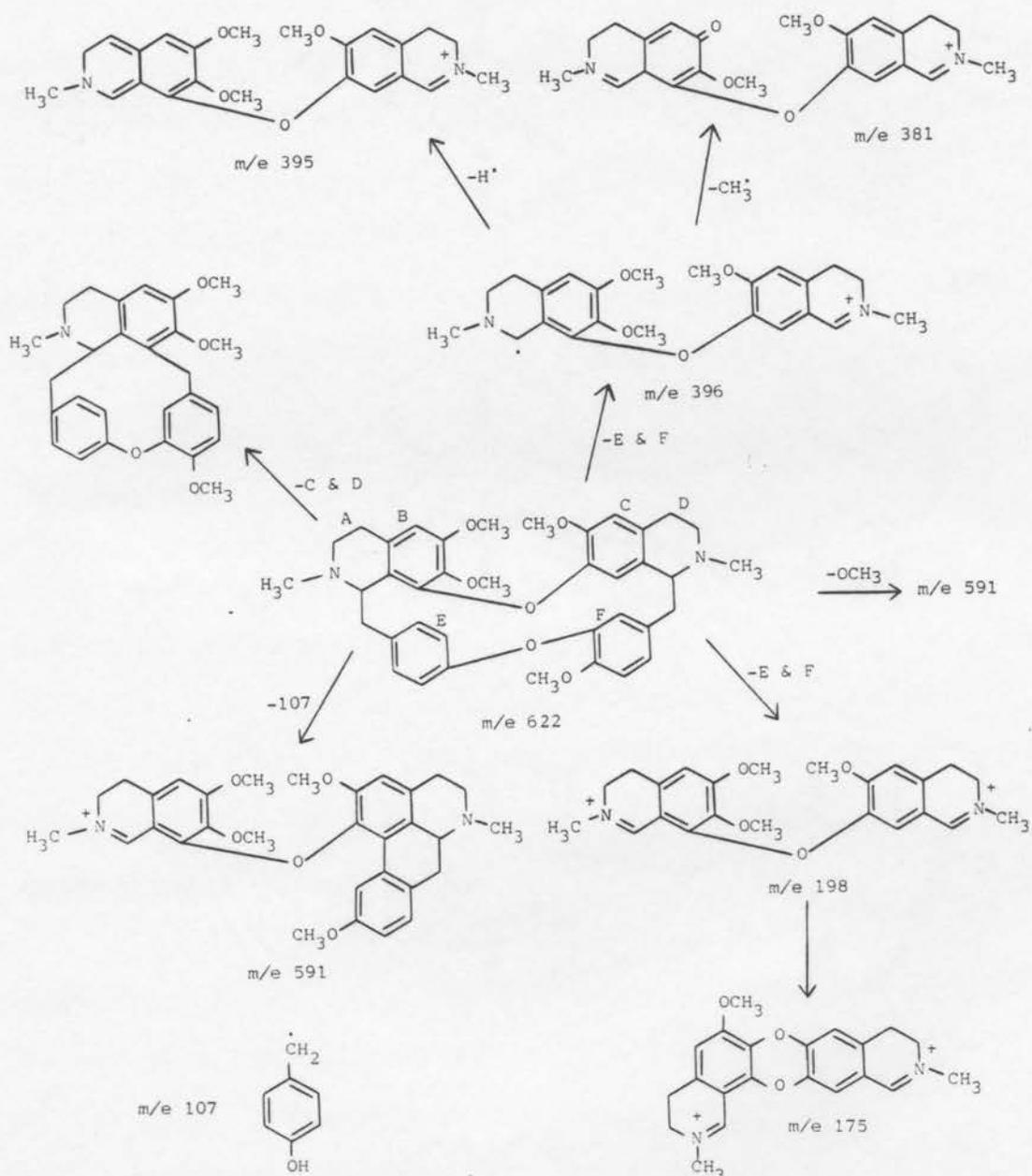
Figure 16   MS Fragmentation of Obaberine

Obaberine
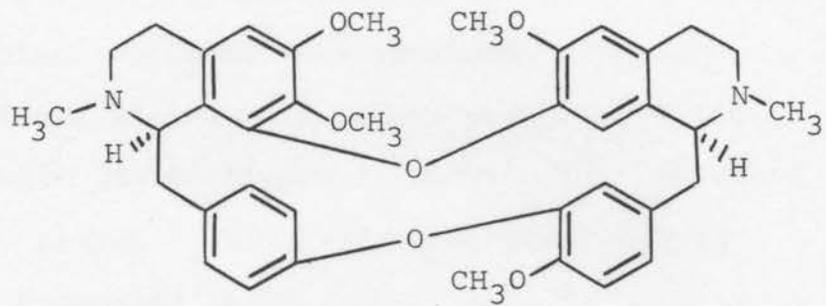
Thalrugosine
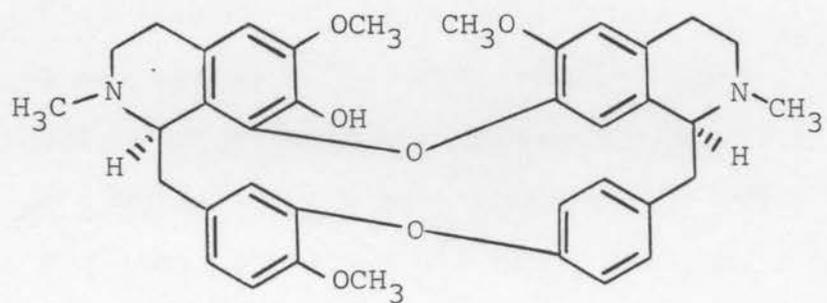
Oxyacanthine
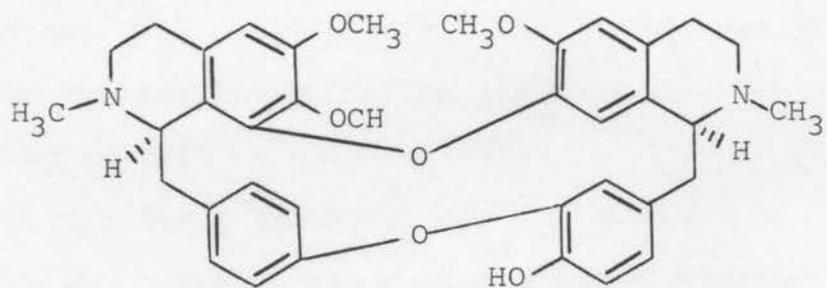
Obamegine
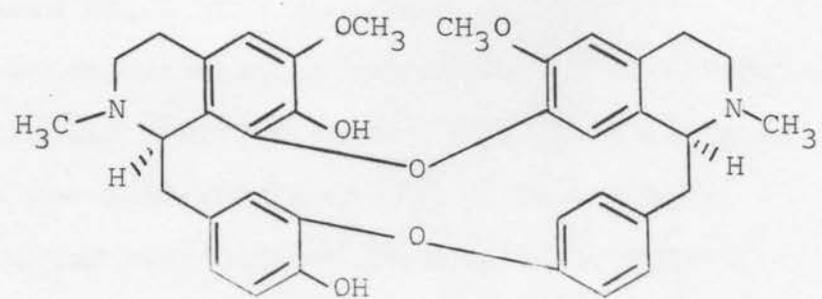
Figure 17

Bisbenzylisoquinoline Alkaloids from M. Repens

Tlc showed no further separation by the alumina column, so all the remaining fractions were combined. This non-phenolic material was placed on a Si gel preparative tlc plate, and the plate was developed in Solvent F. The third band on the prep. plate, R_f 0.3, was removed and eluted with 5% ammonium hydroxide in ethanol. The ethanolic solution was evaporated to give 15 mg of an alkaloid showing in the NMR ($CDCl_3$) (Figure 18) three methoxy absorbances: two at 3.93 and one at 3.77. Two N-methyls were seen at 2.30 and 2.50, with a broad multiplet of aromatic peaks in the region 6.06 to 7.0. A mass spectrum, M^+ 608 (57%), 593 (11%), 577 (4%), 471 (3%), 417 (8%), 381 (88%), 367 (38%), 191 (100%), 174 (32%) and 168 (19%), showed this alkaloid to be another bisbenzylisoquinoline. The NMR, MS and optical rotation, $\angle\alpha\angle_D$ = +130 (c = 0.004, MeOH), match those of the known bisbenzylisoquinoline alkaloid thalrugosine[52] (Figure 17).

Since only a very small amount of the non-phenolic material was left, the phenolic material was next investigated. The phenolic material (0.54 g) was placed on two Si gel prep. plates which were developed in (12:7:1) ethyl acetate:2-propanol:ammonium hydroxide. This solvent gave two main alkaloid positive bands. The two main bands were removed and the alkaloids were eluted from the Si gel using 5% ammonium hydroxide in ethanol. Tlc showed that the top band contained one main alkaloid at R_f 0.38
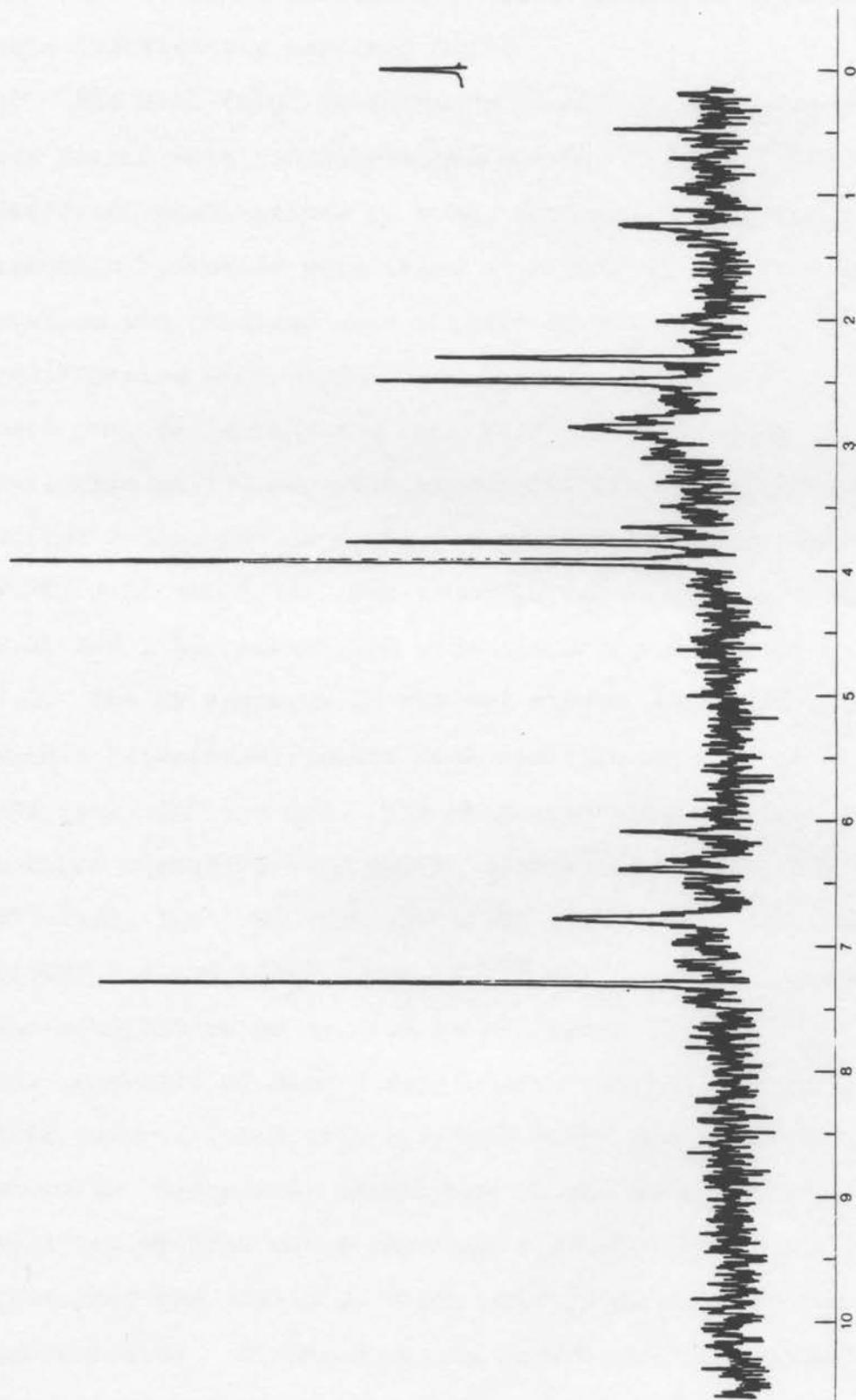
Figure 18 ^{1}H-NMR (CDCl$_3$) of Thalrugosine

in (16:3:1) ethyl acetate:2-propanol:ammonium hydroxide, so this fraction was explored first.

The HPLC (High Performance Liquid Chromatography) unit was fitted with two Waters μ- porasil columns in series. Different combinations of ethyl acetate, 2-propanol and ammonium hydroxide were tried with Band 1, but the best separation was obtained with a ratio of 9:10:1. The columns were equilibrated with 9:10:1, and then 15 injections of Band 1 were run, collecting the main peak (determined by UV detection at 280 nm) each time. An NMR $(CDCl_3)$ (Figure 19) of the evaporated material showed three methoxy peaks at 3.76, 3.60 and 3.15. Two N-methyl resonances were seen at 2.63 and 2.52, along with aromatic protons between 6.3 and 7.2. The UV spectrum in ethanol showed λ max 226 and 284 with a bathochromic shift upon addition of base to λ max 305 (sh), 285 and 234. The MS proved this alkaloid to be another bisbenzylisoquinoline with M^+ 608 (73%), 593 (3%), 577 (2%), 501 (3%), 416 (2%), 395 (51%), 381 (31%), 198 (100%) and 175 (53%). The MS,[50a] NMR[53] and UV[54] prove the alkaloid to be oxyacanthine (Figure 17).

One-half of Band 2 was injected onto a 1.5 by 95 cm MPLC column eluted with (12:7:1) ethyl acetate:2-propanol: ammonium hydroxide. Fractions (5 ml) were collected and analyzed by HPLC using the same solvent. Fractions 30-40 contained one alkaloid, which gave 38 mg of material when concentrated. A mass spectrum showed the fragmentation typical of a bisbenzylisoquinoline alkaloid: M^+ 594 (21%),
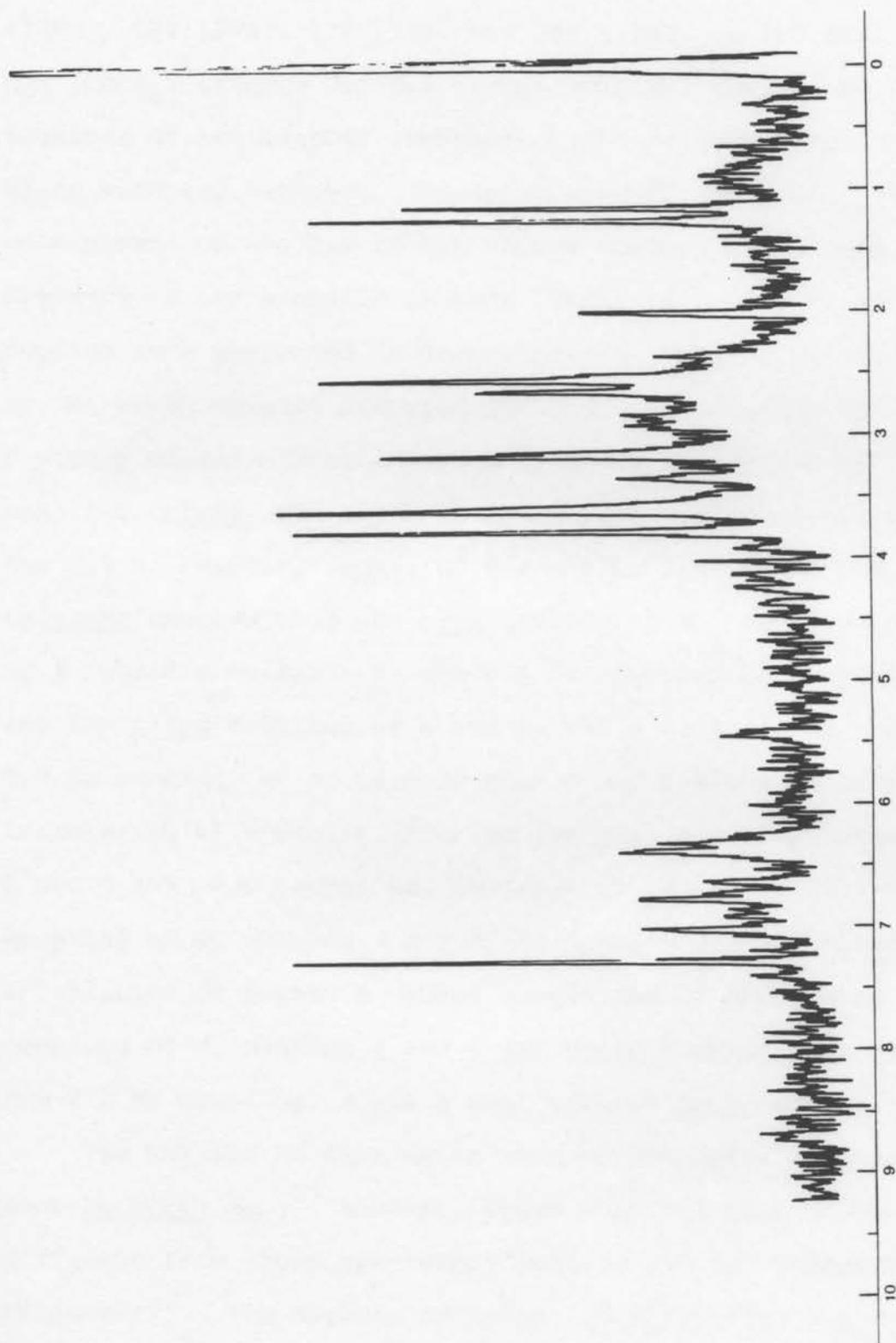
Figure 19 ^{1}H-NMR (CDCl$_3$) of Oxyacanthine

579 (2%), 471 (1%), 403 (6%), 381 (57%), 367 (17%), 192 (100%), 191 (57%), 174 (31%) and 168 (19%). A 360 MHz NMR ($CDCl_3$) (Figure 20) was taken. The NMR showed the presence of two methoxy resonances at 3.94 and 3.79, along with two N-methyl resonances at 2.50 and 2.33. An enlargement of the 6.0 to 7.4 region (Figure 21) showed the presence of ten aromatic protons (Table 2). Decoupling studies were performed to determine the coupling pattern of the seven coupled aromatic protons. Irradiation of proton **a** caused almost complete collapse of the 8.6 Hz coupling (ortho) of proton **b**, and also caused collapse of the 2.9 Hz coupling (meta) of proton **d**. This means that **a** is ortho coupled to **b** and meta coupled to **d**. Irradiation of **b** caused a collapse of the 8.6 Hz coupling of , verifying the ortho coupling of **a** and **b**, and a collapse of the 2.9 Hz coupling of **c**, meaning that **b** and **c** are meta coupled. Irradiation of proton **c** verified the meta coupling between **b** and **c** and also caused the collapse of the large, 8.6 Hz, coupling of **d**, meaning **c** and **d** are ortho coupled. Finally, irradiation of proton **e** caused a collapse of the 8.6 Hz coupling of **f**, meaning **e** and **f** are ortho coupled, and by the 2.2 Hz coupling, **f** and **g** must then be meta coupled.

The NMR and MS data match that of obamegine isolated from Berberis sp.;[55] however, these chemical shifts are different from those previously published[53] for obamegine (Figure 17). The optical rotation, $\angle\alpha\_7_D^{19}$ = +225 (c = 0.013, $CHCl_3$), is somewhat lower than the $\angle\alpha\_7_D^{19}$ = +273
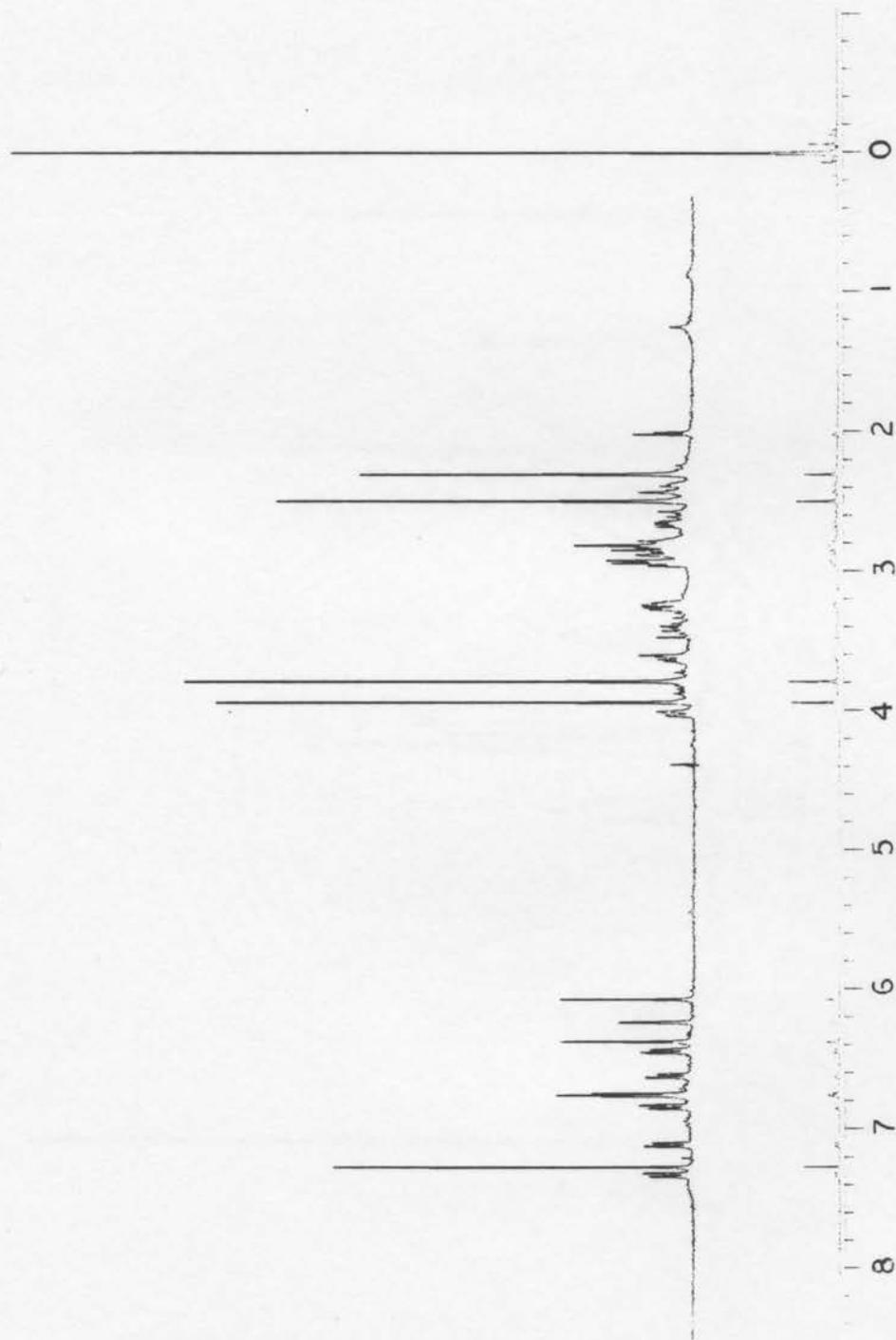
Figure 20 ^{1}H-NMR (CDCl$_3$) of Obamegine
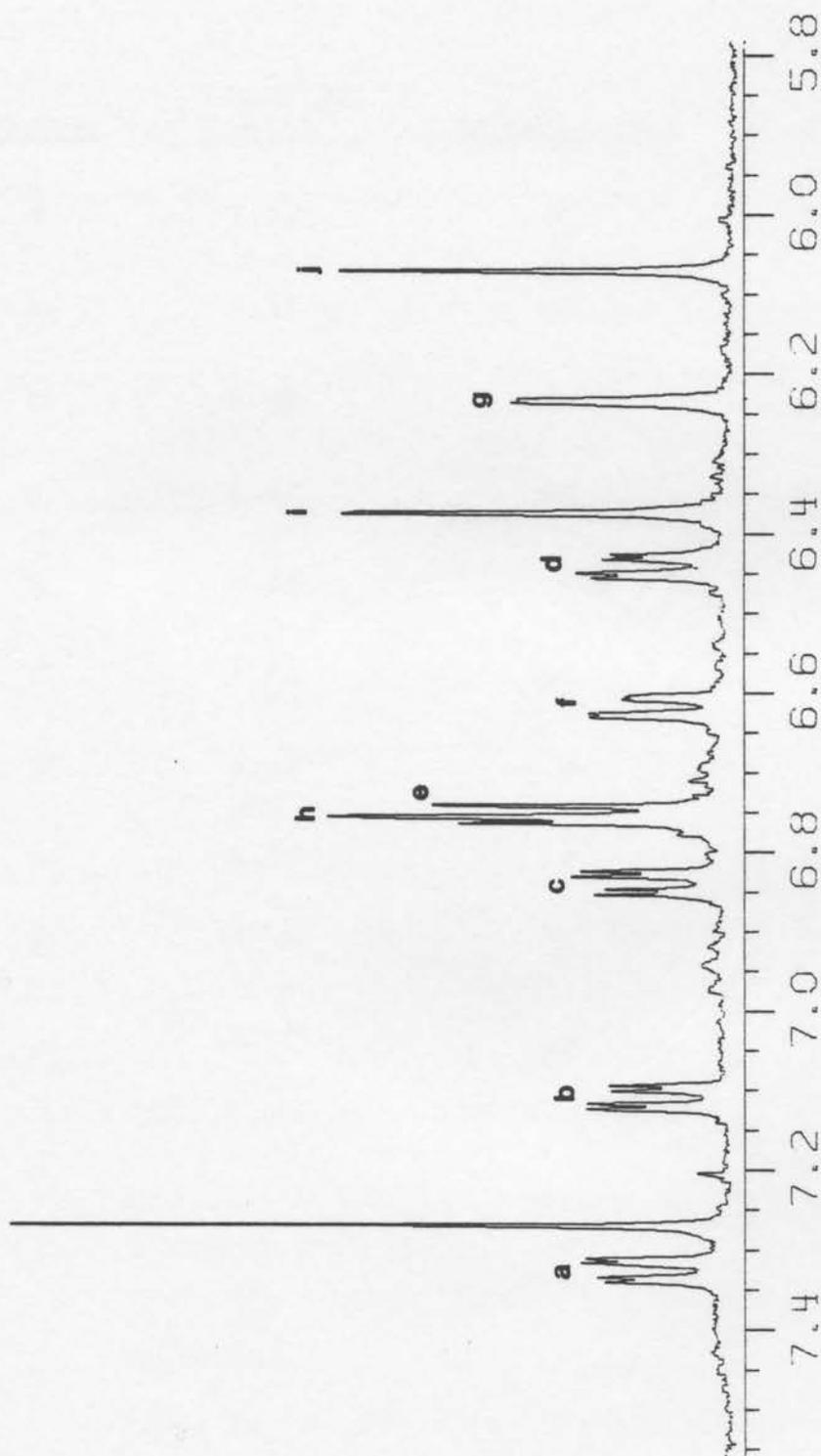
Figure 21 Expansion of the Aromatic Region of Obamegine

Proton	Chemical Shift	Multiplicity	Coupling Constants (Hz)
a	7.33	dd	J=2.9, J=8.6
b	7.11	dd	J=2.9, J=8.6
c	6.84	dd	J=2.9, J=8.6
d	6.44	dd	J=2.9, J=8.6
e	6.75	d	J=8.6
f	6.62	dd	J=2.2, J=8.6
g	6.24	d	J=2.2
h	6.75	s	
i	6.37	s	
j	6.07	s	

Table 2   Aromatic Protons of Obaberine

(CHCl$_3$) observed in the literature,[56] but the NMR distinguishes it from the other possible isomer atherospermoline,[57] which has $\angle\alpha7_D^{18}$ = +202 (CHCl$_3$).

A sample of the bisbenzylisoquinoline fraction, obtained before the separation of the phenolic and non-phenolic fractions (Figure 14), was sent to the National Cancer Institute for antitumor testing. The KB Cell test showed an ED$_{50}$ = 13, while the P388 test showed no activity, T/C% = 104$_{25}$. After the identification of these alkaloids, the activity of the combined fraction was shown to closely match that reported for oxyacanthine, the most active of the four bisbenzylisoquinolines isolated. None of the four compounds showed sufficient activity for further evaluation as antitumor agents.

Tlc of the chloroform portion of the stems (Figure 14) showed it to contain mostly oxyacanthine along with some thalrugosine, obamegine and traces of several more polar alkaloids. Since tlc of M. repens roots showed more of the polar alkaloids, further investigation of the chloroform material was not undertaken.

D. Extraction and Isolation of the Root Constituents

1. Extraction of the Plant Material and Preliminary Treatment

1411 g of M. repens roots were successively extracted in a Soxhlet apparatus with 2.5 l of hexane, ethanol and methanol for 24 hr each. Evaporation of each of these

extracts provided 8.1 g of hexane soluble material, 174 g
of ethanol soluble material as a viscous brown oil and
41 g of methanol soluble material.

The hexane layer showed no alkaloids by tlc, so it was
not investigated. The ethanol soluble material was
treated by a different extraction procedure (Figure 22).
The use of Reinecke's Salt, ammonium tetrathiocyanodi-
ammonochromate, causes precipitation of the quaternary alka-
loids.

2. Isolation of the Chloroform Soluble Component

The chloroform soluble material was separated by flash
chromatography[58] on Si gel with chloroform as the eluent.
The first two fractions provided 0.20 g of material whose
NMR ($CDCl_3$) (Figure 23) showed two methoxy peaks at 3.90 and
3.98. One proton singlets were seen at 5.66, 6.10, 6.63
and 7.20. The broad singlet at 6.10 disappeared after addi-
tion of D_2O. The NMR also showed a two proton singlet at
5.90 along with two doublets at 6.85 and 7.03, each having
a 8.0 Hz coupling. The UV (EtOH) showed absorptions with
λ max 215, 282 and 373. Addition of base caused no change in
the spectrum, but addition of acid resulted in λ max 214, 226
(sh), 263 and 348. A ^{13}C-NMR showed nineteen peaks at
149.53, 147.89, 146.69, 146.14, 129.21, 128.45, 124.42,
118.70, 115.02, 114. 49, 107.95, 104.98, 104.39, 101.07,
73.57, 60.89, 56.23, 51.85 and 30.24 ppm.

The NMR and UV spectra correlate well with berberinol[59]
(Figure 24) except that the ^{13}C-NMR of berberinol should

173.8 g EtOH material

dissolve in 2.5 l 2% citric acid
extract twice with 750 ml EtOAc

| aqueous layer | EtOAc layer |

make basic to pH 9 with
concentrated NH_4OH

concentrate to 11.3 g

extract three times with
with 1 l Et_2O

| Et_2O layer | aqueous layer |

concentrate to 12.8 g

extract three times with
750 ml $CHCl_3$

| basic aqueous layer | $CHCl_3$ layer |

acidify to pH 5 with
concentrated HCl

concentrate to 2.04 g

treat three times with 500 ml
5% Reinecke's salt, filtering
after each treatment

| solid precipitate | aqueous solution |

dry to give 37.5 g

Figure 22
Extraction of Ethanol Soluble M. Repens Roots Material

D₂O
SHAKE
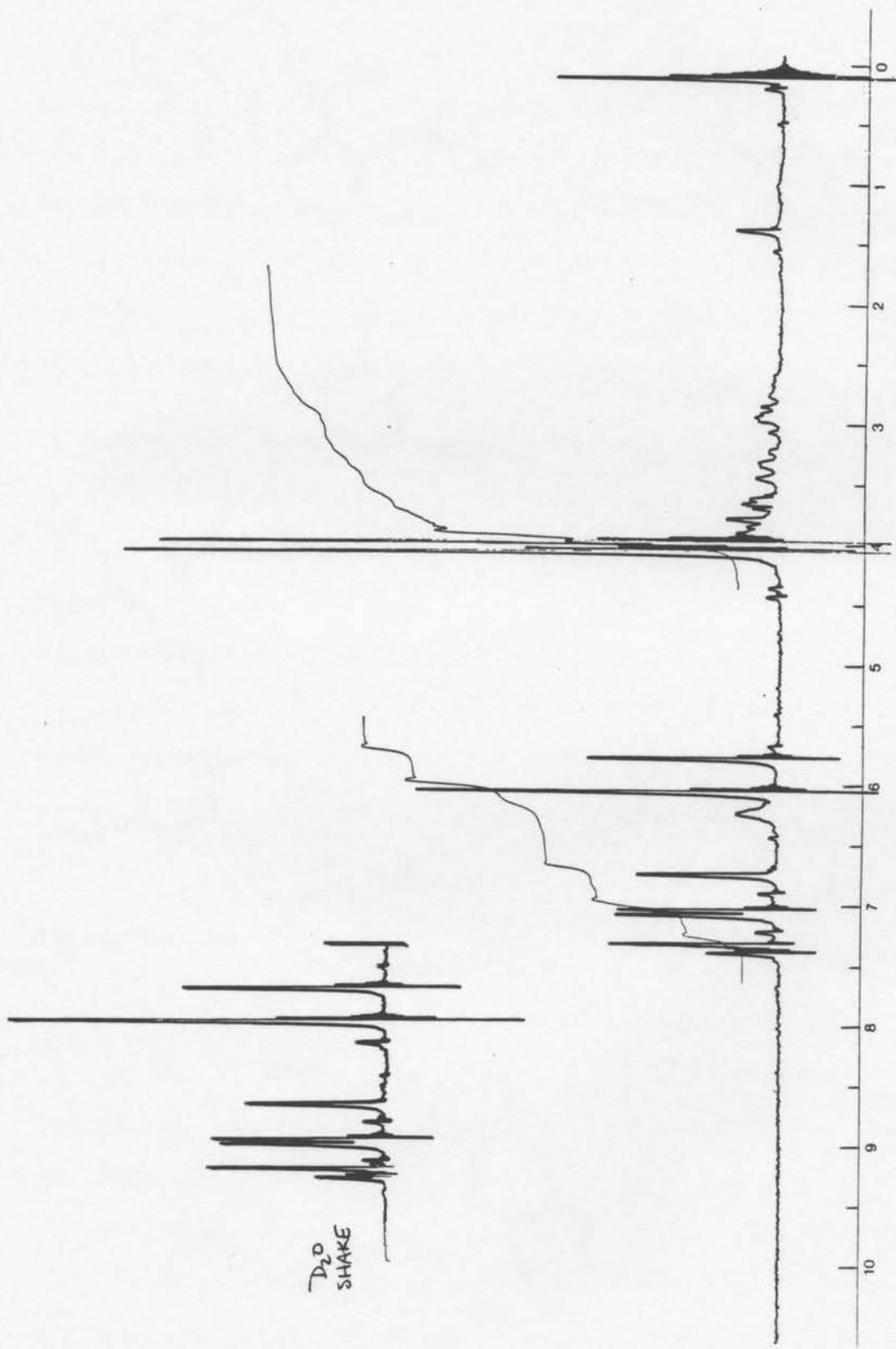
Figure 23 ^{1}H-NMR (CDCl$_3$) of Berberinol

Berberinol

Berberine

Syringaresinol

Magnoflorine

Jatrorrhizine

Columbamine

Palmatine
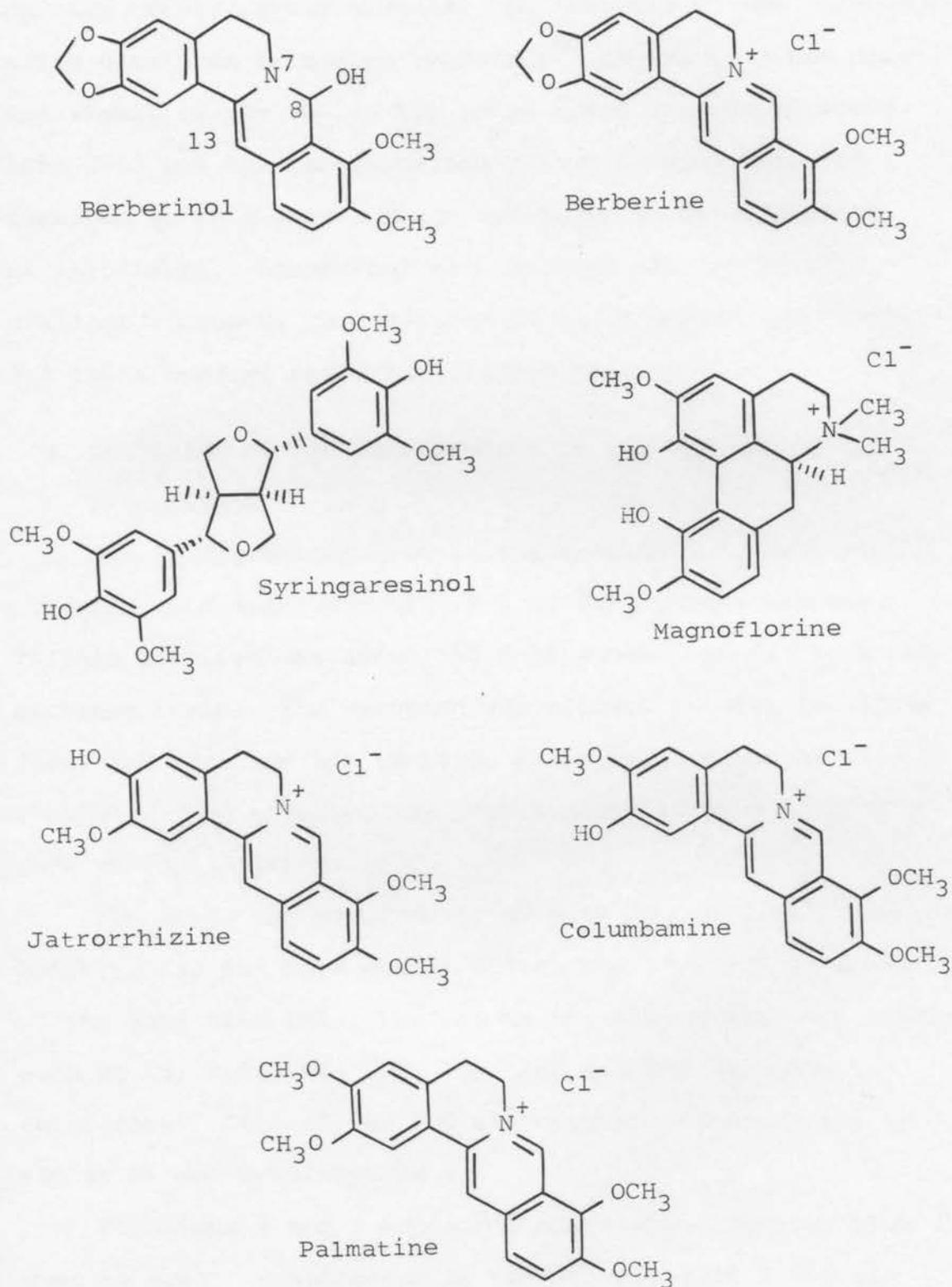
Figure 24 Constituents of M. Repens Roots

contain twenty carbon signals. An analysis of the ^{13}C-NMR
using data from reference compounds[60] showed only one car-
bon signal in the 96-102 ppm range where two should occur.
Both C-13 and the carbon of the methylenedioxy ring are
expected in this area. These two signals must therefore
be coincident. Berberinol is generally considered an
artifact formed by the addition of water across the reactive
7-8 imine bond of berberine (Figure 24).

3. Isolation of the Constituents of the Reinecke's Salt
   Precipitate

The 37.5 g of material from the Reinecke's Salt preci-
pitation were dissolved in 1.8 l of 50% aqueous acetone.
To this solution was added 365 g of Dowex 1-X8 Cl$^-$ form ion
exchange resin. The solution was allowed to stir for three
days, and then the ion exchange resin was removed by fil-
tration. The solution was concentrated to give 15 g of a
dark yellowish solid.

The solid was adsorbed to 30 g of acidic alumina (Baker)
Activity 1), and this was placed on top of a 900 g column
of the same material. The column was eluted with two liters
each of 2%, 3.5%, 6%, 10%, 18%, 35% and 60% methanol in
chloroform. Each of the 750 ml fractions was analyzed by
tlc on Si gel developed in S_4.

Fractions 2 and 3 contained spots that appeared blue
when sprayed. Evaporation of the solvent gave 0.5 g of
material which was purified by flash chromatography on Si
gel to give 0.12 g. An NMR (CDCl$_3$) (Figure 25) showed a
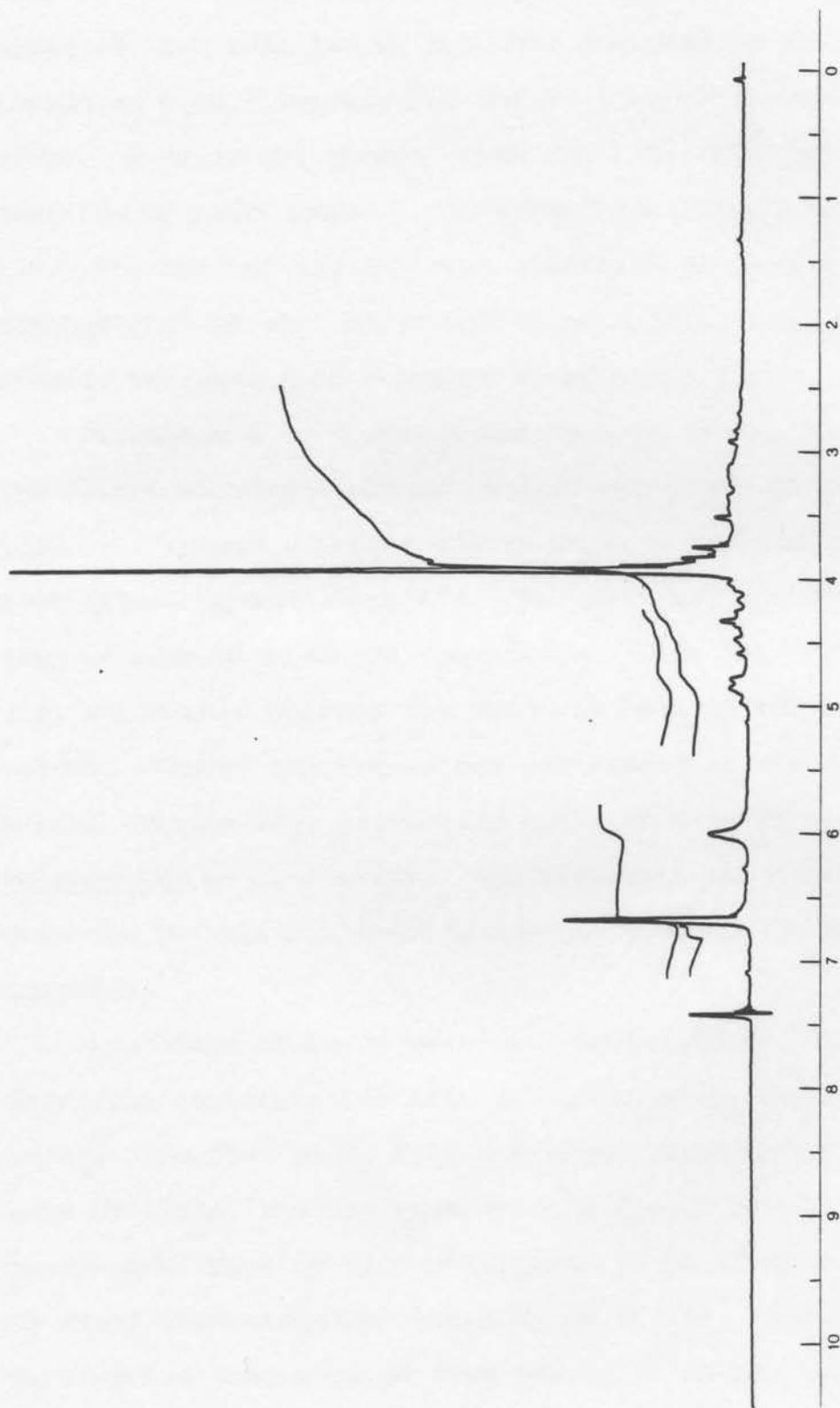
Figure 25 ^{1}H-NMR (CDCl$_3$) of Syringaresinol

peak in the methoxy region at 3.88, a peak at 5.93 which dis-
appeared upon addition of D_2O, and one peak in the aromatic
region at 6.60. Integration showed a 6:1:2 ratio of these
peaks. A UV (EtOH) showed λ max 281 (sh), 273 and 241.
Addition of base caused a shift to λ max 282 (sh), 262 and
228. The NMR and tlc in three different solvent systems
matched that of syringaresinol (Figure 24), a lignan pre-
viously isolated from a number of Berberis sp.[61]

Fractions 6 to 9 showed one spot at low R_f on tlc.
The fractions were combined to give two grams of an alka-
loid that showed a bright yellow color under long wave UV.
A UV (EtOH) showed λ max 430, 350, 267, 229 and 204. Addi-
tion of base shifted the spectrum to λ max 355, 275 and
215, while acid shifted the spectrum back to the neutral
values. The UV and tlc values correspond to standard ber-
berine (Figure 24), previously isolated from Zanthoxylum
monophyllum by I. Sharifi. The structure was confirmed
when the IR spectrum exactly matched that of the published
spectrum.[62]

Fractions 15 to 20 were next investigated. These
fractions contained two main alkaloids at R_f 0.30 and 0.53
in S_4. The spot at R_f 0.30 was bright blue under long
wave UV light, and the spot at 0.53 turned red in the
presence of base on tlc. Fractions 15 to 20 were purified
by flash chromatography using S_4 on Si gel. The higher
R_f material was isolated from the flash column and
crystallized from methanol to which several drops of

concentrated hydrochloric acid had been added. The UV
(EtOH) (Figure 26) of the crystals showed λ max 430, 390,
272 (sh), 262, 240 and 228. Addition of strong base
shifted the spectrum to λ max 388, 286 (sh), 244 and 230,
which reverted to the original values upon addition of
strong acid. This UV spectrum was indicative of a proto-
berberine alkaloid. An NMR ($CDCl_3$-d_6-DMSO) (Figure 27)
showed the presence of three methoxy peaks and six aromatic
protons (Table 3). The UV and NMR spectra are similar to
those of jatrorrhizine[63] (Figure 24), although the NMR
shifts in the literature are somewhat different, having
been recorded in CD_3OD. The IR spectrum of the alkaloid
matched that of jatrorrhizine,[62] confirming the structure.

The fractions containing the R_f 0.30 material were
also evaporated. A prep. tlc plate was run in S_4 to purify
some of the material. The band at R_f 0.30 was scraped off
the plate and eluted with 5% ammonium hydroxide in ethanol.
A UV (EtOH) (Figure 28) showed peaks at λ max 323, 280, 230
and 213 (sh). Addition of base enhanced the peak at 212 but
did not otherwise change the spectrum. Addition of acid
changed the UV to λ max 303, 269, 225 and 211 (sh). The
acidic UV spectrum was that of a 1,2,10,11-substituted apor-
phine. The lack of change between the neutral and basic
spectra could be due to the presence of a zwitterionic (phen-
oxide) form of the alkaloid. The NMR (D_2O) (Figure 29)
showed the presence of two N-methyl groups at 2.58 and 3.06.
Two methoxy peaks were evident at 3.73 and 3.83, along with
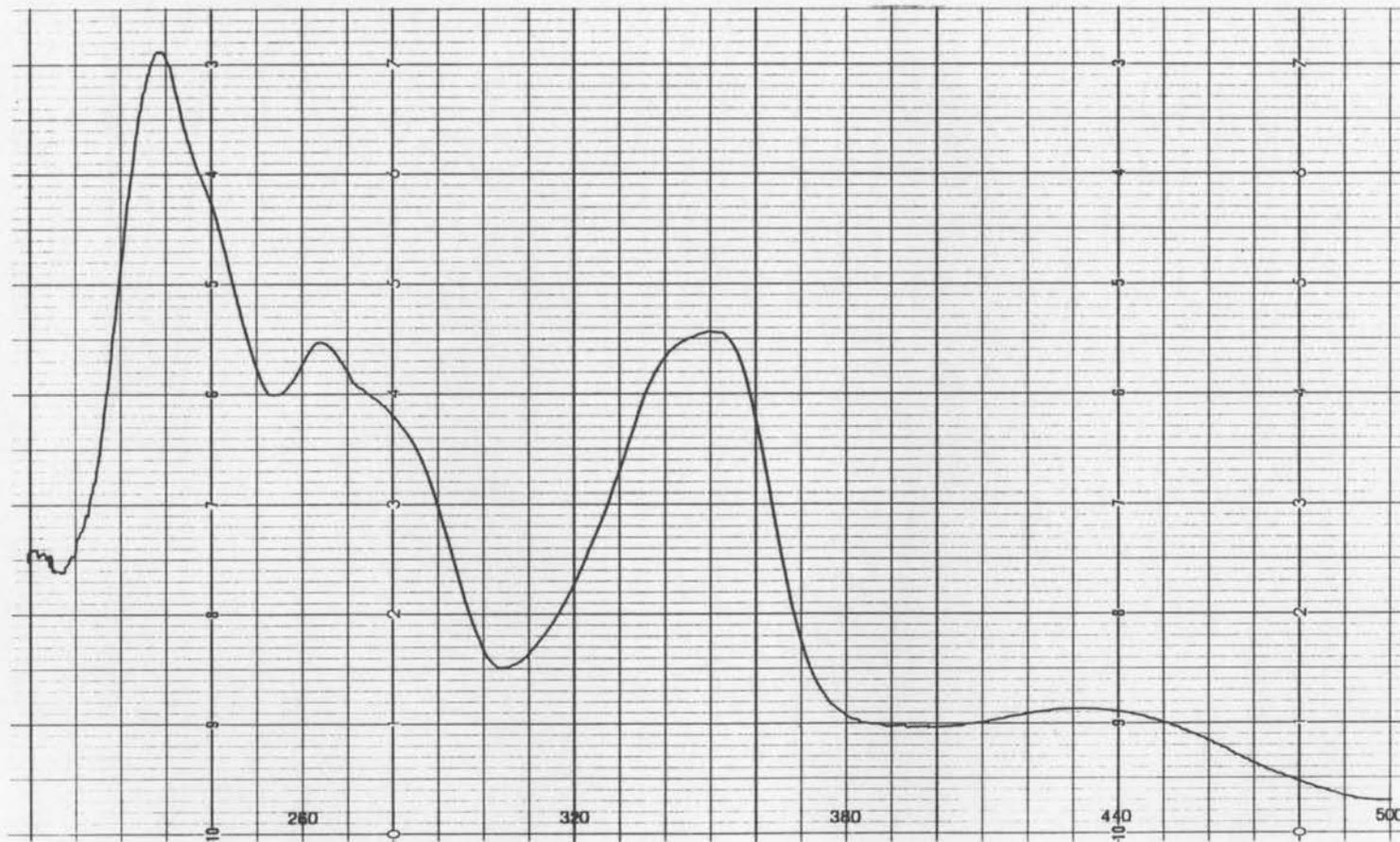
Figure 26a UV (EtOH) of Jatrorrhizine

59
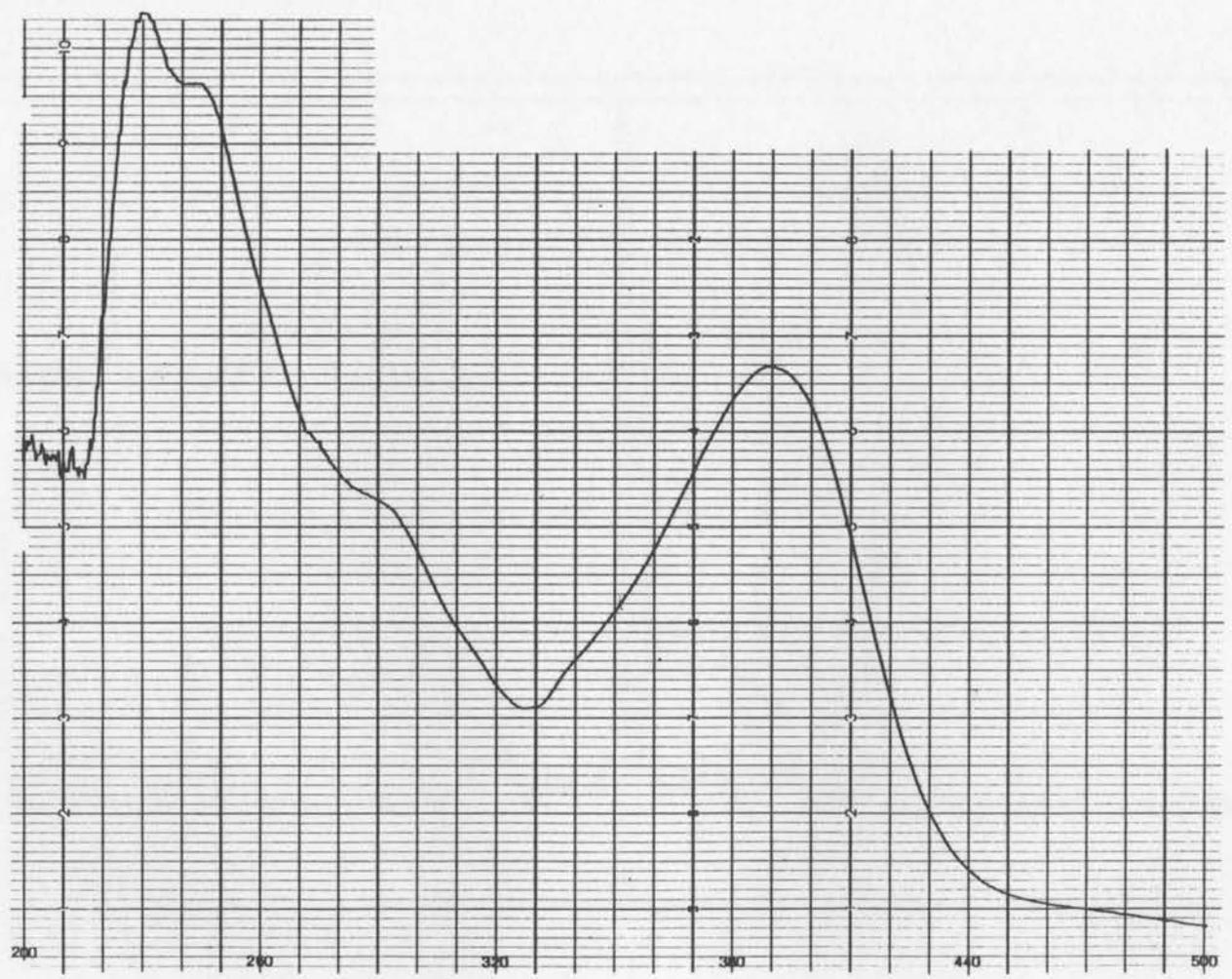
Figure 26b UV (EtOH + KOH) of Jatrorrhizine
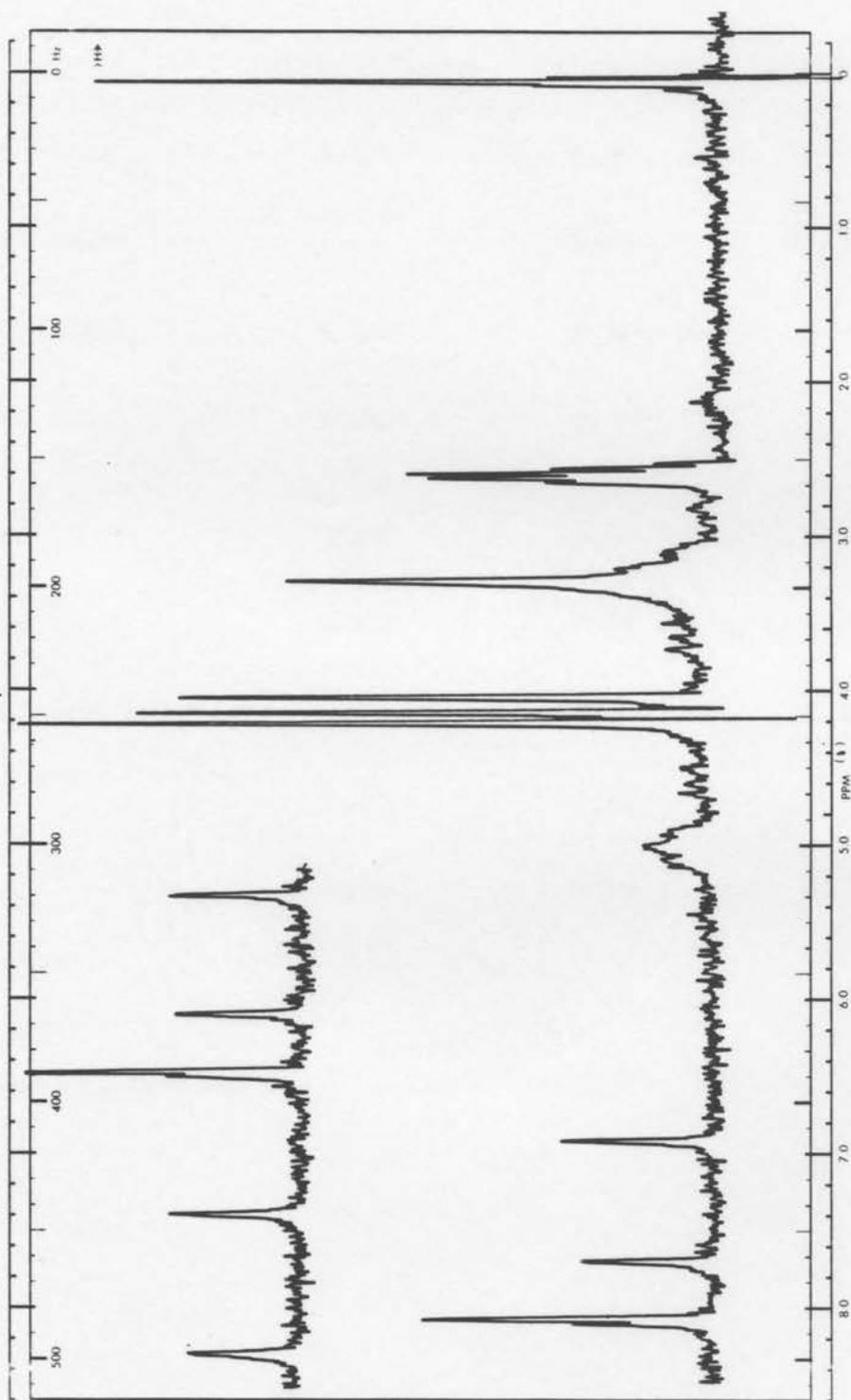
Figure 27 ^{1}H-NMR (CDCl$_3$-d$_6$-DMSO) of Jatrorrhizine

	Jatrorrhizine	Palmatine	Columbamine
C-2 $-OCH_3$	4.0	4.0	–
C-3 $-OCH_3$	–	3.91	3.90
C-9 $-OCH_3$	4.10*	4.10*	4.10*
C-10 $-OCH_3$	4.16*	4.16*	4.16*
H-8	9.83	9.86	9.86
H-13	8.93	8.96	8.66
H-11 and H-12	8.03	8.06	8.03
H-1	7.65	7.66	7.83
H-4	6.86	6.96	6.90

*May be reversed

Table 3 Chemical Shifts of Protoberberine Alkaloids
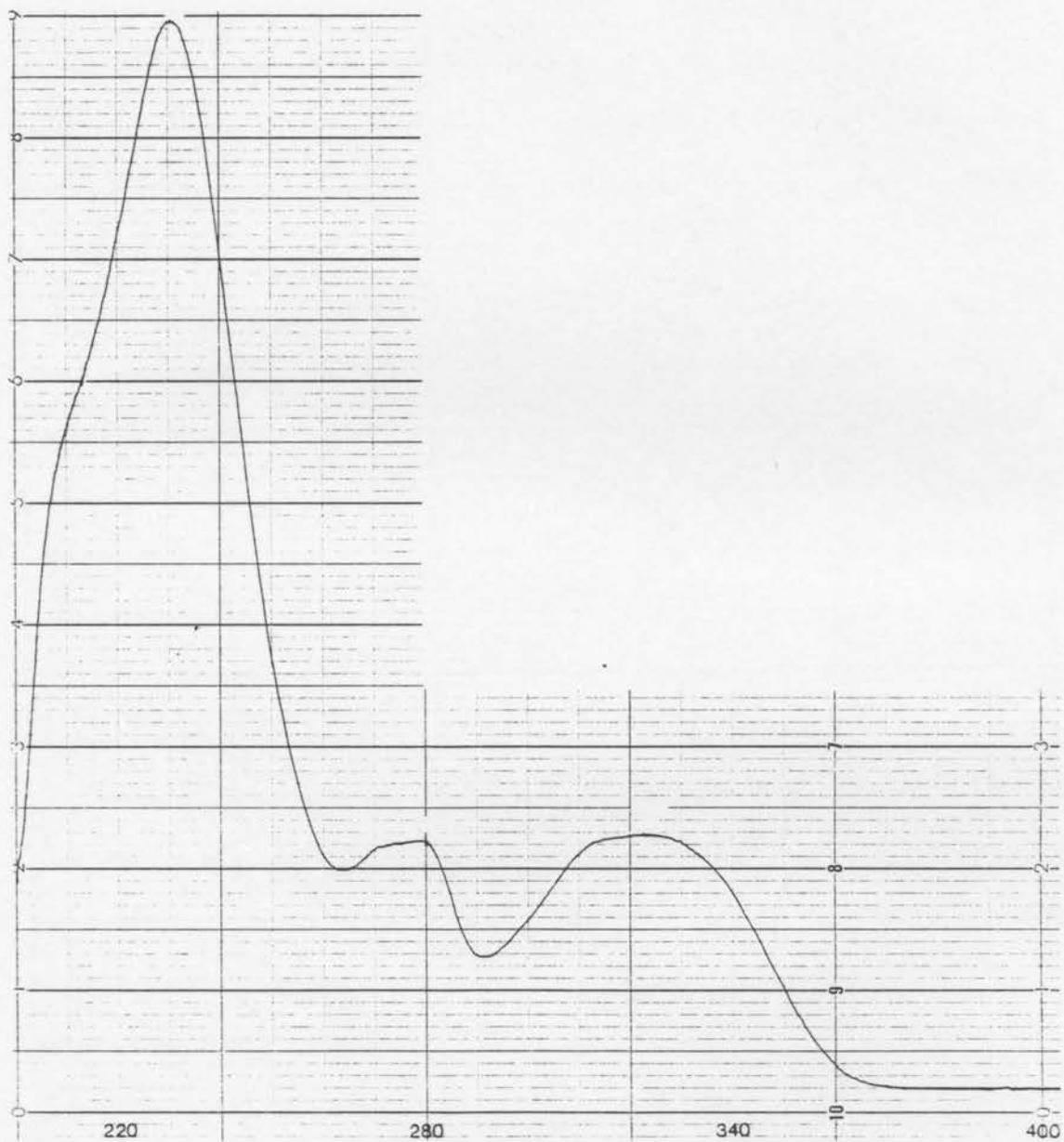
Figure 28a UV (EtOH) of Magnoflorine
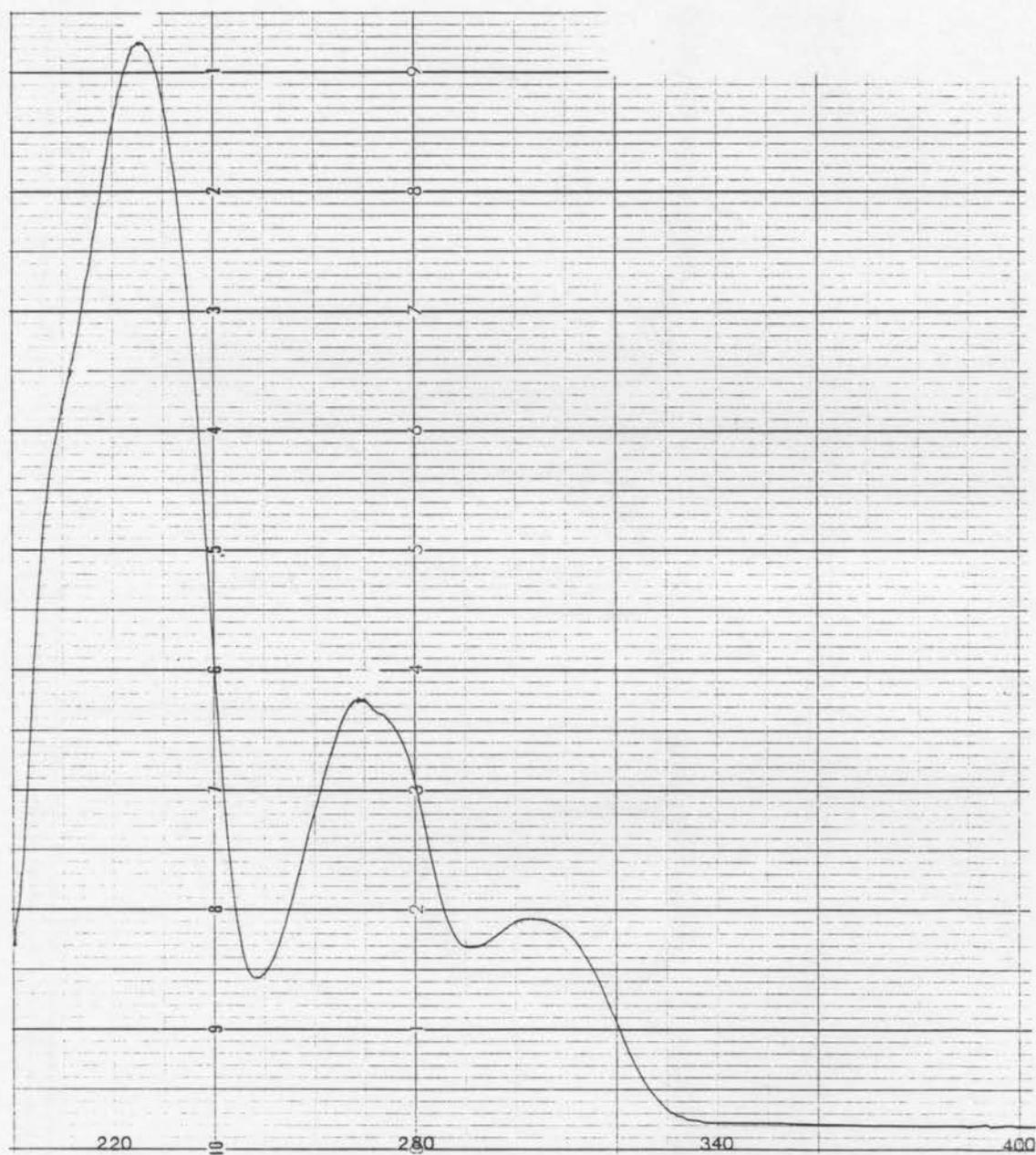
Figure 28b UV (EtOH + HCl) of Magnoflorine
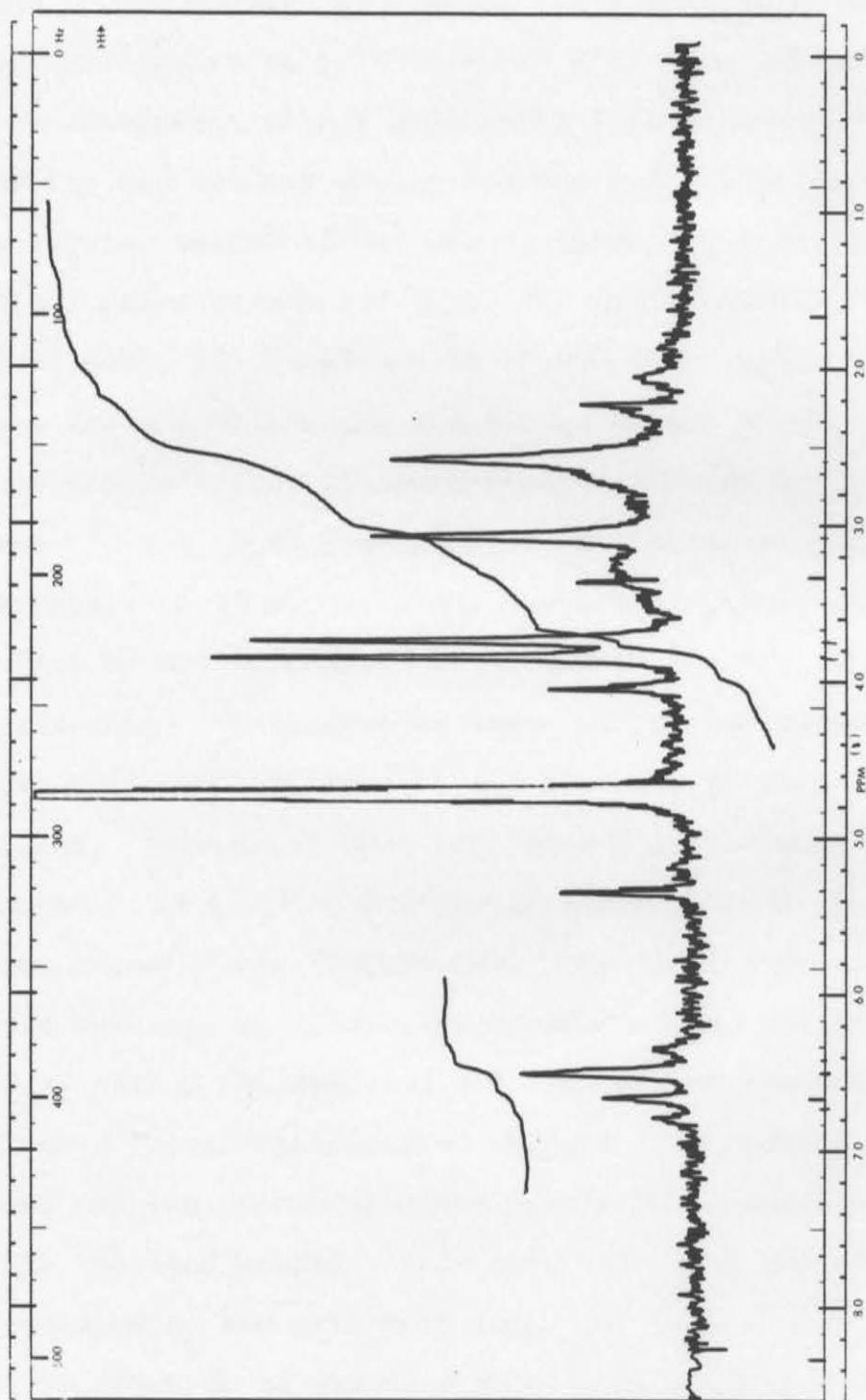
Figure 29 ^{1}H-NMR (D_2O) of Magnoflorine

three aromatic protons - a one proton singlet at 6.50, a one proton doublet at 6.40 (J = 7.5 Hz) and another one proton doublet at 6.72 (J = 7.5 Hz). The NMR and UV data are consistent with a quaternary 1,2,10,11-aporphine, having two methoxy groups and two hydroxy groups. The molecular weight of 342 was confirmed by a mass spectrum. Other peaks at m/e 327 (6%), 312 (3%), 298(6%), 284 (18%), 271 (64%), 255 (30%) and 58 (100%) were apparent. Since the NMR and UV spectra did not match any of the known quaternary 1,2,10,11-aporphines, a ^{13}C-NMR was obtained. The ^{13}C-NMR (D_2O) proton noise decoupled spectra showed the presence of 20 carbon atoms whose multiplicities were determined by off-resonance decoupling (Table 4). The absence of methoxy ^{13}C-resonances above 60 ppm indicated that the two hindered positions, 1 and 11, must be phenolic positions. This meant that positions 2 and 10 must be the methoxy substituted positions, and therefore the alkaloid was magnoflorine (Figure 24). The inconsistent NMR data was resolved by dissolving a sample of magnoflorine in D_2O, taking the spectrum and then adding several drops of NaOD. The aromatic region changed from showing the usual one and two proton singlets to the exact pattern seen in the isolated sample. This must mean that the alkaloid was isolated as the phenoxide ion.

Fraction 12 showed a spot at R_f 0.40 that was reddish-brown when exposed to basic tlc solvents. This fraction was concentrated and purified on a prep. tlc plate using

Chemical Shift	Multiplicity
152.72	s
151.43	s
150.58	s
149.63	s
125.85	s
123.32	s
120.86	s
116.78	d
115.56	s
110.33	d
109.19	d
70.76	d
62.08	t
56.29	q
55.95	q
53.79	q
43.53	q
31.59	t
24.61	t

Table 4 ^{13}C-NMR of Magnoflorine

S_4. The alkaloid positive band was eluted with 5% ammonium hydroxide in ethanol and concentrated to give 0.04 g. A UV (EtOH) (Figure 30) showed λ max 433, 350, 272 (sh), 265, 227 (sh) and 211. Addition of base shifted the UV spectrum to λ max 368, 330, 274, 242 and 225. The UV spectrum indicated another protoberberine alkaloid, and this was confirmed by the NMR ($CDCl_3$-d_6-DMSO) (Figure 31), which showed three methoxy resonances along with six aromatic protons (Table 4). The UV spectrum and tlc in several solvent systems correspond to columbamine[65] (Figure 24).

There are four protoberberine alkaloids which commonly occur together - berberine, palmatine, columbamine and jatrorrhizine. Since three of these had already been isolated in our work it was decided to recheck the material for palmatine. Palmatine (Figure 24) can be separated from berberine on tlc with cyclohexane:diethylamine (9:1).[63] Such a tlc of the isolated berberine showed a very small spot at an R_f corresponding to palmatine. A prep. tlc plate using cyclohexane:diethylamine was developed and the band corresponding to palmatine was eluted. The tlc values and molecular weight determined by chemical ionization mass spectroscopy were identical to a sample of palmatine that was synthesized by methylation of jatrorrhizine (see below).

Fractions 10 and 11 from the acidic alumina column were concentrated and chromatographed on a cellulose column using 0.1 N hydrochloric acid as the eluent to give 0.10 g of alkaloid positive material. This material was then

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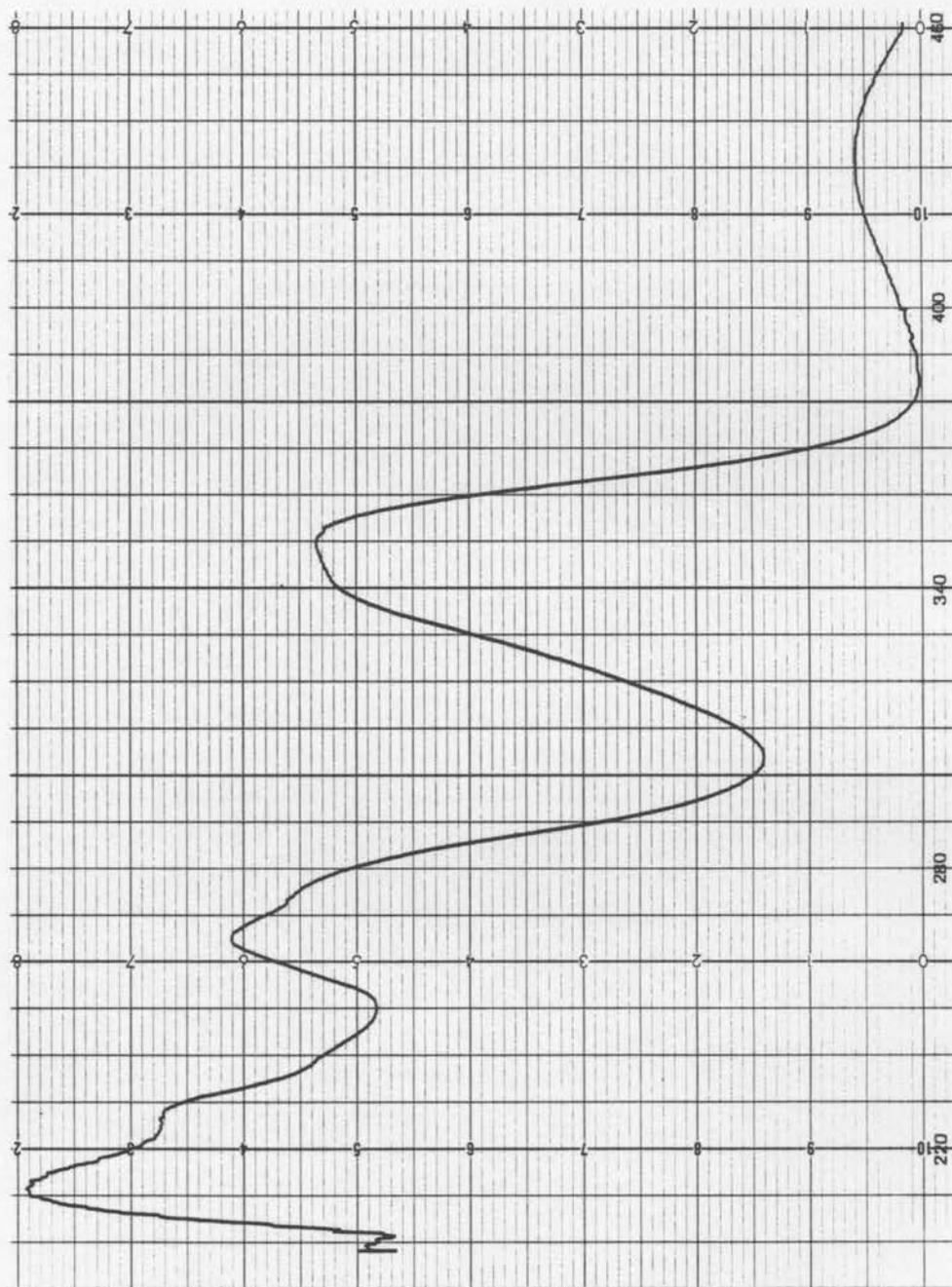
Figure 30a UV (EtOH) of Columbamine
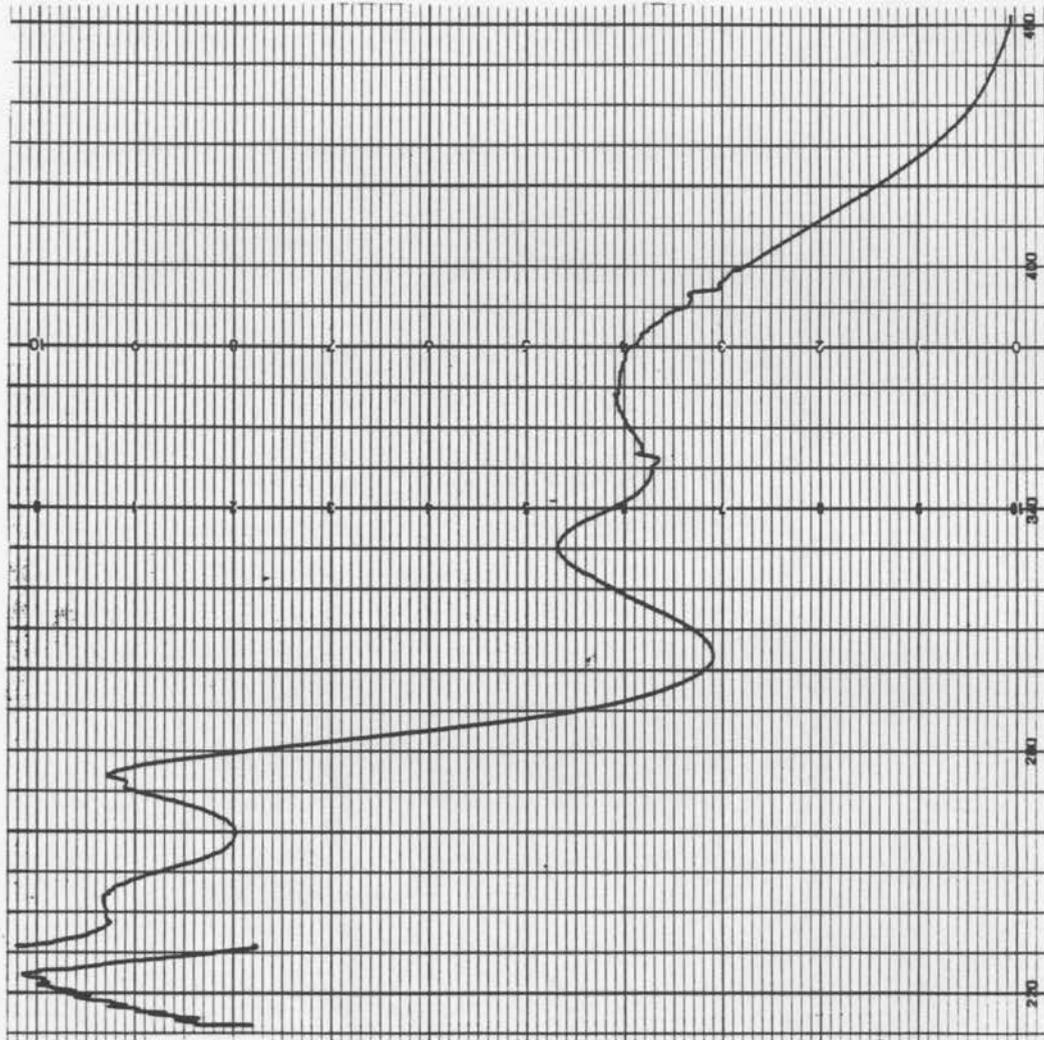
Figure 30b UV (EtOH + KOH) of Columbamine
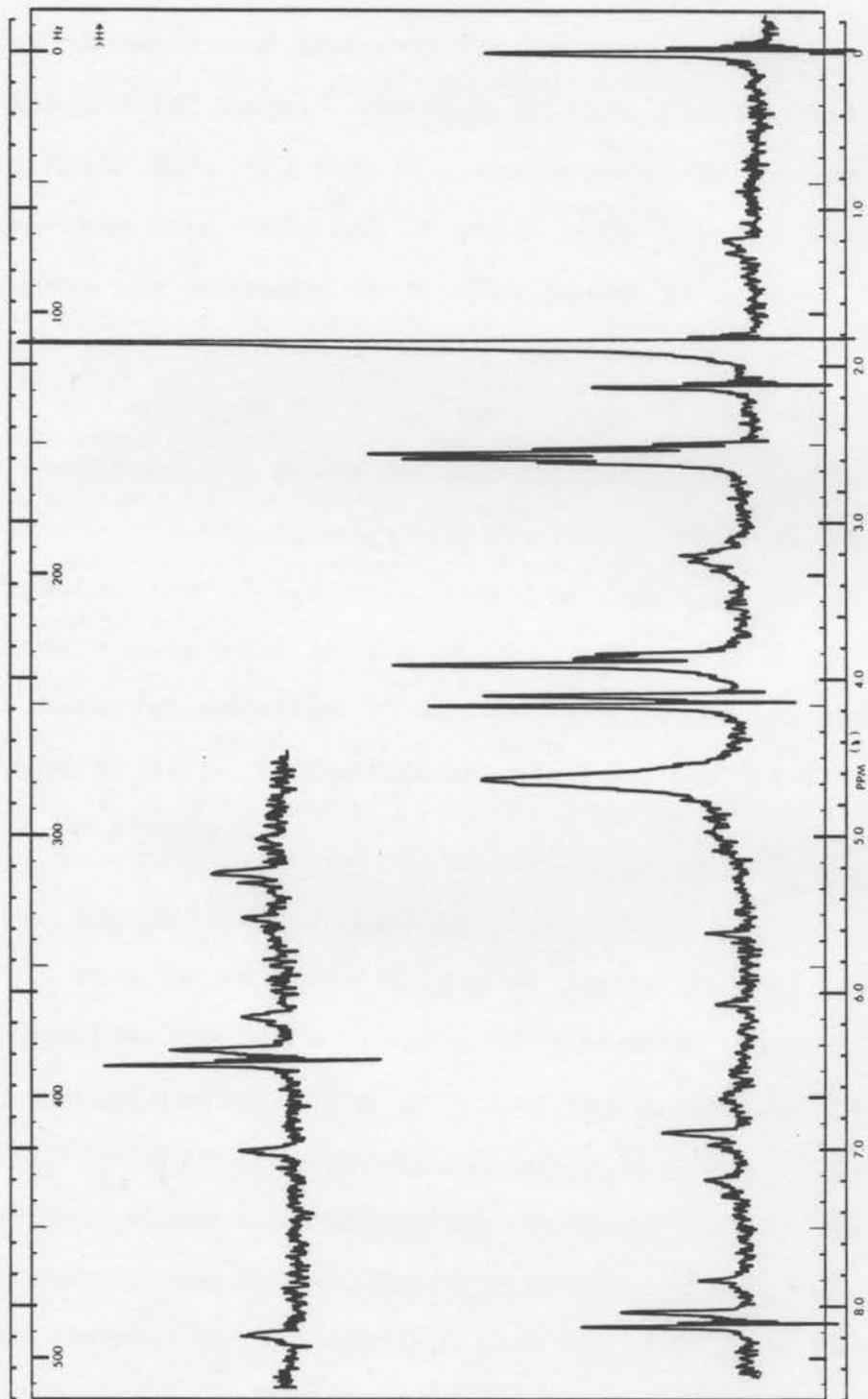
Figure 31 ^{1}H-NMR (CDCl$_3$-d$_6$-DMSO) of Columbamine

chromatographed on the MPLC using Si gel and 1% hydrochloric acid in methanol as the eluent. Fraction 6 (TS-4-40-14) was concentrated and a UV (EtOH) showed λ max 314, 255, 229 (sh) and 221 (sh). Addition of base shifted the spectrum to λ max 313, 255 and 217, which reverted to the original spectrum upon addition of acid. The 360 MHz NMR (CD_3OD) showed the presence of singlet peaks at 3.36, 4.08, 4.13, 4.44 and 7.72. Two doublets were apparent at 8.21 (J = 7.2 Hz) and 8.36 (J = 7.2 Hz). The ^{13}C-NMR showed the presence of ten peaks at 159.64, 154.61, 136.80, 134.94, 125.48, 124.49, 107.73, 106.39, 57.5 and 57.1 ppm. A chemical ionization mass spectrum peak at 190 (M+1) indicated a molecular weight of 189. Due to the small amount of material obtained (5 mg) and the inconsistency of the spectral data, a reasonable structure for TS-4-40-14 could not be proposed.

4. Synthesis of Palmatine Iodide

To a solution of 0.14 g of jatrorrhizine in dimethyl-formamide was added 0.12 g of potassium carbonate and 1 ml of methyl iodide. The solution was heated for two hours at 100°C under a nitrogen atmosphere, at which time the excess methyl iodide was removed by evaporation, and the potassium carbonate was removed by filtration. The dimethylformamide was removed by evaporation, and the resulting brownish solid was crystallized from water to give yellow needles, melting point 242°-244°C (d) (lit.[62] mp 239°-241°C), which showed

an NMR (CDCl$_3$-d$_6$-DMSO) (Figure 32) containing 4 methoxy signals and six aromatic protons (Table 4).

5. Isolation of Ether Soluble Constituents

The 12.9 g of ether soluble material was divided into a phenolic and a non-phenolic fraction in the same manner as the stems. This provided 3.0 g of non-phenolic material and 8.9 g of phenolic material. Tlc of the non-phenolic fraction showed the presence of three previously isolated alkaloids - obaberine, thalrugosine and oxyacanthine - so it was not investigated further.

The 8.9 g of phenolic material was chromatographed on the MPLC using (45:5:1) ethyl acetate:2-propanol:ammonium hydroxide as the solvent. Fractions were collected on the basis of UV absorption at 280 nm. Tlc of these fractions revealed the presence of three main alkaloids - thalrugosine, oxyacanthine and obamegine. Isolation and purification of these alkaloids was not pursued.

III. Discussion

A total of 13 alkaloids were isolated and identified from M. repens. These 13 alkaloids belong to three related structural classes - the aporphines, the protoberberines and the bisbenzyltetrahydroisoquinolines. Five of the 13 alkaloids are aporphine alkaloids. The aporphine alkaloids have a wide range of physiological activity. Most of this activity is centered upon the central nervous system. Isocorydine exhibits sedative effects in mice; corydine acts
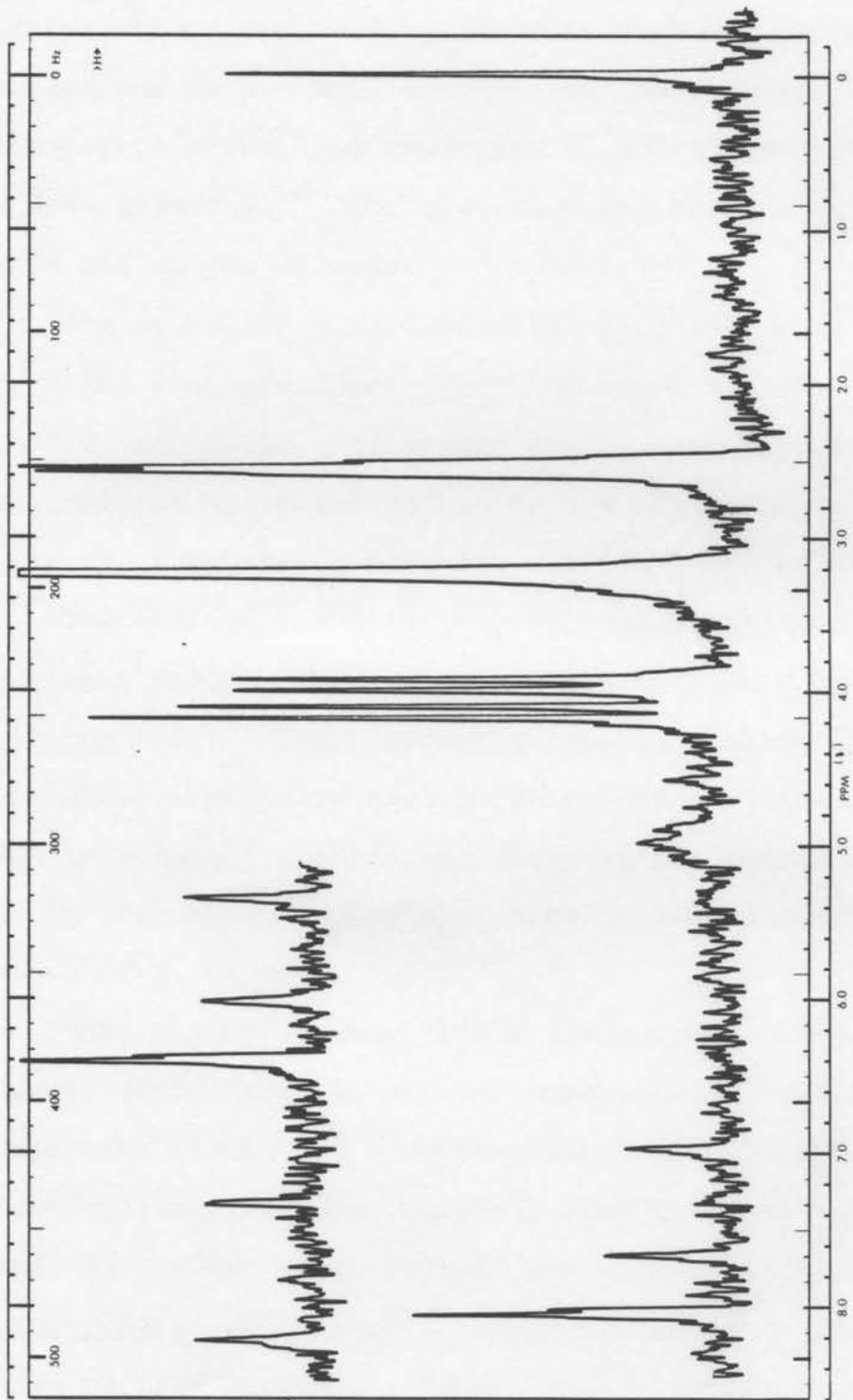
Figure 32 ^{1}H-NMR (CDCl$_3$-d$_6$-DMSO) of Palmatine

as an irritant and a depressor; glaucine has an anti-
tussive effect and causes reduction of blood glucose levels
in cats and rats. Both corydine and isocorydine have an
adrenolytic effect and are known to act cholinergically,
as does glaucine.[64] The physiological effects of thalipor-
phine are as yet unknown.

The aporphine alkaloids of M. repens may be responsible
for other biological activity. Apomorphine, 10,11-
dihydroxyaporphine, has strong emetic activity; although
the isolated aporphine alkaloids are not known for this
activity, the Ramah Navaho did use the plant as an emetic.

Isoboldine, 1,9-dihydroxy-2,10-dimethoxyaporphine, is
an insect feeding deterrent in the leaves of Cocculus
trilobus D.C.[65] Its structural relation to the isolated
alkaloids, especially thaliporphine and glaucine, might
mean they have a similar activity. This idea is augmented
by the presence of these aporphine alkaloids only in the
leaves of M. repens.

Some bisbenzylisoquinoline alkaloids are known to
possess antimicrobial, antiinflammatory and anesthetic
properties along with a curare-like activity. Two of the
bisbenzylisoquinolines isolated, obamegine and thalrugo-
sine, have shown antimicrobial activity in vitro against
Mycobacterium smegmatis.[52] Another, oxyacanthine, is
known to have hypotensive activity.[66] Although the bis-
benzylisoquinoline fraction of M. repens did not show

antitumor activity, related alkaloids like thalicarpine do show this activity.

The protoberberine alkaloids show three main types of activity - antimicrobial, uterine stimulatory and anti-neoplastic. Berberine shows low toxicity coupled with emetic activity, antibacterial activity, blocking of acetylcholine response, antiarrythmic activity, anti-diarrheal activity and stimulation of uterine muscle con-tractions among other activities.[64,67] Palmatine, jatror-rhizine and columbamine have shown many of these same effects, although in most cases they appear to be less active than berberine. These activities and the large amounts of these alkaloids present in the roots and stems of M. repens could explain its practical use by the Navaho to treat infections and diarrhea. The antimicrobial activity of these alkaloids and their presence in the roots and stems of M. repens could be the plant's primary defense against soil microbes.

Taxonomically, M. repens is in the same subsection, Euaquifoliatae, as M. aquifolium and is therefore very closely related. Five of the seven alkaloids isolated from M. aquifolium - berberine, jatrorrhizine, corydine, iso-corydine and oxyacanthine - are also found in M. repens. Of the other alkaloids, isoboldine, isolated from M. aquifolium, has the same oxygenation pattern as both thaliporphine and glaucine from M. repens. This is also shown by the bisbenzylisoquinolines, where oxyacanthine has

the same oxygenation pattern as obaberine, and berbamine
has the same pattern as does thalrugosine and obamegine.
The protoberberines also show this pattern, verifying the
close taxonomic relationship of the two species.

This similarity of alkaloidal components is also
shown by the other _Mahonia_ _sp_. studied, although most of
those species show fewer alkaloids. The fewer number of
alkaloids isolated in most cases seems to be due to a
study of only one part of the plant or due to less exten-
sive studies of the plant extracts.

Not only are the alkaloids from _M_. _repens_ similar to
alkaloids isolated from other _Mahonia_ _sp_., but they are
also either the same or similar to the alkaloids of _Berberis_
_sp_. This data does not add support to the separation of
Berberis and _Mahonia_ into two distinct genera, but further
study of the constituents of _Mahonia_ _sp_. is necessary to
clarify this question.

CHAPTER IV

THE SYNTHESIS OF N-METHYLISOCORYTUBERINE

I. Introduction

Aporphine alkaloids have been found in more than
fifteen plant families and are the second largest group of
isoquinoline alkaloids. All known, naturally occurring
aporphines are substituted at the C-1 and C-2 positions.
Almost any other position on the ring system may also be
substituted, but most substituents occur at positions C-9,
C-10 and C-11.

One of the most common aporphine alkaloids found in
nature is magnoflorine (Figure 33). Magnoflorine and N,N-
dimethyllindcarpine (Figure 33) have been described many
times in the literature, but the physical data reported for
the two alkaloids was very similar. In an effort to re-
solve this problem, the preparation of magnoflorine, N,N-
dimethyllindcarpine and two other isomers, N-methyliso-
corytuberine (Figure 33) and N,N-dimethylhernovine (Figure
33) was considered important. The synthesis of N-methyliso-
corytuberine was undertaken and is described here. The
differences between magnoflorine and N,N-dimethyllindcar-
pine have been resolved elsewhere[68] through semi-synthesis.

Synthetic approaches to the aporphine ring system
generally involve the preparation of an appropriately sub-
stituted tetrahydrobenzylisoquinoline which then undergoes
a cyclization to the aporphine nucleus. Three general
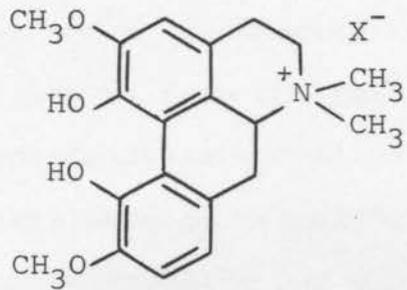
Magnoflorine
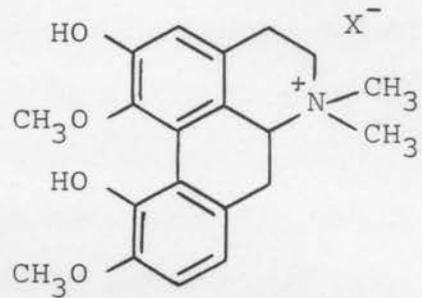
N,N-Dimethyllindcarpine
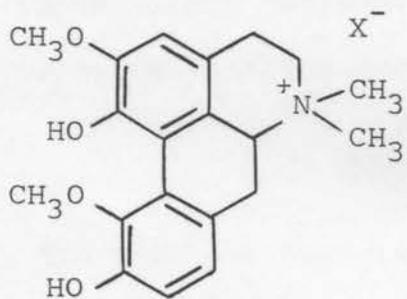
N-Methylisocorytuberine
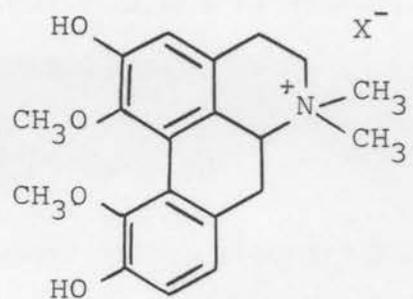
N,N-Dimethylhernovine

Figure 33 Quaternary 1,2,10,11-Aporphines

approaches to the tetrahydrobenzylisoquinoline moiety are employed: the Bischler-Napieralski reaction, the Pictet-Spengler reaction and the Pomeranz-Fritsch reaction (Figure 34). The Bischler-Napieralski reaction employs the condensation of a β-phenethylamine and a carboxylic acid or acid chloride to form the amide, which is cyclized to a 3,4-dihydrobenzylisoquinoline by phosphorus oxychloride. The Pictet-Spengler reaction involves the condensation of a β-phenethylamine and an aldehyde to produce the intermediate imine, which may be cyclized in acidic solution to produce the tetrahydrobenzylisoquinoline. The Pomeranz-Fritsch reaction involves the condensation of an aromatic aldehyde and an aminoacetal to produce the benzalaminoacetal, which is cyclized in the presence of acid to form the isoquinoline nucleus. The isoquinoline is subsequently alkylated to form the benzylisoquinoline.

II. Results and Discussion

The Bischler-Napieralski reaction was chosen as the initial route to N-methylisocorytuberine. The synthesis of the bottom half of N-methylisocorytuberine was attempted initially. 2-Nitrovanillin acetate ($\underline{2}$),[69] prepared from vanillin ($\underline{1}$), was converted to 2-nitrobenzylvanillin ($\underline{3}$).[70] Sodium borohydride reduction of 2-nitrobenzylvanillin provided 4-benzyloxy-3-methoxy-2-nitrobenzyl alcohol ($\underline{4}$) which was converted directly to 4-benzyloxy-3-methoxy-2-nitrobenzyl chloride ($\underline{5}$) by reaction with thionyl
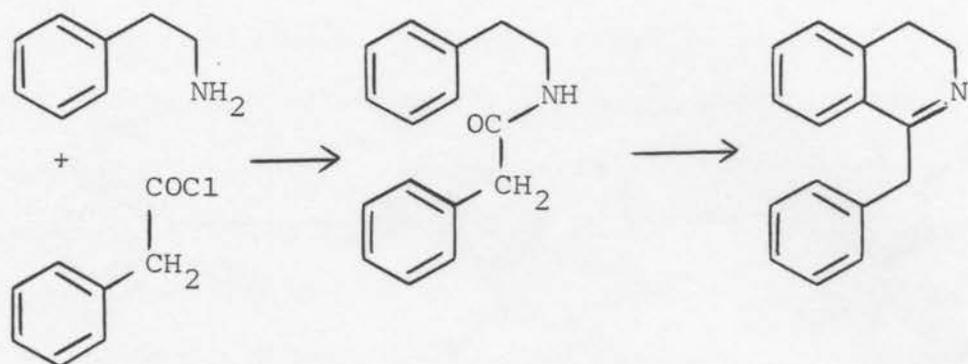
Bischler-Napieralski Reaction
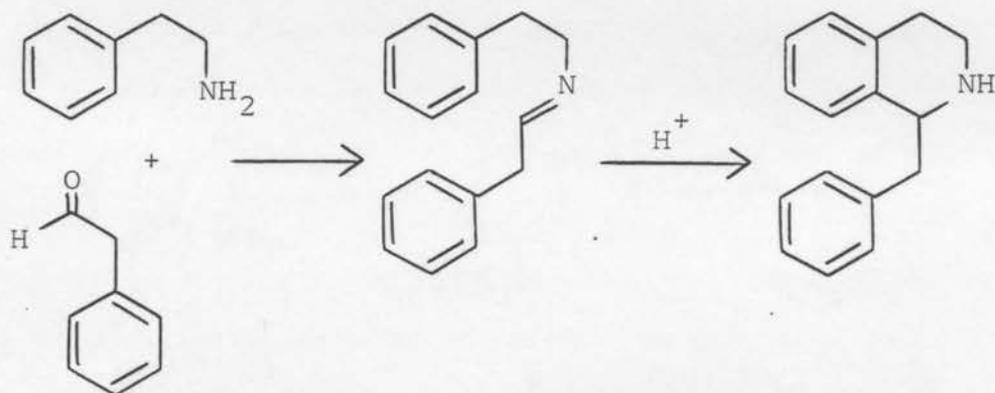
Pictet-Spengler Reaction
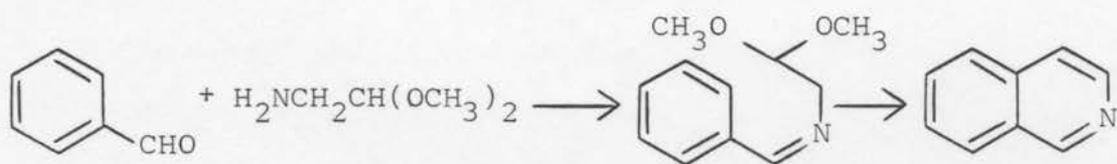
Pomeranz-Fritsch Reaction

Figure 34

Synthetic Routes to Tetrahydrobenzylisoquinolines
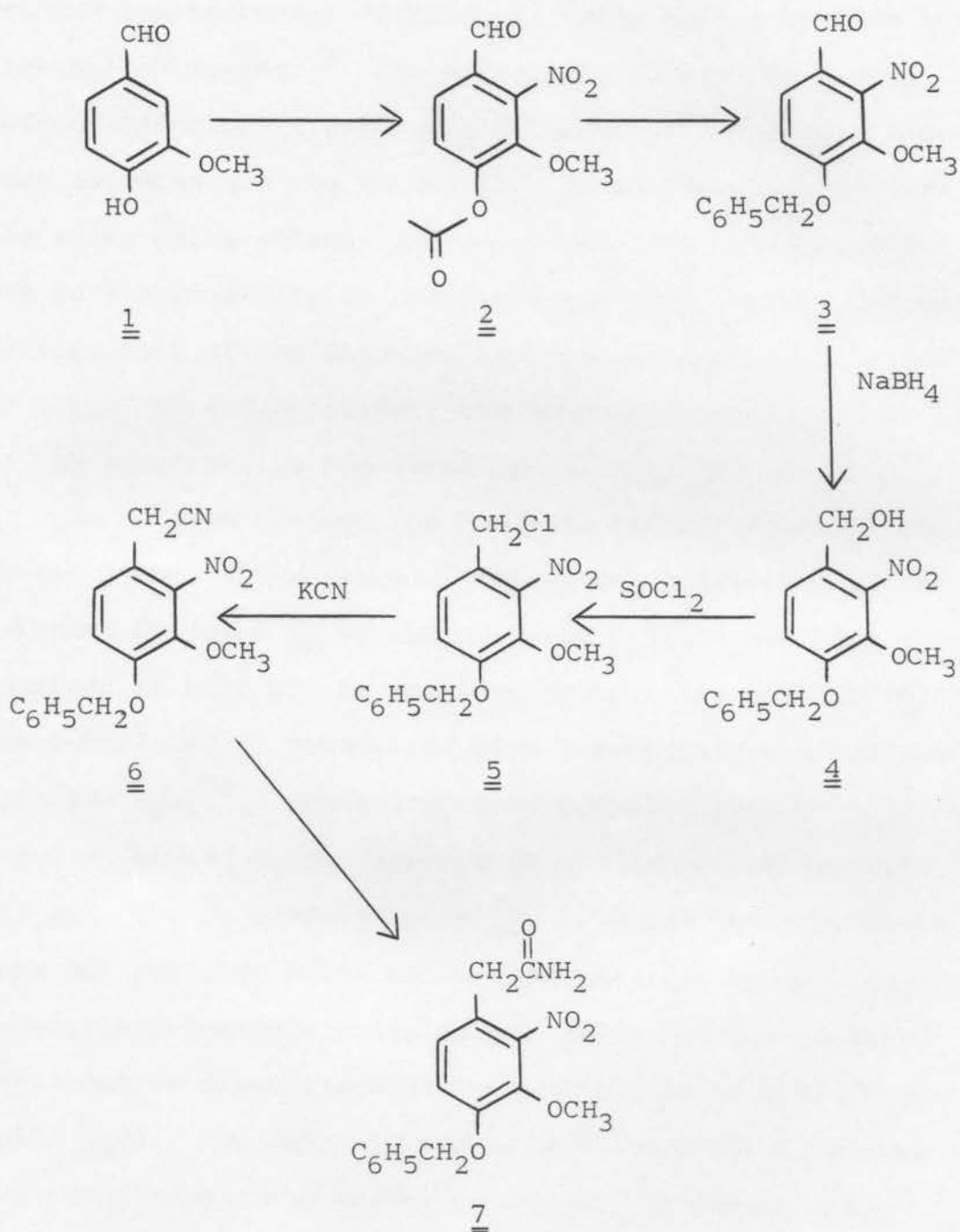
Figure 35

Synthetic Scheme for the Attempted Preparation of
N-Methylisocorytuberine by the Bischler-Napieralski Reaction

chloride.[71] The chloride 5 was converted to 4-benzyloxy-3-methoxy-2-nitrobenzyl cyanide (6) using sodium cyanide in dimethyl sulfoxide.[72] The hydrolysis of cyanide 6 with sodium hydroxide in water was attempted.[73] The only product isolated was the amide (7). An attempt to hydrolyze the amide using ethanol and water was also unsuccessful. Due to the inability to hydrolyze the cyanide and reported[74] difficulties of the Bischler-Napieralski cyclization with an ortho nitro substituent, the Bischler-Napieralski route to the isoquinoline was abandoned.

As an alternative, the Pomeranz-Fritsch reaction was investigated. This method involved the alkylation of the Reissert compound 16 by the chloride 5 which had been synthesized as part of the previous route. Isovanillin (8) was benzylated in ethanol to give 3-benzyloxy-4-methoxybenzaldehyde (9).[75] 7-Benzyloxy-6-methoxyisoquinoline (13) was synthesized by an adaption of the method of Stermitz[76] et. al. The intermediates to 13, although new substances, were not purified prior to further reaction in most cases. 3-Benzyloxy-4-methoxybenzaldehyde and a 10% excess aminoacetaldehyde dimethylacetal were condensed to provide the imine (10). The imine 10 was allowed to crystallize and was then washed with hexane to remove the excess aminoacetaldehyde dimethylacetal. This proved easier than purification by distillation.[76] The imine (10) was reduced with platinum (IV) oxide and hydrogen to provide the amine (11). The amine 11 was reacted with p-toluenesulfonyl

84
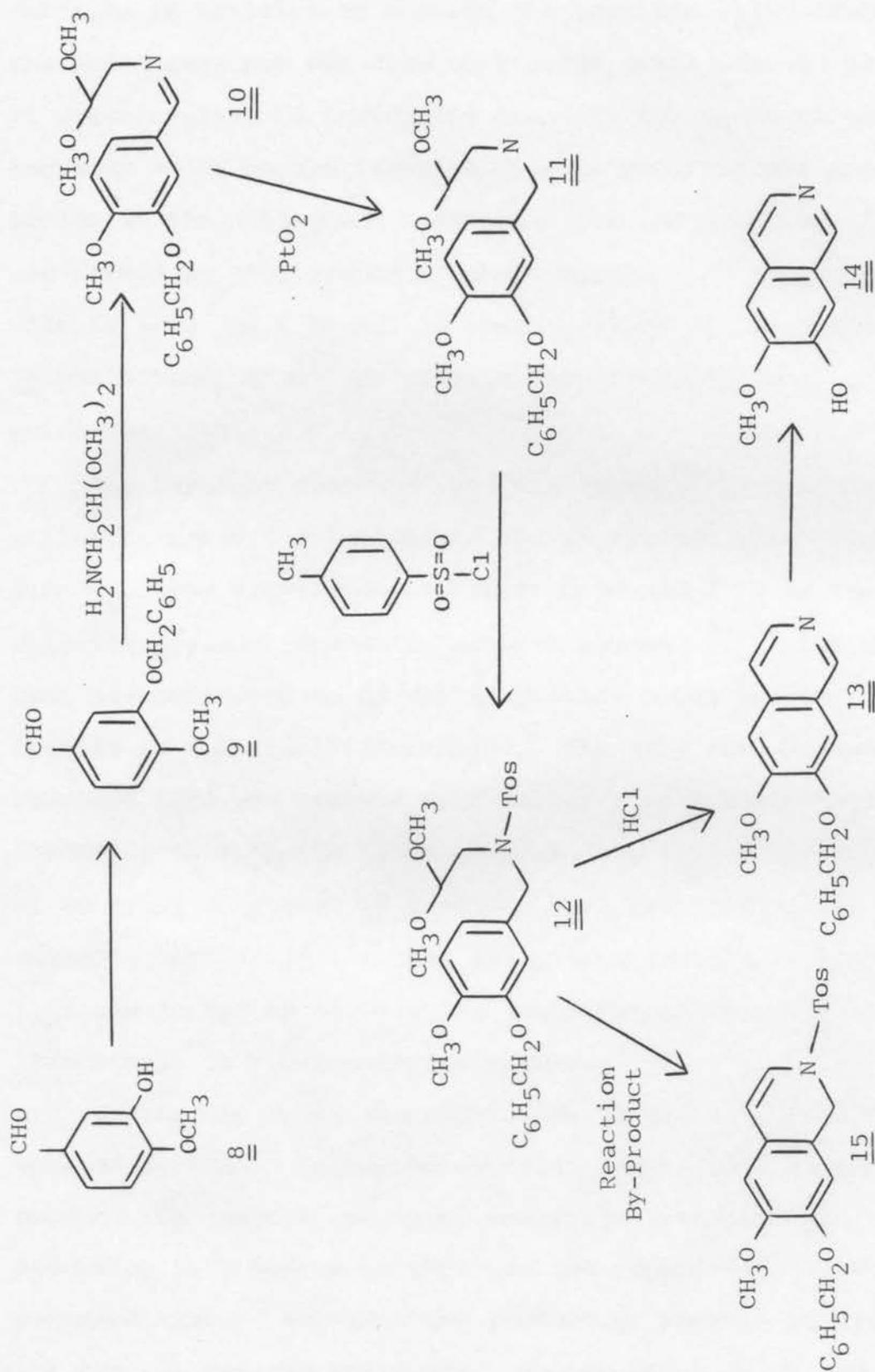
Figure 36 Synthetic Scheme for the Preparation of 7-Benzyloxy-6-methoxyisoquinoline

chloride in pyridine to provide the tosylate (12). When the above sequence was done on a large scale (ca. 50 g), it was necessary to purify the tosylate by column chromatography or by crystallization from methanol to get good yields in the subsequent reaction. The isoquinoline (13) was formed by cyclization of the tosylate in 6 N hydrochloric acid and dioxane. A small portion of isoquinoline 13 was debenzylated[77] to provide 7-hydroxy-6-methoxyisoquinoline (14).

The Reissert compound (16) was formed[74] by the reaction of benzoyl chloride and sodium cyanide with isoquinoline 13. The alkylation of Reissert compound 16 by the chloride 5 was attempted by several methods,[78,74] but the best method proved to be the alkylation using sodium hydride in N,N-dimethylformamide. The alkylated Reissert compound (17) was reacted with Triton B in N,N-dimethylformamide to form the benzylisoquinoline (18). Purification of benzylisoquinoline 18 provided as a by-product, the isoquinaldonitrile (19).[79] The benzylisoquinoline methiodide (20) was formed by methylating benzylisoquinoline 18 with iodomethane in N,N-dimethylformamide.

Hydrogenation of the methiodide 20 was attempted by several methods. Sodium borohydride reduction[74] failed to produce the desired tetrahydrobenzylisoquinoline (21). Reduction with sodium borohydride and copper (II) 2,4-pentanedionate[80] provided one product of unknown structure, but not the desired structure. Sodium borohydride and
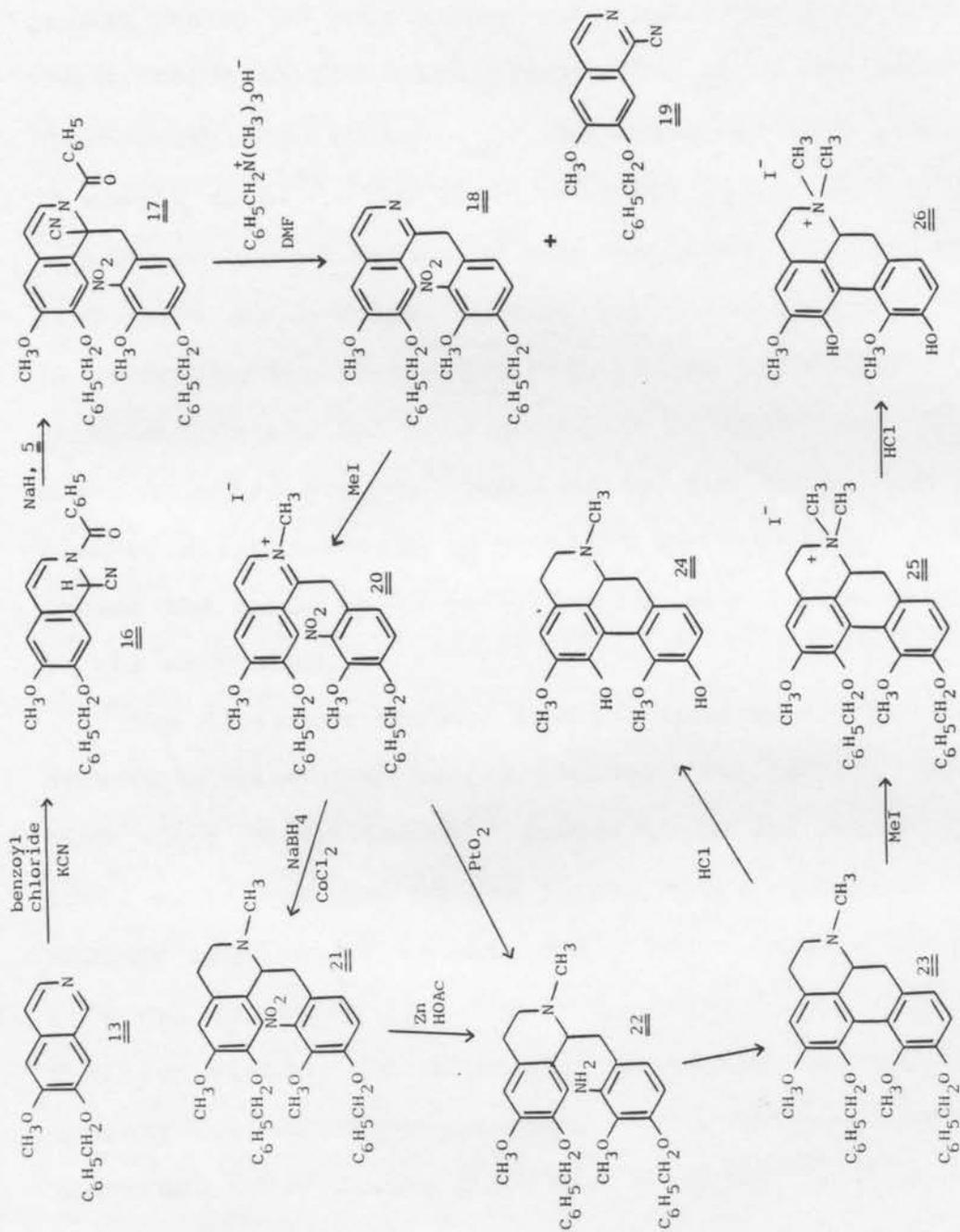
Figure 37 Synthetic Scheme for the Preparation of N-Methylisocorytuberine

cobalt (II) chloride hexahydrate[81] reduced a model compound, 1-(3-methoxybenzyl)-7-methoxyisoquinoline methiodide, in 95% yield, so this method was used. The reduction of the nitrotetrahydrobenzylisoquinoline 21 to the aminotetrahydrobenzylisoquinoline (22) was attempted with zinc metal in acetic acid.[74] The yield of amine 22 was very poor so another method of reduction was developed. Since platinum (IV) oxide and hydrogen reduced the model compound, 1-(3-methoxybenzyl)-7-methoxyisoquinoline methiodide, in excellent yield, and this procedure is also known to reduce aromatic nitro groups,[82] this method was used. The reduction of nitro compound 20 provided the amine 22. NMR showed the amine to be sufficiently pure to use directly in the next step.

Two different Pschorr cyclizations were tried to determine which gave better yields. The Pschorr cyclization using copper powder[83] proved to be the better cyclization, so it was used on the larger scale reaction. The Pschorr cyclization in both small scale cases provided the dibenzyl aporphine 23. The large scale (ca. 3.5 g) Pschorr reaction yielded the monobenzyl aporphine, 10-benzyloxy-1-hydroxy-2,11-dimethoxyaporphine. Debenzylation is known to sometimes occur during a Pschorr reaction.[74] A small amount of 23 was heated with hydrochloric acid to provide isocorytuberine (24). The NMR and mass spectrum of the purified isocorytuberine matched that of isocorytuberine synthesized previously in low yield by phenol oxidation.[43,84]

The protected aporphine 23 was methylated with iodo-
methane to provide the aporphine methiodide (25). The apor-
pine methiodide was heated in hydrochloric acid to provide
N-methylisocorytuberine (26). This is the first reported
synthesis of N-methylisocorytuberine. N-methylisocory-
tuberine has not as yet been reported as a natural product,
but isocorytuberine has recently been isolated from Glaucium
fimbrilligerum.[85]

N-Methylisocorytuberine may be distinguished from
magnoflorine and N,N-dimethyllindcarpine on the basis of tlc
R_f values. In S_4 the R_f values for magnoflorine, N,N-
dimethyllindcarpine and N-methylisocorytuberine are 0.31,
0.17 and 0.12 respectively. In formamide, magnoflorine
has an R_f 0.62, while both N,N-dimethyllindcarpine and N-
methylisocorytuberine have an R_f of 0.50.

The ^{1}H-NMR may also be used to distinguish between
magnoflorine and N-methylisocorytuberine. Magnoflorine
shows two methoxy resonances at 3.83 and 3.73 ppm, while
the two methoxy peaks of N-methylisocorytuberine are seen
at 3.87 and 3.45 ppm. The high field (3.45 ppm) peak of
N-methylisocorytuberine is characteristic of a methoxy in
the shielded C-11 position of an aporphine nucleus. The
^{13}C-NMR also shows this effect. The methoxy absorbances of
magnoflorine occur at 56.29 and 56.95 ppm, while those of
N-methylisocorytuberine are at 55.71 and 60.97 ppm. The
two isomers may also be distinguished by the chemical
shift pattern of the aromatic carbons. This is especially

evident in the case of the oxygen substituted carbon atoms. These four carbons in magnoflorine occur at 152.72, 151.43, 150.58 and 149.63. In N-methylisocorytuberine there is a wider chemical shift range: 154.50, 150.82, 144.75 and 143.35. Neither a ^{1}H-NMR in deuterium oxide nor a ^{13}C-NMR of N,N-dimethyllindcarpine was available for comparison.

III. Experimental

A. Attempted Synthesis of N-Methylisocorytuberine via the Bischler-Napieralski Reaction

2-Nitrovanillin acetate (2). 2-Nitrovanillin acetate was prepared by the method of Pschorr and Sumuleanu.[69] Vanillin was acetylated using potassium hydroxide and acetic anhydride in water to give vanillin acetate (91% yield): mp 75.5°-76.5°C (lit.[86] mp 77°C). Vanillin acetate was then nitrated with fuming red nitric acid in carbon tetrachloride solution below 0°C. After 2 hr of stirring, the solution was quenched with ice water. Filtration provided 2-nitrovanillin acetate (67.5% yield): mp 76°-81.5°C (lit.[69] mp 85°-87°C); NMR (CDCl$_3$) 2.40 (s, 3H, Ac), 3.93 (s, 3H, -OCH$_3$), 7.42 (d, 1H, J = 8 Hz), 7.70 (d, 1H, J = 8 Hz), 9.90 (s, 1H, -CHO).

2-Nitrobenzylvanillin (3). 2-Nitrobenzylvanillin was prepared from 2-nitrovanillin acetate by the procedure of MacDonald.[70] Crystallization of the solid from acetic acid and water provided yellowish plates of 2-nitrobenzylvanillin (40% yield): mp 110.5°-112.5°C (lit.[70] mp 111.5°-112.5°C);

NMR (CDCl$_3$) 4.0 (s, 3H, -OCH$_3$), 5.30 (s, 2H, -OCH$_2$Ph), 7.16 (d, 1H, J = 8 Hz), 7.43 (bs, 5H, Ar), 7.63 (d, 1H, J = 8 Hz), 9.80 (s, 1H, -CHO).

4-Benzyloxy-3-methoxy-2-nitrobenzyl alcohol (4). Sodium borohydride (4.59 g) was added in portions to a solution of 3 in 175 ml of diglyme. After addition was complete, the solution was heated for 15 min at 100°C, diluted with water and then extracted with chloroform. Evaporation of the chloroform provided a solution of 4 in diglyme which was used directly for the next step.

4-Benzyloxy-3-methoxy-2-nitrobenzyl chloride (5). The alcohol was converted to the chloride by reaction with thionyl chloride.[71] Upon standing, the chloride crystallized from the residual diglyme to give a yellow solid (77.7% yield): mp 71°-72.5°C; NMR (CDCl$_3$) 3.92 (s, 3H, -OCH$_3$), 4.46 (s, 2H, -CH$_2$Cl), 5.10 (s, 2H, -OCH$_2$Ph), 7.07 (s, 2H, Ar), 7.37 (s, 5H, Ar); IR (CHCl$_3$) 1550, 1510, 1380 cm^{-1}.

4-Benzyloxy-3-methoxy-2-nitrobenzyl cyanide (6). Reaction of the chloride and sodium cyanide in dimethyl sulfoxide provided the cyanide.[72] Crystallization from ethanol gave yellow crystals (81% yield): mp 93°-97°C; NMR (CDCl$_3$) 3.65 (s, 2H, -CH$_2$CN), 3.95 (s, 3H, -OCH$_3$), 5.10 (s, 2H, -OCH$_2$Ph), 6.90-7.20 (m, 2H, Ar), 7.33 (bs, 5H, Ar); IR (neat) 2280 (-C≡N) cm^{-1}.

Attempted hydrolysis of 4-benzyloxy-3-methoxy-2-nitrobenzyl cyanide. The cyanide (10.6 g) was placed in a

round bottomed flask with sodium hydroxide (23 g) and 60 ml of water. The reaction was brought to reflux and maintained there for 24 hr.[73] A solid material was detected in the flask during the period of reflux. Isolation of this solid and crystallization from ethanol provided red cyrstals: mp 169°-170.5°C; NMR (d_6-acetone) 3.8 (s, 1H, -OCH$_3$), 5.22 (s, 2H, -OCH$_2$Ph), 6.47 (d, 1H, J = 8 Hz), 7.30-7.57 (m, 5H, Ar), 7.67 (d, 1H, J = 8 Hz), 8.90 (bs, 2H, -NH$_2$); IR (CDCl$_3$) 3500, 3380 (-CONH$_2$); 1675, 1600 (-CO-); 1630 cm^{-1}. This data suggested the material to be the amide, an intermediate hydrolysis product. None of the acid was obtained from the reaction. On the assumption that the acid was not forming because the amide was insoluble, the reaction was next tried on the amide using (1:1) ethanol to water as the solvent. The amide was soluble, but after 24 hr of heating at reflux, no recognizable product was obtained.

B. Synthesis of N-Methylisocorytuberine via the Pomerantz-Fritsch Isoquinoline Cyclization.

3-Benzyloxy-4-methoxybenzaldehyde (9). This was prepared by the benzylation of isovanillin according to the method of Uff.[75] Crystallization of the chloroform soluble material from ethanol gave the benzyloxy compound (78% yield): NMR (CDCl$_3$) 3.95 (s, 3H, -OCH$_3$), 5.18 (s, 2H, -OCH$_2$Ph), 7.04 (d, 2H, J = 8.5 Hz), 7.33-7.56 (m, 6H, Ar), 9.83 (s, 1H, -CHO).

7-Benzyloxy-6-methoxy isoquinoline (13). 3-Benzyloxy-4-methoxybenzaldehyde (6.28 g) and a 10% excess of aminoacetaldehyde dimethylacetal were allowed to reflux in benzene using a Dean-Stark trap until the stoichiometric amount of water had been collected. Washing with hexane provided a white solid: mp 81.5°-8.35°C; NMR (CDCl$_3$) 3.37 $\angle$s, 6H, -CH(OCH$_3$)$_2$_$\overline{7}$, 3.73 (d, 2H, N-CH$_2$-, J = 5 Hz), 3.87 (s, 3H, -OCH$_3$), 4.64 $\angle$t, 1H, CH$_2$-CH (OCH$_3$)$_2$, J = 5 Hz$\overline{7}$, 5.15 (s, 2H, -OCH$_2$Ph), 6.87 (d, 2H, J = 8 Hz), 7.13-7.53 (m, 7H, Ar), 8.17 (s, 1H, -CH=N); IR (KBr) 1652 (C=N) cm^{-1}. The imine was reduced to the amine using 1% by weight platinum (IV) oxide in ethanol solution at 50 psi hydrogen in a Parr apparatus. Filtration and evaporation gave a light yellow oil: NMR (CDCl$_3$) 1.55 (s, 1H, -NH), 2.68 $\angle$d, 2H, -NH-CH$_2$-CH(OCH$_3$)$_2$, J = 5 Hz$\overline{7}$, 3.30 $\angle$s, 6H, -CH(OCH$_3$)$_2$_$\overline{7}$, 3.68 (s, 2H, -CH$_2$NH-), 3.82 (s, 3H, -OCH$_3$), 4.43 $\angle$t, 1H, -NH-CH$_2$-CH(OCH$_3$)$_2$, J = 5 Hz$\overline{7}$, 6.83-6.92 (m, 3H), 7.23-7.50 (m, 5H, Ar); IR (neat) 3360 (N-H); 1615 and 1600 (Ar) cm^{-1}. The amine was reacted with p-toluenesulfonyl chloride in pyridine to yield the tosylate. A yellowish solid was isolated: mp 49°-51°C; NMR (CDCl$_3$) 2.37 (s, 3H, -CH$_3$), 3.15 $\angle$d, 2H, -CH$_2$CH(OCH$_3$)$_2$, J = 5 Hz$\overline{7}$, 3.17 $\angle$s, 6H, -CH (OCH$_3$)$_2$_$\overline{7}$, 3.80 (s, 3H, -OCH$_3$), 4.30 $\angle$t, 1H, -CH$_2$CH(OCH$_3$)$_2$, J = 5 Hz$\overline{7}$, 4.37 (s, 2H, -CH$_2$-N), 4.97 (s, 2H, -OCH$_2$Ph), 6.73 (s, 3H, Ar), 7.17-7.47 (m, 7H, Ar), 7.70 (d, 2H, J = 8 Hz). The tosylate was dissolved in 75 ml of peroxide free dioxane and 15 ml of 6 N hydrochloric acid. The

solution was allowed to reflux for 6 hr in the dark under a nitrogen atmosphere. Chromatography of <u>13</u> on Si gel with chloroform:methanol (25:1) provided a solid (57% yield from <u>9</u>): mp 150°-153°C; ^{1}H-NMR (CDCl$_3$) 3.80 (s, 3H, -OCH$_3$), 5.02 (s, 2H, -O<u>CH$_2$</u>Ph), 6.76 (s, 1H, H-5 or H-8), 6.94 (s, 1H, H-8 or H-5), 7.00-7.28 (m, 6H, Ar), 8.07 (d, 1H, H-3, J = 6 Hz), 8.66 (s, 1H, H-1); ^{13}C-NMR (CDCl$_3$) 55.82, 70.47, 104.93, 106.85, 118.90, 124.30, 127.08, 127.83, 128.36, 132.24, 135.86, 141.61, 148.97, 149.61, 152.97 ppm; IR (CHCl$_3$) 1627 (-CH=N-), 1502, 1480, 1250, 1145, 865 cm^{-1}.

7-Benzyloxy-1,2-dihydro-6-methoxy-2-p-toluenesulfonyl isoquinoline (<u>15</u>). When a large scale cyclization (~30 g) was purified by column chromatography, a small amount of a by-product was isolated: NMR (CDCl$_3$) 2.36 (s, 3H, -CH$_3$), 3.94 (s, 3H, -OCH$_3$), 4.64 (s, 2H, 1-CH$_2$), 5.22 (s, 2H, -O<u>CH$_2$</u>Ph), 5.96 (d, 1H, H-4, J = 9 Hz), 6.72 (s, 1H, H-5 or H-8), 6.76 (s, 1H, H-8 or H-5), 6.89 (d, 1H, H-3, J = 9 Hz), 7.40-7.68 (m, 7H, Ar), 7.89 (d, 2H, J = 8 Hz).

7-Hydroxy-6-methoxyisoquinoline (<u>14</u>). 7-Benzyloxy-6-methoxyisoquinoline was heated at reflux under a nitrogen atmosphere for 6 hr in 6 <u>N</u> hydrochloric acid.[77] Addition of ammonium hydroxide yielded a tan solid: NMR (CD$_3$OD) 4.03 (s, 3H, -OCH$_3$), 7.20 (s, 1H, H-5 or H-8), 7.33 (s, 1H, H-8 or H-5), 7.63 (d, 1H, H-4, J = 7 Hz), 8.03 (d, 1H, H-3, J = 7 Hz), 8.89 (bs, 1H, H-1).

2-Benzoyl-7-benzoxy-1-cyano-1,2-dihydro-6-methoxy-isoquinoline (<u>16</u>). The Reissert compound <u>16</u> was formed[74]

by the addition of 1.75 ml (15.1 mmol) of benzoyl chloride to a vigorously stirred mixture of 2.0 g (7.54 mmol) of the isoquinoline, 15 ml of methylene chloride, 1.77 g (27.1 mmol) of sodium cyanide and 3.5 ml of water. After 4 hr of stirring, the solution was separated and the organic phase concentrated. Crystallization from ethanol provided $\underline{16}$ (56% yield): mp 154.5°-157.5°C (decomp.); NMR (CDCl$_3$) 3.86 (s, 3H, -OCH$_3$), 5.13 (s, 2H, -O-C$\underline{H}_2$-Ph), 5.91 (d, 1H, H-3, J = 7.5 Hz), 6.37 (s, 1H, H-1), 6.50 (d, 1H, H-4, J = 7.5 Hz), 6.70 (s, 1H, H-5 or H-8), 6.82 (s, 1H, H-8 or H-5), 7.27-7.53 (m, 10H, Ar); IR (CHCl$_3$) 2400 (-C≡N), 1670 (N-CO-Ph), 1630, 1510, 1345 cm^{-1}.

Anal. Calcd. for C$_{25}$H$_{20}$N$_2$O$_3$: C, 75.74; H, 5.08; N, 7.07. Found: C, 75.60; H, 4.89; N, 7.04.

2-Benzoyl-1-(4-benzyloxy-3-methoxy-2-nitrobenzyl)-7-benzyloxy-1-cyano-1,2-dihydro-6-methoxyisoquinoline ($\underline{17}$). 0.126 g (3 mmol, 57% in mineral oil) of sodium hydride was washed with hexane and then stirred in a nitrogen atmosphere with 5 ml of N,N-dimethylformamide. A mixture of 0.81 g (1.77 mmol) of the Reissert compound $\underline{16}$ and 0.78 g (2.65 mmol) of the chloride $\underline{5}$ in 45 ml of N,N-dimethylformamide was added to the sodium hydride while external cooling in an ice bath was maintained.[74] The solution was quenched with water and extracted with benzene. The benzene was concentrated to give a yellow oil. This material was used unpurified in the next step.

1-(4-Benzyloxy-3-methoxy-2-nitrobenzyl)-7-benzyloxy-6-methoxyisoquinoline (18). The alkylated Reissert compound 17 was dissolved in 25 ml of N,N-dimethylformamide and stirred in a nitrogen atmosphere. 1.4 ml (40% in MeOH) of benzyltrimethylammonium hydroxide (Triton B) was added to the solution, which immediately turned black. After 30 min of stirring, the amine was precipitated with 2 ml of concentrated hydrochloric acid, washed with ice water and diethyl ether and then dried. Crystallization from ethanol provided 0.72 g (75.6% yield) of the benzylisoquinoline: mp 154.5°-155°C; NMR (CDCl$_3$) 3.97 (s, 6H, 6 and 3', -OCH$_3$), 4.33 (s, 2H, -CH$_2$-N), 5.0 (s, 2H, -OCH$_2$Ph), 5.20 (s, 2H, -OCH$_2$Ph), 6.53 (d, 1H, H-6' or H-7', J = 8.5 Hz), 6.77 (d, 1H, H-7' or H-6', J = 8.5 Hz), 7.02 (s, 1H, H-5 or H-8), 7.17-7.50 (m, 12H, Ar), 8.29 (d, 1H, H-3, J = 6 Hz); IR (CHCl$_3$) 1630, 1550, 1520, 1490, 1450, 1390 cm^{-1}.

Anal. Calcd. for C$_{32}$H$_{28}$N$_2$O$_6$: C, 71.63; H, 5.26; N, 5.22. Found: C, 71.37; H, 5.23; N, 4.98.

7-Benzyloxy-1-cyano-6-methoxyisoquinoline (19). 19[79] was isolated as the major by-product when the mother liquor from 18 was chromatographed on Si gel with toluene:ethyl acetate (4:1). NMR (CDCl$_3$) 4.0 (s, 3H, -OCH$_3$), 5.27 (s, 2H, -OCH$_2$Ph), 7.07 (s, 1H, H-5 or H-8), 7.27-7.73 (m, 7H, Ar), 8.38 (d, 1H, H-3, J = 6 Hz); IR (KBr) 2230 (-C≡N), 1620, 1560, 1500, 1480, 1430 cm^{-1}.

1-(4-Benzyloxy-3-methoxy-2-nitrobenzyl)-7-benzyloxy-6-methoxyisoquinoline methiodide (20). 1.08 g (2.01 mmol)

of the benzylisoquinoline $\underline{18}$, 15 ml of N,N-dimethylforma-
mide and 9 ml of iodomethane were heated on a steam bath for
4 hr. The N,N-dimethylformamide (DMF) was concentrated,
and the yellow solid washed with diethyl ether to remove
all the DMF. 1.4 g (2.01 mmol, 100% yield) of the methio-
dide was obtained: NMR (d_6-DMSO) 3.97 (s, 3H, N^+-CH_3),
4.10 (s, 3H, -OCH_3), 4.27 (s, 3H, -OCH_3), 4.92 (bs, 2H,
-$\underline{CH_2}$-N), 5.22 (s, 2H, -$O\underline{CH_2}$Ph), 5.33 (s, 2H, -$O\underline{CH_2}$Ph),
6.31 (d, 1H, H-5' or H-6', J = 9 Hz), 7.10-7.50 (m, 11H,
Ar), 7.77-7.93 (m, 2H, Ar), 8.37 (d, 1H, H-4, J = 7 Hz),
8.68 (d, 1H, H-3, J = 7 Hz).

$\underline{1-(4-Benzyloxy-3-methoxy-2-nitrobenzyl)-7-benzyloxy-6-}$
$\underline{methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline}$ ($\underline{21}$).
0.104 g (0.19 mmol) of the methiodide $\underline{\underline{20}}$, 0.095 g (0.399
mmol) of cobalt (II) chloride hexahydrate and 10 ml of
methanol were stirred under a nitrogen atmosphere. 0.07 g
(1.9 mmol) of sodium borohydride was added in small portions
to the solution. The solution was stirred for 3 hr, con-
centrated, and 15 ml of 1 $\underline{N}$ hydrochloric acid were added.
The acidic solution was extracted with chloroform, and the
chloroform soluble material was chromatographed on alumina
with chloroform to provide 0.045 g of isoquinoline $\underline{21}$ (43%
yield): NMR ($CDCl_3$) 2.33 (s, 3H, N-CH_3), 2.5-3.7 (m, 7H),
3.80 (s, 3H, -OCH_3), 3.93 (s, 3H, -OCH_3), 4.93 (s, 2H,
-$O\underline{CH_2}$Ph), 5.03 (s, 2H, -$O\underline{CH_2}$Ph), 6.33 (s, 1H, H-5 or H-8),
6.53 (s, 1H, H-8 or H-5), 6.62 (d, 1H, H-5' or H-6', J =

8.5 Hz), 6.88 (d, 1H, H-6' or H-5', J = 8.5 Hz), 7.33-7.50 (m, 10H, Ar).

1-(2-Amino-4-benzyloxy-3-methoxybenzyl)-7-benzyloxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (<u>22</u>). 0.77 g of <u>20</u>, 15 ml of ethanol and 0.07 g of platinum (IV) oxide were placed in a pressure bottle. 50 psi hydrogen was maintained with stirring at room temperature for 24 hr. The solution was filtered through Celite, concentrated, dissolved in chloroform and washed with saturated sodium bicarbonate to give (after Si gel chromatography with chloroform:methanol 15:1) 0.35 g of <u>22</u> (0.63 mmol, 57% yield): NMR (CDCl$_3$) 2.47 (s, 3H, -NCH$_3$), 2.53-3.7 (m, 7H), 3.87 (s, 6H, -OCH$_3$), 4.98 (s, 2H, -OCH$_2$Ph), 5.05 (s, 2H, -OCH$_2$Ph), 6.27 (s, 1H, H-5' or H-6'), 6.32 (s, 1H, H-6' or H-5'), 6.40 (s, 1H, H-5 or H-8), 6.57 (s, 1H, H-8 or H-5), 7.33-7.57 (m, 10H, Ar); IR (CHCl$_3$) 3420, 3340 (NH$_2$), 3000, 2930, 1610, 1495, 1465, 1370, 1250 cm^{-1}; CI MS 565 (1%), 553 (4%), 525 (M+1, 23%), 281 (100%), 192 (71%).

1,10-Dibenzyloxy-2,11-dimethoxyaporphine (<u>23</u>). 0.35 g (0.63 mmol) of amine <u>22</u>, 8.0 ml of glacial acetic acid[83] and 1 ml of concentrated sulfuric acid were stirred in an argon atmosphere with external ice cooling. 0.13 g of sodium nitrite in 2 ml of water was added to the solution, and stirring at 5°C continued for 45 min. The ice bath was removed and the sulfamic acid, 0.6 g of freshly prepared copper powder[87] and 40 ml of acetone were added. The solution was heated at reflux for 45 min, cooled to room temperature, made basic

with ammonium hydroxide and extracted with chloroform. The chloroform was chromatographed on Si gel with ethyl acetate: methanol (5:1) to give 0.019 g (0.037 mmol, 5.9% yield) of the aporphine: NMR (CDCl$_3$) 2.57 (s, 3H, NCH$_3$), 2.7-3.3 (m, 7H), 3.75 (s, 3H, -OCH$_3$), 3.90 (s, 3H, -OCH$_3$), 4.73 (bs, 1H, -O-$\underline{H}$CH-Ph), 4.83 (bs, 1H, -O-HC$\underline{H}$-Ph), 5.09 (bs, 2H, -O$\underline{CH_2}$Ph), 6.67 (s, 1H, H-3), 6.80 (s, 2H, H-8 and H-9), 7.03 (bs, 5H, Ar), 7.17-7.5 (m, 5H, Ar); UV (EtOH) λ max 304 (sh), 274, 226 (sh), 214 nm.

When the reaction was scaled up to 3.5 g, the product isolated was 10-benzyloxy-1-hydroxy-2,11-dimethoxyaporphine. The C-1 benzyloxy had hydrolyzed during the reaction: NMR (CDCl$_3$) 2.60 (s, 3H, -N-CH$_3$), 2.6-3.4 (m, 7H), 3.73 (s, 3H, -OCH$_3$), 3.87 (s, 3H, -OCH$_3$), 5.10 (s, 2H, -O$\underline{CH_2}$Ph), 6.63 (s, 1H, H-3), 6.85 (d, 1H, H-8 or H-9), 7.05 (d, 1H, H-9 or H-8), 7.27-7.50 (m, 5H, Ar).

The second Pschorr cyclization was that of Cava and Lakshmikantham.[74] 0.62 g (0.91 mmol) of 20 was hydrogenated in ethanol with platinum (IV) oxide and the resulting amine was used without purification. Amine 22 was dissolved in 1 ml concentrated hydrochloric acid and 15 ml water. The solution was cooled in an ice bath under an argon atmosphere. 0.1 g of sodium nitrite in 2 ml water was added dropwise, and the mixture stirred for 1 hr. At the end of 1 hr, excess sulfamic acid was added and the mixture allowed to stir at room temperature overnight. The mixture was heated on a steam bath for 15 min. Excess zinc dust was added

and heating continued for a further 15 min. The solution was cooled, made basic with ammonium hydroxide and extracted with chloroform. The chloroform was chromatographed to give 0.021 g (0.041 mmol, 4.5% yield) of the aporphine.

Isocorytuberine (24). 0.019 g (0.037 mmol) of the dibenzyloxyaporphine was dissolved in 1 ml of toluene and 5 ml of 6 N hydrochloric acid. The solution was heated at reflux for 45 min, at which time it was cooled and then extracted with chloroform. The acidic aqueous phase was made basic with ammonium hydroxide and then extracted with chloroform. The chloroform extract of the basic aqueous phase was concentrated to give 0.010 g (0.032 mmol, 86% yield): NMR (CDCl$_3$) (Figure 38) 2.56 (s, 3H, NCH$_3$), 2.5-3.2 (m, 7H), 3.68 /s, 3H, (C-11, -OCH$_3$)/, 3.91 /s, 3H, (C-2, -OCH$_3$)/, 6.66 (s, 1H, H-3), 6.85 (d, 1H, H-9, J = 8 Hz), 7.01 (d, 1H, H-8, J = 8 Hz); UV (EtOH) (Figure 39) λmax 307, 297 (sh), 273, 266, 234 nm; MS, M+ 327, 296, 295, 279, 149. These data match those of isocorytuberine synthesized previously by phenol oxidation (NMR[84a] and MS[84b]).

1,10-Dibenzyloxy-2,11-dimethoxy aporphine methiodide (25). 0.020 g (0.039 mmol) of aporphine 23 was dissolved in 1 ml of N,N-dimethylformamide and 1 ml of methyl iodide was added. The solution was allowed to reflux for 5 hr, and then the excess methyl iodide and DMF were removed. Si gel chromatography with chloroform provided 0.018 g (0.027 mmol, 71% yield) of the methiodide: NMR (CDCl$_3$) 3.08
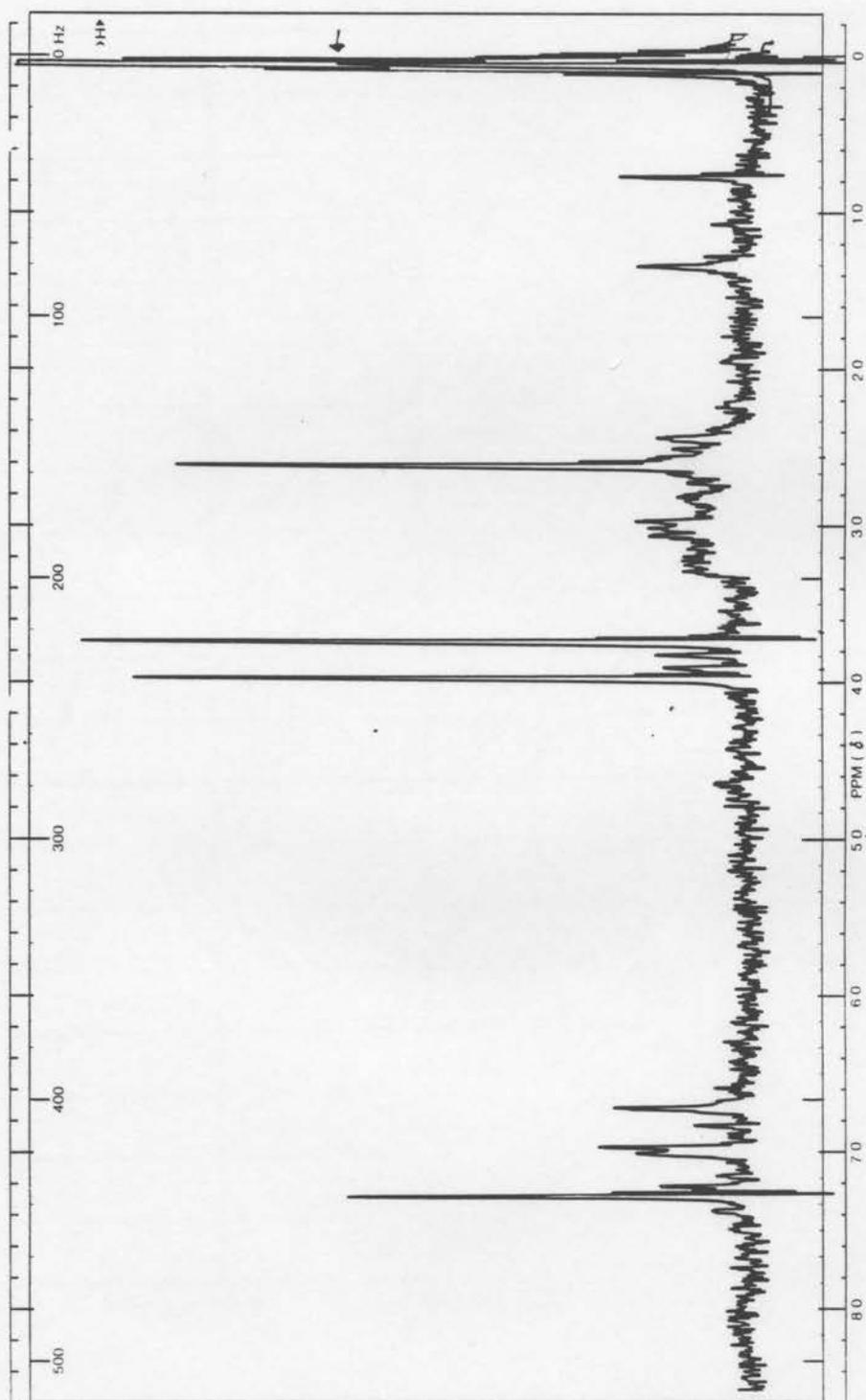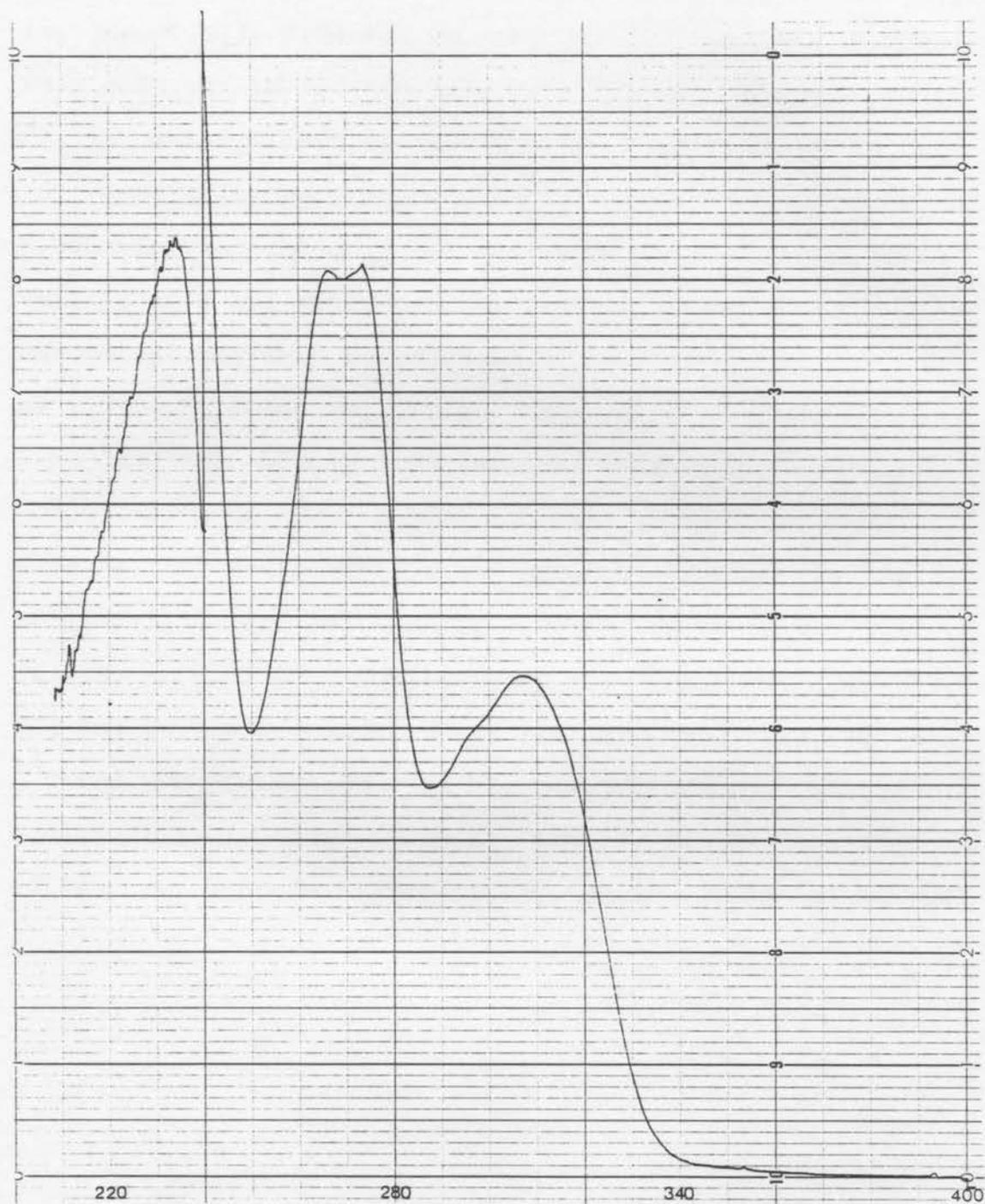
Figure 38 ^{1}H-NMR (CDCl$_3$) of Isocorytuberine

Figure 39 UV (EtOH) of Isocorytuberine

(s, 3H, -NCH$_3$), 3.63 (s, 3H, -NCH$_3$), 3.3-3.8 (m, 7H), 3.70 (s, 3H, -OCH$_3$), 3.88 (s, 3H, -OCH$_3$), 4.77 (s, 1H, -O-$\underline{H}$CH-Ph), 4.82 (s, 1H, -O-HC$\underline{H}$-Ph), 5.02 (s, 2H, -OC$\underline{H}_2$Ph), 6.70 (s, 1H, H-3), 6.78-7.08 (m, 7H, Ar), 7.18-7.35 (m, 5H, Ar).

N-Methylisocorytuberine (26). 0.018 g (0.027 mmol) of dibenzyloxyaporphine 25 was dissolved in 5 ml 6 N hydrochloric acid and allowed to reflux for 3 hr. Si gel chromatography with S$_4$ provided 0.010 g (80% yield) of isocorytuberine methiodide: NMR (D$_2$O) (Figure 40) 2.68 (bs, 3H, -NCH$_3$), 3.23 (bs, 3H, -NCH$_3$), 3.45 (s, 3H, -OCH$_3$), 3.87 (s, 3H, -OCH$_3$), 6.60 (s, 2H, H-9 and H-8), 6.83 (s, 1H, H-3); NMR (CD$_3$OD) (Figure 41) 2.57 (bs, 3H, -NCH$_3$), 3.37 (bs, 3H, -NCH$_3$), 3.62 (s, 3H, -OCH$_3$), 3.92 (s, 3H, -OCH$_3$); UV (EtOH) (Figure 42) λmax 307, 273, 267, 227 nm; UV (EtOH + NaOH) λmax 341, 279 (sh), 243, 223 nm; ^{13}C-NMR (CH$_3$OD) 154.50, 150.82, 144.75, 143.35, 125.13, 124.38, 121.63, 120.87, 120.41, 120.17, 118.07, 110.77, 69.84, 61.32, 60.97, 55.71, 53.38, 43.10, 30.43, 23.89 ppm.
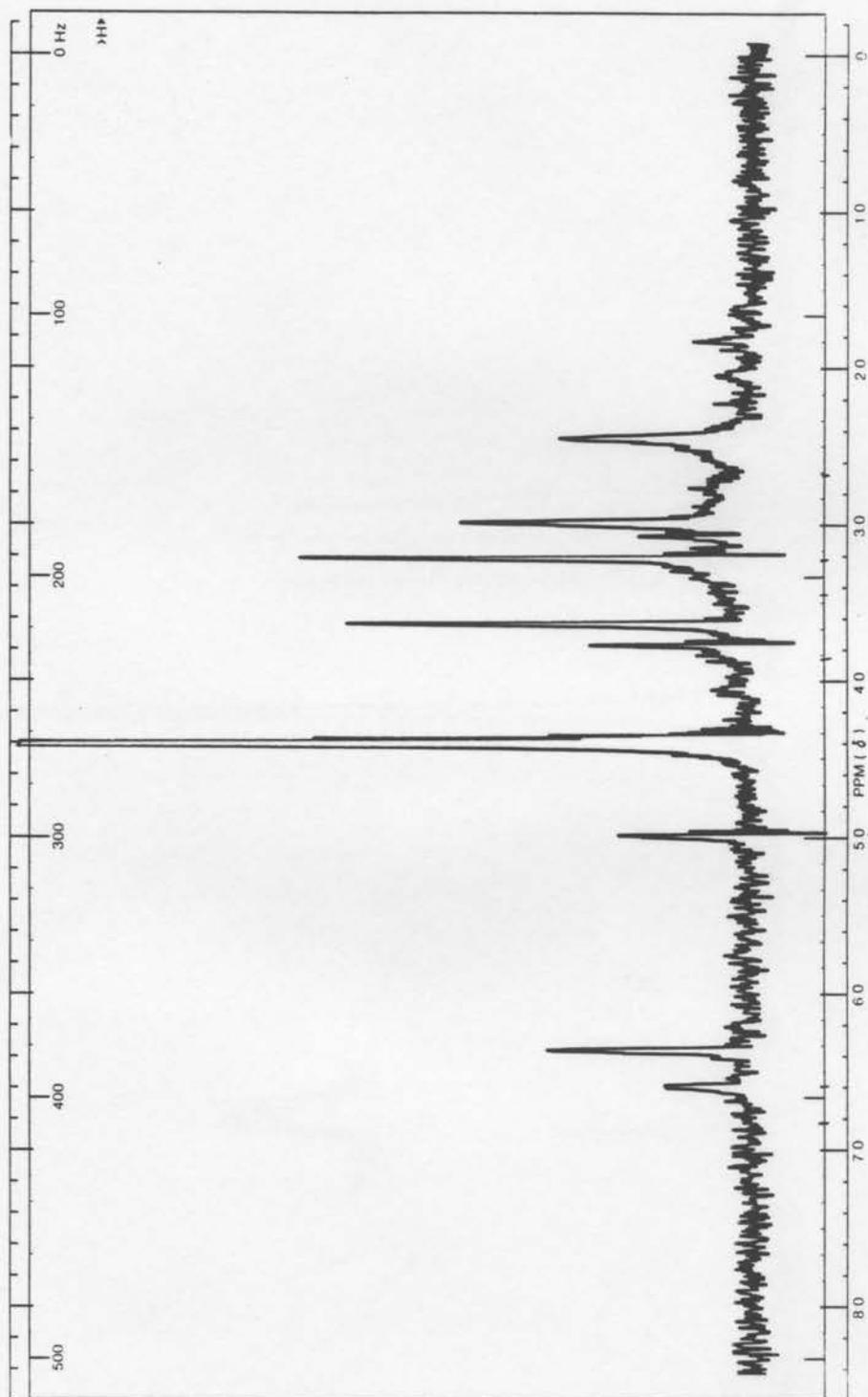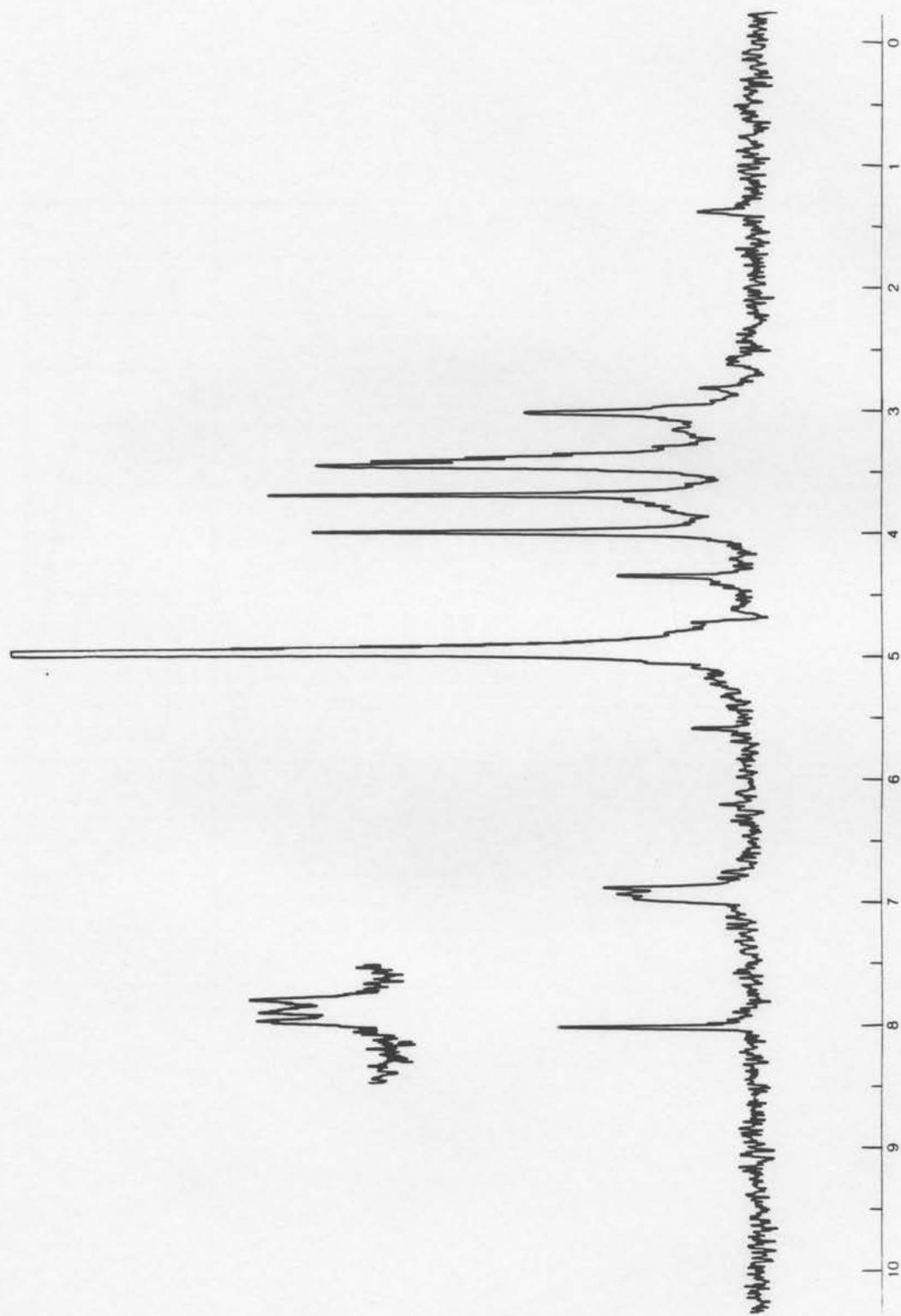
Figure 40 ^{1}H-NMR (D_2O) of N-Methylisocorytuberine

Figure 41 ^{1}H-NMR (CD$_3$OD) of N-Methylisocorytuberine
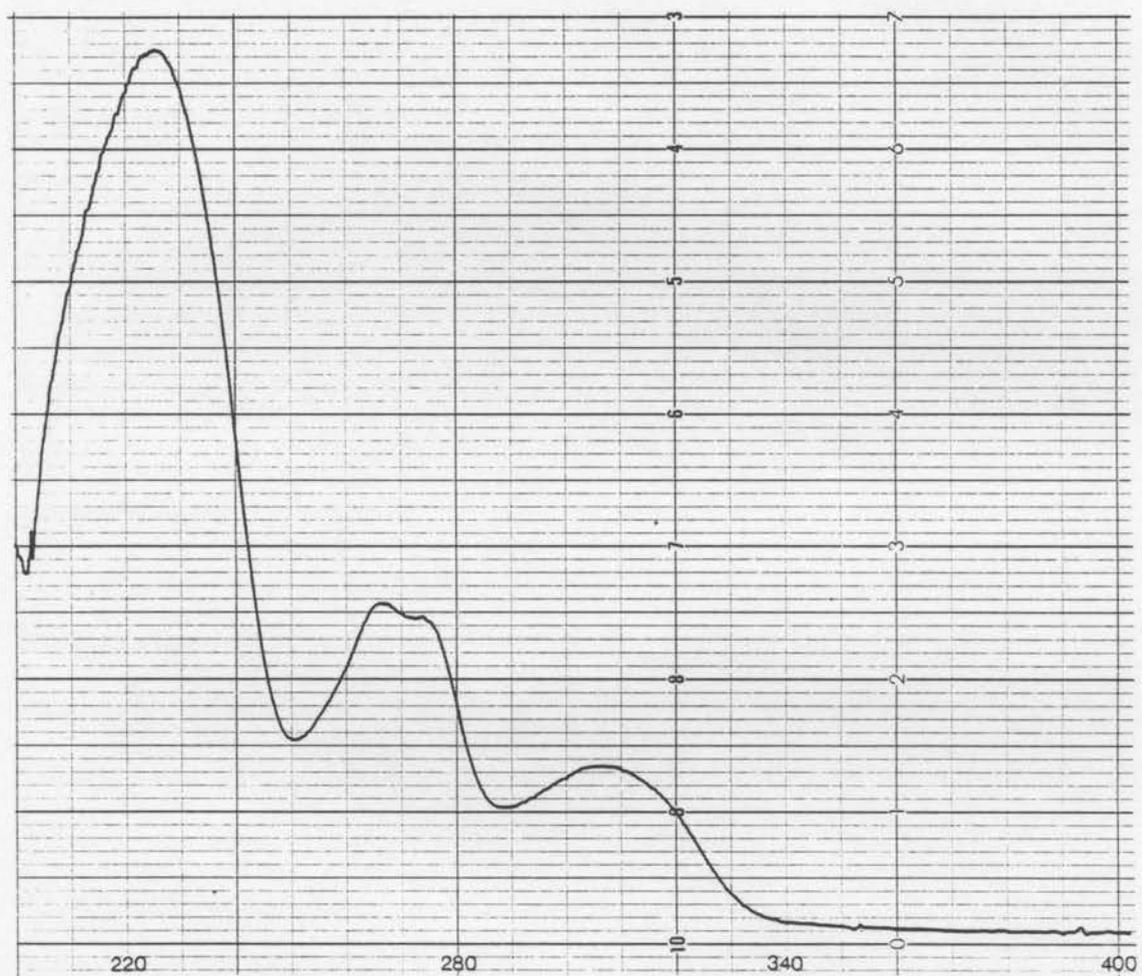
Figure 42a   UV (EtOH) of N-Methylisocorytuberine
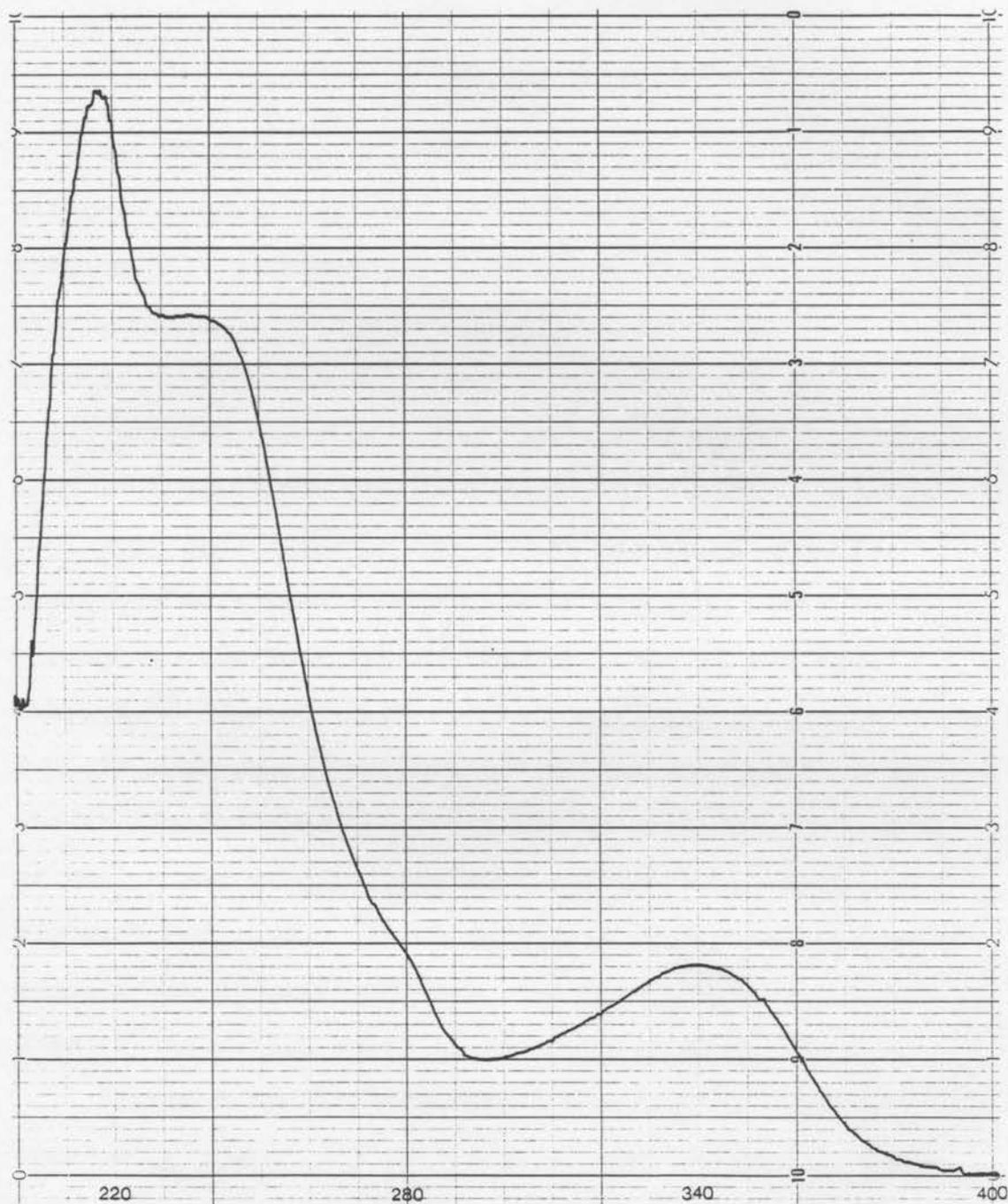
Figure 42b UV (EtOH + KOH) of N-Methylisocorytuberine

CHAPTER V

THE CONSTITUENTS OF <u>ONCIDIUM</u> <u>CEBOLLETA</u>

I. <u>Introduction</u>

The Orchidaceae is the largest family of flowering
plants. Orchids are very widespread, being abundant in the
tropics, but increasingly less common in temperate and sub-
arctic regions. There are an estimated 25,000 species of
orchids divided into 750 genera, although new species are
still being discovered. The family is economically impor-
tant as ornamentals and as a source of vanilla flavoring.

<u>Oncidium</u> is one of the larger genera of orchids.
There are more than 400 species of <u>Oncidium</u> distributed
throughout the West Indies, Mexico, Central and South
America.[88] One of these species, <u>Oncidium</u> <u>cebolleta</u> (Jacq.)
Sw., is interesting because of its use as a hallucinogen.
The Tarahumara Indians of the Rio Chínipas barranca area
of Mexico use <u>O</u>. <u>cebolleta</u> as an important temporary re-
placement of peyote.[89] <u>O</u>. <u>cebolleta</u> is not only found in
Mexico, but it also occurs in the West Indies, Central
America and as far south as Paraguay in South America.

Although the hallucinogenic agent of <u>O</u>. <u>cebolleta</u> is
unknown, <u>O</u>. <u>cebolleta</u> and 20 other species of <u>Oncidium</u> are
reported to contain unknown alkaloids.[90]

II. Results

A. Plant Material, Preliminary Extraction and Alkaloid
   Testing

O. cebolleta was collected in 1978 and identified by
Robert A. Bye, Jr., Department of Environmental, Population
and Organismic Biology, University of Colorado, while in
the Rio Chínipas region of Mexico. The plant material was
allowed to air dry before being cut into small pieces.

As a preliminary test for alkaloids, 5 g of the dried
plant material was extracted successively in a Soxhlet
apparatus with hexane, chloroform and methanol. An acid-
base extraction was performed on the chloroform and methanol
extracts. The combined chloroform and n-butanol extracts
of the basic aqueous solution were chromatographed on Si
gel tlc plates. Visualization of the tlc plates with iodo-
platinic acid revealed the presence of three alkaloid
positive spots in the methanol extract at R_f 0.45, 0.53 and
0.66 in toluene:n-butanol:acetic acid:water (15:18:10:5).
The hexane and chloroform extracts did not show an alkaloid
positive reaction.

Since alkaloids were found in the small scale extrac-
tion by tlc, 114 g of O. cebolleta were extracted with 2.5 l
each of hexane, chloroform and methanol in a Soxhlet appar-
atus. Evaporation of the extracts provided 3.1 g of hexane
soluble material, 4.0 g of chloroform soluble material and
4.3 g of methanol soluble material.

B. Attempted Isolation of the Alkaloidal Material from the
   Methanol Extract

The 4.3 g of methanol soluble material were dissolved
in 150 ml of 2 N sulfuric acid and extracted with chloro-
form. The acidic aqueous phase was then made basic to pH 9
with ammonium hydroxide and extracted twice with 100 ml of
chloroform, and then three times with 150 ml of n-butanol.
The chloroform and n-butanol extracts were concentrated and
spotted on a tlc plate. Development of the tlc plate and
visualization with iodoplatinic acid showed no alkaloid
positive spot in either extract.

C. Isolation of the Chloroform Soluble Constituents

1. Initial Column Chromatography

Since we were unable to isolate alkaloids from the
methanol extract, the constituents of the chloroform ex-
tract were examined. Three grams of the chloroform soluble
material were injected on a 37 by 350 mm Michel-Miller High
Performance Low Pressure Liquid Chromatography (HPLPLC)
column. The HPLPLC column was eluted with 33%, 50%, 67%
ethyl acetate in hexane, and finally with pure ethyl ace-
tate. Fractions were collected on the basis of UV analysis
at 280 nm. Fractions 1-22 were analyzed by tlc in hexane:
ethyl acetate (2:1). On this basis, fractions 14-18 were
combined, but showed only nonaromatic protons. These
fractions were not explored further. Fractions 22-43 were
developed in hexane:ethyl acetate (2:1). Fractions 25 and

26 had the same R_f value and were combined to give 84 mg of material. Fractions 27, 28 and 29 were also similar, so they were combined to give 141 mg.

2. Isolation of the Components from Fractions 27-29

The 141 mg from fractions 27-29 were injected on a 22 by 130 mm Michel-Miller HPLPLC column and eluted with chloroform. Fractions 4 to 7 were combined and chromatographed on a 0.25 mm Si gel prep. plate to give 16 mg of TS-0-9-13. An NMR ($CDCl_3$) (Figure 43) showed peaks at 3.95 (s, 3H, $-OCH_3$), 4.05 (s, 3H, $-OCH_3$), 4.08 (s, 3H, $-OCH_3$), 5.87 (bs, 1H), 5.90 (bs, 1H), 7.13 (s, 1H), 7.22 (s, 1H), 7.45 (s, 2H) and 9.00 (s, 1H). The two peaks at 5.87 and 5.90 disappeared after addition of D_2O. The IR ($CHCl_3$) showed the presence of a hydroxyl function with peaks at 3480 and 3400, along with other peaks at 2290, 2910, 2825, 1610, 1562, 1500, 1470, 1260, 1205 and 1105 cm^{-1}. The UV (EtOH) (Figure 44) showed λ max 361, 344, 328, 309 (sh), 298 (sh), 289 (sh), 284, 261, 240 (sh), 218 and 205 nm. Addition of base caused a bathochromic shift to λ max 385, 368, 325, 311, 273 and 219 nm, confirming the presence of a phenolic hydroxy function. The MS established a molecular weight of 300 (100%) and showed other peaks at 285 (45%) and 242 (31%). In a Nuclear Overhauser Effect (NOE) experiment, irradiation of the methoxy group at 4.08 ppm caused a 26% enhancement in the proton signal at 9.00 ppm, establishing their close proximity. TS-0-9-13 was acetylated by stirring in pyridine and acetic anhydride for 12 hr. Evaporation of
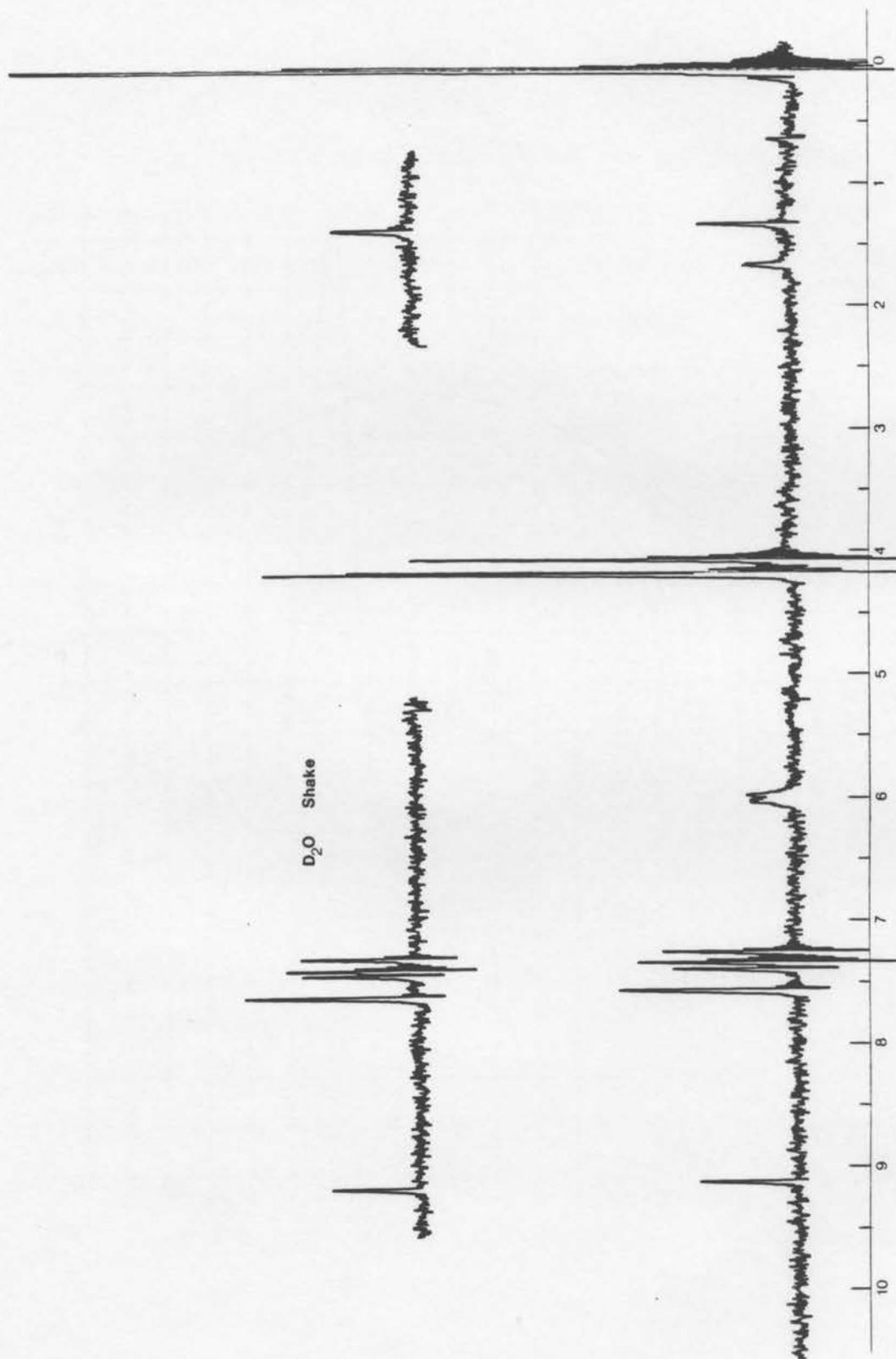
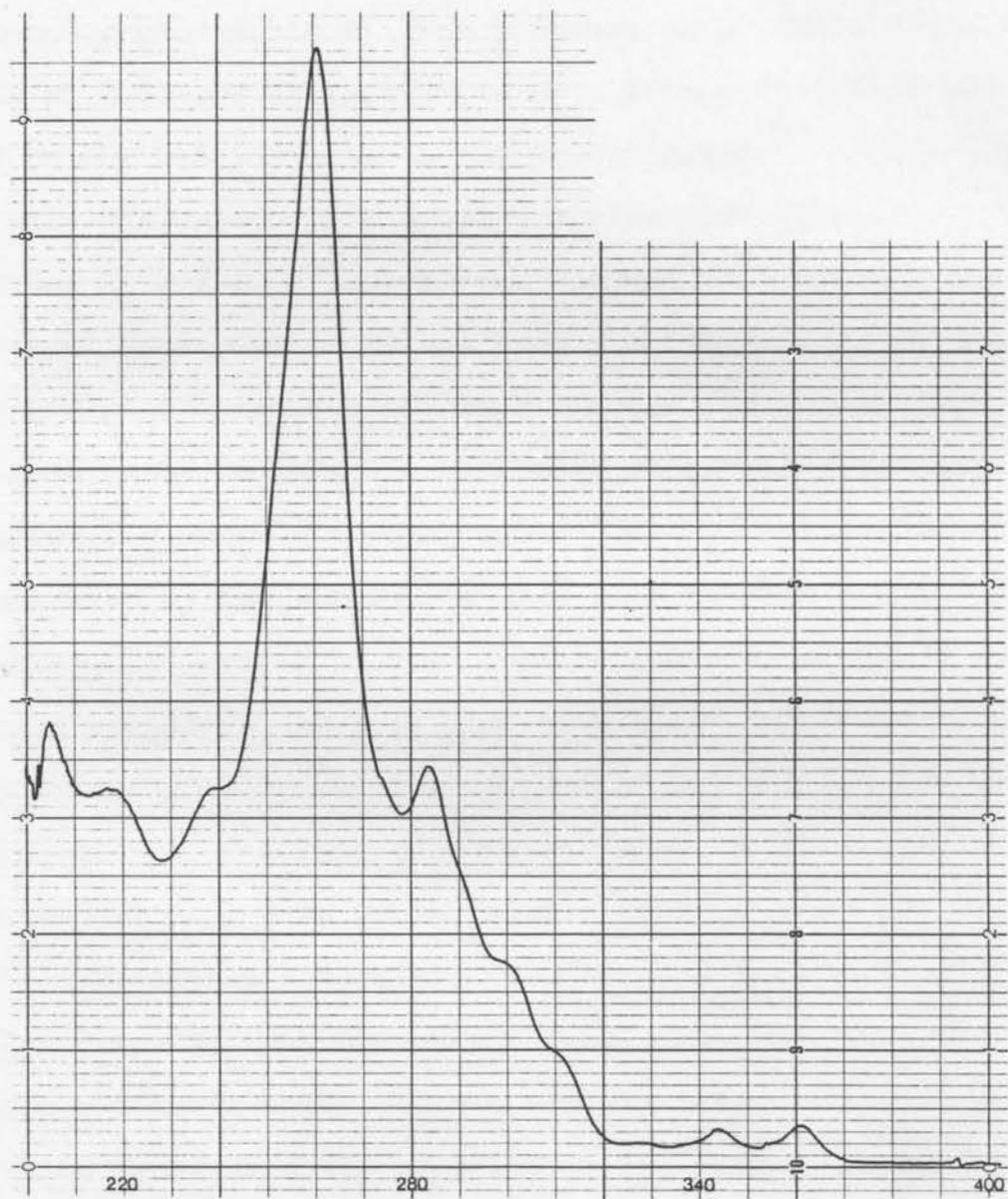Figure 43 NMR (CDCl$_3$) of TS-0-9-13

Figure 44   UV (EtOH) of TS-0-9-13

the solvent provided TS-0-9-13A whose NMR (CDCl$_3$) showed peaks at 2.40 (bs, 6H, OAc), 4.06 (bs, 9H, -OCH$_3$), 7.40 (s, 1H), 7.57 (bs, 3H) and 9.30 (s, 1H).

The UV and NMR data show TS-0-9-13 to be a dihydroxy-trimethoxyphenanthrene. The presence of a single downfield peak at 9.00 ppm is indicative of a proton at either the C-4 or the C-5 position on the phenanthrene.[92] Since there is only one proton, the other position must be either hydroxy or methoxy substituted. If C-4 is a hydroxy substituent, acetylation of the phenanthrene usually shifts the H-5 proton upfield by about 0.6 ppm.[93] Acetylation of TS-0-9-13 shifted the H-5 proton downfield to 9.30 ppm, meaning C-4 must be methoxy substituted. This was substantiated by the NOE enhancement of H-5. The H-9 and H-10 protons are present in most phenanthrenes as either a two proton singlet or as a pair of doublets. The presence of a two proton singlet usually means that C-1 and C-8 are unsubstituted, while a pair of doublets usually means that either C-1 or C-8 is substituted. In TS-0-9-13, H-9 and H-10 appeared as a singlet, meaning TS-0-9-13 is a 2,3,4,6,7-dihydroxytrimethoxyphenanthrene, with C-4 substituent being a methoxy group. There are six possible structures fitting these data (27-32 below). 27,[92], 28,[94a] 29[94b] and 30[94c] have all previously been isolated from Combretum sp.
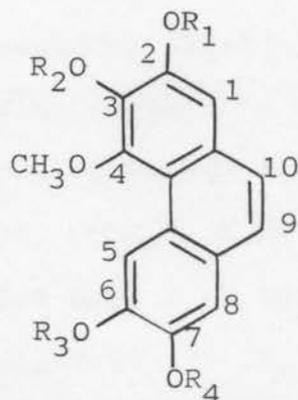
27 $R_1 = R_2 = CH_3$, $R_3 = R_4 = H$

28 $R_2 = R_4 = CH_3$, $R_1 = R_3 = H$

29 $R_2 = R_3 = CH_3$, $R_1 = R_4 = H$

30 $R_1 = R_4 = CH_3$, $R_2 = R_3 = H$

31 $R_1 = R_3 = CH_3$, $R_2 = R_4 = H$

32 $R_3 = R_4 = CH_3$, $R_1 = R_2 = H$

TS-0-9-13 did not correspond to any of the previously known phenanthrenes, so it must be either 31 or 32. Structure 31 has the same substituent pattern on the top ring as does 30. One would expect that if TS-0-9-13 were 31, the C-2 methoxy, the C-4 methoxy and H-1 chemical shifts would be the same as or very similar to those peaks in 30. That these peaks are not similar suggests that TS-0-9-13 is structure 32, 2,3-dihydroxy-4,6,7-trimethoxyphenanthrene. This assignment was reinforced by the reaction of TS-0-9-13 when sprayed with Chloramine-T.[95] TS-0-9-13 showed a reddish-purple color, which is probably due to the formation of the ortho quinone. Spraying TS-0-9-13 with ferric chloride produced a dark brown spot.

Fractions 18-20 were rechromatographed by Si gel plc using chloroform:methanol (25:1). The bottom band was removed and eluted with chloroform to give 10 mg of TS-0-11-17. An NMR (d_6-acetone)(Figure 45) showed peaks at 3.92 (s, 3H, -OCH_3), 4.09 (s, 3H, -OCH_3), 6.84 (d, 1H, J = 2.4 Hz), 6.92 (d, 1H, J = 2.4 Hz), 7.20 (d, 1H, J = 9.4 Hz), 7.57 (d, 1H, J = 9.1 Hz), 7.95 (d, 1H, J = 9.1 Hz) and 9.17 (d, 1H, J = 9.4 Hz). The UV (EtOH) (Figure 46) showed peaks at 327 (sh), 309, 295 (sh), 285 (sh), 263, 228 (sh) and 210 nm. Addition of base caused a bathochromic shift to 309 (sh), 299 (sh), 283, 269 and 220. The IR (neat) showed a broad peak at 3360 along with other peaks at 2920, 2840, 1610, 1465, 1350, 1300, 1205 and 1150. Acetylation of TS-0-11-17 provided TS-0-11-17A: NMR (d_6-acetone) 2.35 (s, 3H, -OAc), 2.39 (s, 3H, -OAc), 4.00 (s, 3H, -OCH_3), 4.18 (s, 3H, -OCH_3), 7.15 (d, 1H, J = 2.4 Hz), 7.41 (d, 1H, J = 2.4 Hz), 7.44 (d, 1H, J = 9.4 Hz), 7.84 (d, 1H, J = 9.1 Hz), 8.21 (d, 1H, J = 9.1 Hz) and 9.47 (d, 1H, J = 9.4 Hz). The molecular formula of $C_{20}H_{18}O_6$ for TS-0-11-17A was confirmed by an exact mass of 354.1096. The NMR showed the presence of two protons at 6.84 and 6.92 ppm which are _meta_ coupled, two protons at 7.20 and 9.17 which are _ortho_ coupled, and the final two aromatic protons at 7.57 and 7.95 ppm which are also _ortho_ coupled. There was only one proton in the downfield (H-4 or H-5) range, and this proton is _ortho_ coupled, meaning that there is an H-6 proton and that C-4 is substituted. Acetylation of TS-0-11-17 did not shift
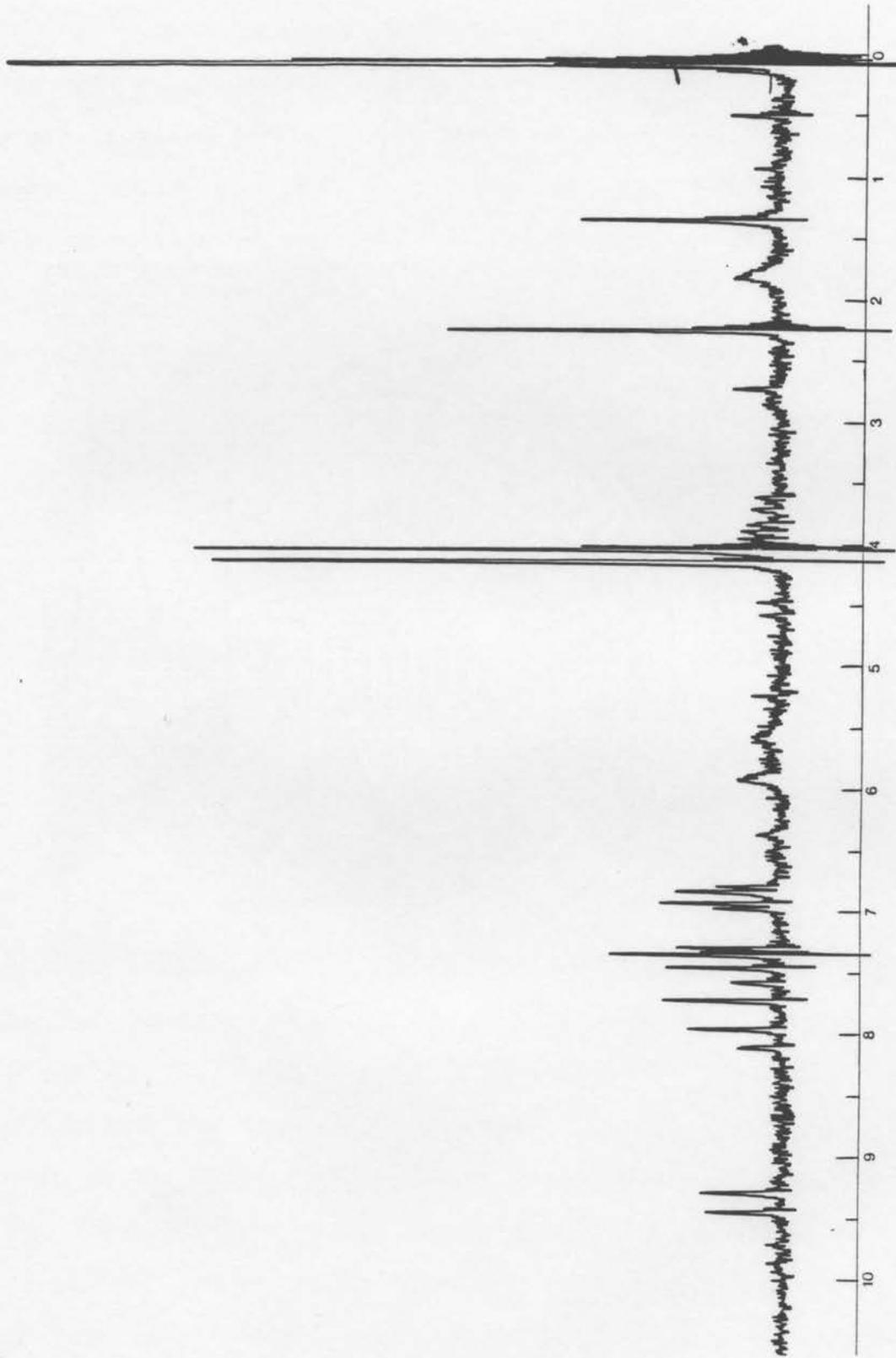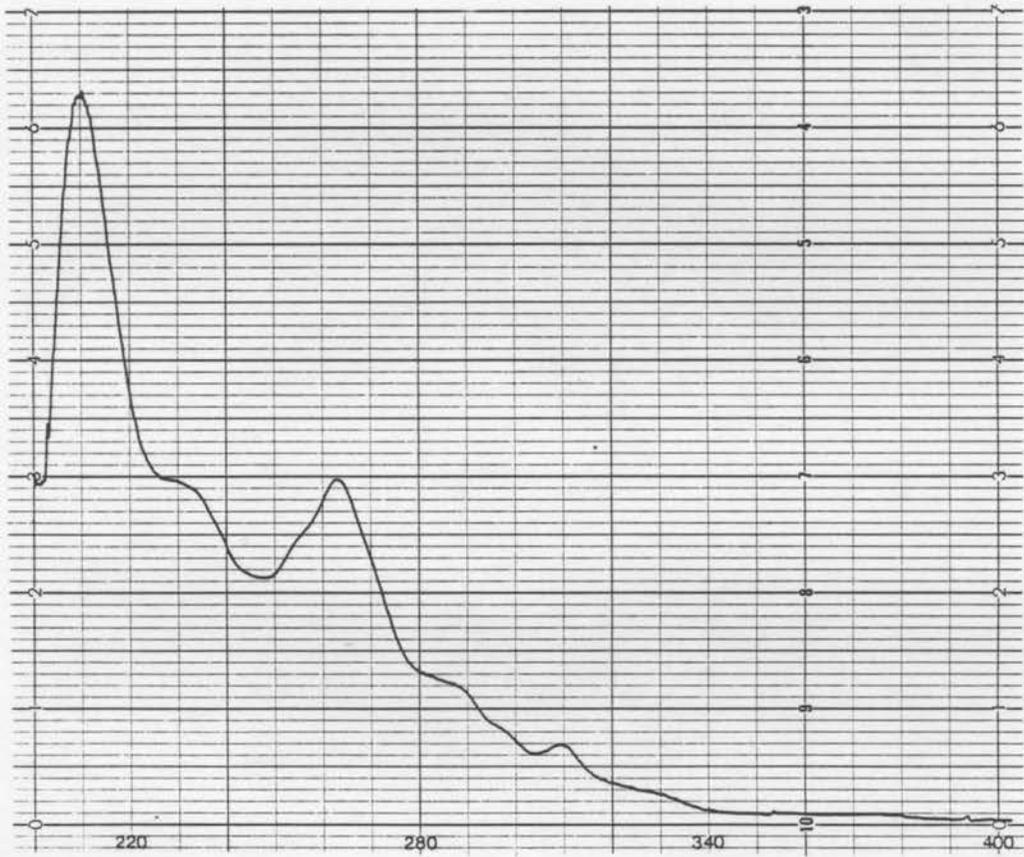
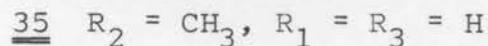Figure 45   NMR (d_6-acetone) of TS-0-11-17

Figure 46   UV (EtOH) of TS-0-11-17

the H-5 proton upfield, so C-4 is methoxy substituted. H-6
is not coupled to any protons except H-5, so C-7 and C-8
are hydroxy or methoxy substituted. This leads to the
ortho coupling shown by H-9 and H-10 (7.57 and 7.95). The
other two protons at 6.84 and 6.92 are meta coupled, imply-
ing TS-0-11-17 is a 2,4,7,8-tetrasubstituted phenanthrene.
Placement of one methoxy substituent at C-4 gave three
possible structures for TS-0-11-17 (33-35 below). The

$\underline{33}$ $R_1 = CH_3$, $R_2 = R_3 = H$

$\underline{34}$ $R_3 = CH_3$, $R_1 = R_2 = H$

$\underline{35}$ $R_2 = CH_3$, $R_1 = R_3 = H$
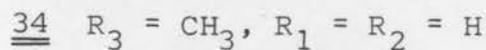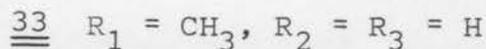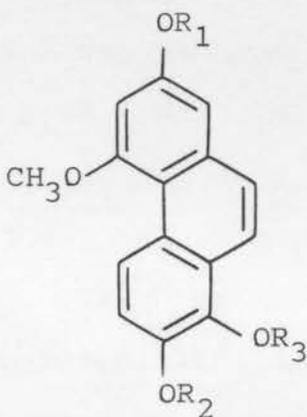
structure of TS-0-11-17 could not be decided on the basis
of the spectral data, but TS-0-11-17 sprayed a light yellow-
green with Chloramine-T and a purple-brown color with ferric
chloride. The absence of a strong color reaction with
Chloramine-T made $\underline{33}$ unlikely. 2,7-Dihydroxyphenanthrene
was synthesized (see D), and showed no color reaction with
ferric chloride. Since $\underline{34}$ should have color reactions
similar to 2,7-dihydroxyphenanthrene, and since TS-0-11-17
sprayed brown with ferric chloride, $\underline{35}$

(2,8-dihydroxy-4,7-dimethoxyphenanthrene) is the most likely structure for TS-O-11-17.

3. Isolation of the Components from Fractions 25 and 26

Fractions 25-26 showed three distinct spots on tlc. The 84 mg of material were chromatographed on a 22 by 130 mm Michel-Miller HPLPLC column using chloroform as the eluent. Fractions were collected on the basis of UV analysis at 280 nm. Fractions 8-12 were combined to give 25 mg (TS-O-6-5). TS-O-6-5 was purified by plc with chloroform: methanol (24:1) to give 8 mg: NMR $(CDCl_3)$(Figure 47) 3.97 (s, 3H, $-OCH_3$), 4.10 (s, 3H, $-OCH_3$), 7.17 (s, 1H), 7.17 (dd, 1H, J = 10 Hz and J = 1 Hz), 7.26 (d, 1H, J = 1 Hz), 7.50 (s, 2H) and 9.35 (d, 1H, J = 10 Hz). The UV (EtOH)(Figure 48) showed peaks at λmax 365, 347, 330, 304, 293 (sh), 284, 259, 232 and 211. Addition of base caused a bathochromic shift to λmax 393, 376, 320, 307, 270 and 217. The IR (neat) showed a broad peak at 3360 with other peaks appearing at 2920, 1610, 1500, 1455, 1280 and 1215. The MS established a molecular weight of 270 (100%) with other peaks at 255 (42%) and 212 (40%). The molecular formula, $C_{15}H_{14}O_4$, was confirmed by an exact mass of 270.0920 $\pm$ 0.0040. TS-O-6-5 was acetylated to form TS-O-6-5A. A 360 MHz NMR $(CDCl_3)$ of TS-O-6-5A showed peaks at 2.39 (s, 3H, OAc), 2.42 (s, 3H, -OAc), 4.02 (s, 3H, $-OCH_3$), 4.05 (s, 3H, $-OCH_3$), 7.38 (s, 1H), 7.38 (dd, 1H, J = 10 Hz and J = 1 Hz), 7.58 (d, 1H, J = 1 Hz), 7.60 (s, 2H), 9.59 (d, 1H, J = 10 Hz). In an NOE experiment, each of the methoxy and acetoxy resonances were singly
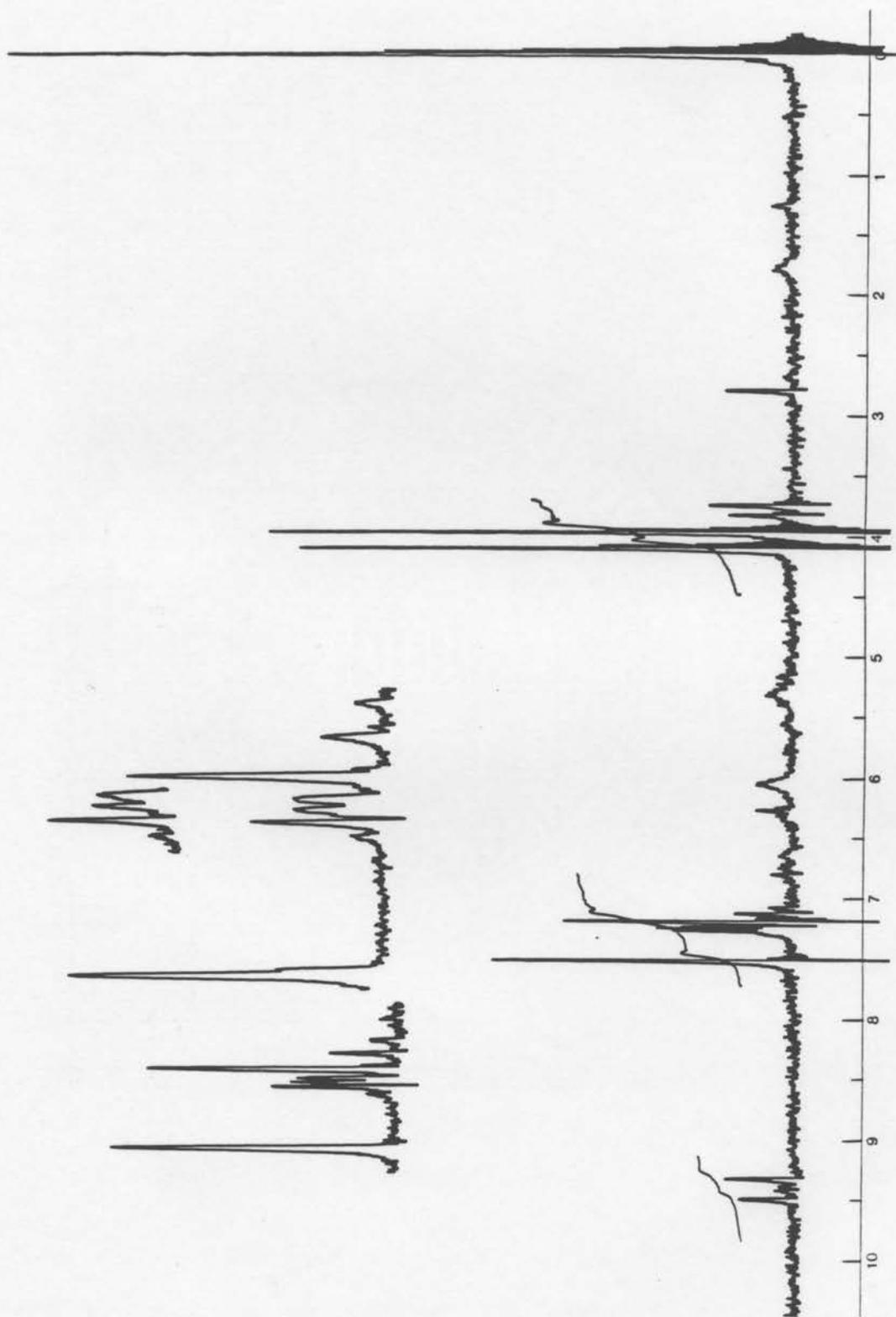
Figure 47 NMR (CDCl$_3$) of TS-0-6-5
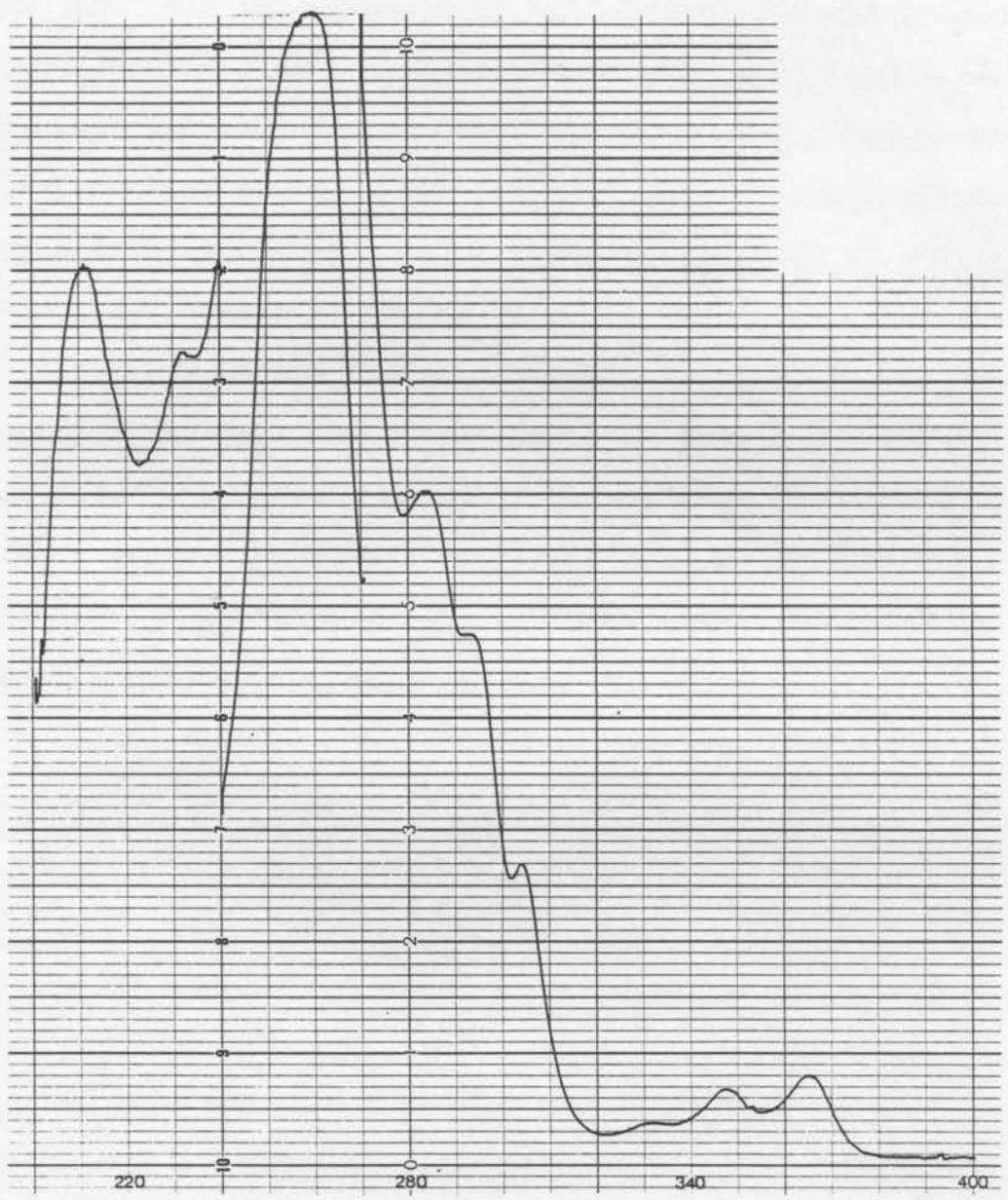
Figure 48   UV (EtOH) of TS-0-6-5

irradiated. Irradiation of the high field methoxy, 4.02
ppm, showed a 15% enhancement of the proton resonance at
9.59 ppm. The NMR spectrum of TS-0-6-5 showed the presence
of small amounts of another compound with a peak at 2.77
ppm, indicative of a 9,10-dihydrophenanthrene. The presence
of two methoxy peaks in the impurity and the inability to
separate the impurity on tlc indicated it might be the 9,10-
dihydro derivative of TS-0-6-5.

Analysis of the NMR spectrum of TS-0-6-5 in the same
manner as the previous phenanthrenes, identified TS-0-6-5
as a 2,3,4,7-dihydroxydimethoxyphenanthrene with a C-4
methoxy group. There are three possible structures for
TS-0-6-5 (36-38 below). 36, 3,7-dihydroxy-2,4-dimethoxy-
phenanthrene, was synthesized (see E) and compared to

36 R_1 = CH_3, R_2 = R_3 = H

37 R_3 = CH_3, R_1 = R_2 = H

38 R_2 = CH_3, R_1 = R_3 = H
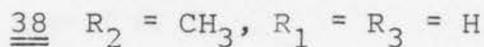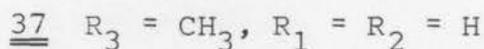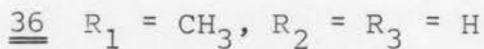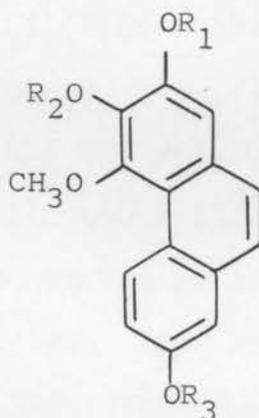
TS-0-6-5. Differences in the R_f values and the chemical
shifts in the NMR spectrum proved these two compounds to be
nonidentical. 37 also seemed unlikely, because TS-0-6-5

sprayed a light brown color with Chloramine-T, and the ortho-dihydroxy compounds spray much darker. The assignment of TS-0-6-5 as 38 (2,7-dihydroxy-3,4-dimethoxyphenanthrene) is reinforced by the color results when the tlc plate was sprayed with ferric chloride. 2,7-dihydroxyphenanthrene has the same phenolic substituent pattern as does 38, and neither showed a color reaction when sprayed with ferric chloride.

Fractions 3-7 from the 22 by 130 mm Michel-Miller column were recombined and purified by plc. Development of the plc plate in chloroform provided two main bands. The lower band was extracted with chloroform to provide 4 mg of TS-0-6-14. The higher R_f band was extracted with chloroform and provided 7 mg of TS-0-6-36.

An NMR ($CDCl_3$) (Figure 49) of TS-0-6-14 showed peaks at 3.96 (s, 6H, $-OCH_3$), 4.10 (s, 3H, $-OCH_3$), 7.18 (s, 1H), 7.28 (d, 1H, J = 9.4 Hz) and 7.58 (d, 1H, J = 9.4 Hz), 7.85 (d, 1H, J = 9.4 Hz), and 9.18 (d, 1H, J = 9.4 Hz). The UV (EtOH) (Figure 50) showed peaks at λ max 367, 349, 309, 295, 287, 265 and 216. Upon addition of base the spectrum shifted to λ max 312, 282 (sh), 274 and 224. The IR (neat) showed a broad peak at 3360 with additional peaks at 2920, 2840, 1600, 1470, 1300 and 1210. The MS showed a molecular weight of 300. Acetylation of TS-0-6-14 provided TS-0-6-14A whose NMR ($CDCl_3$) showed peaks at 2.40 (bs, 6H, -OAc), 4.02 (s, 6H, $-OCH_3$), 4.04 (s, 3H, $-OCH_3$), 7.42 (d, 1H, J = 9 Hz), 7.45 (s, 1H), 7.70 (d, 1H, J = 8 Hz), 8.12 (d, 1H, J = 8 Hz)
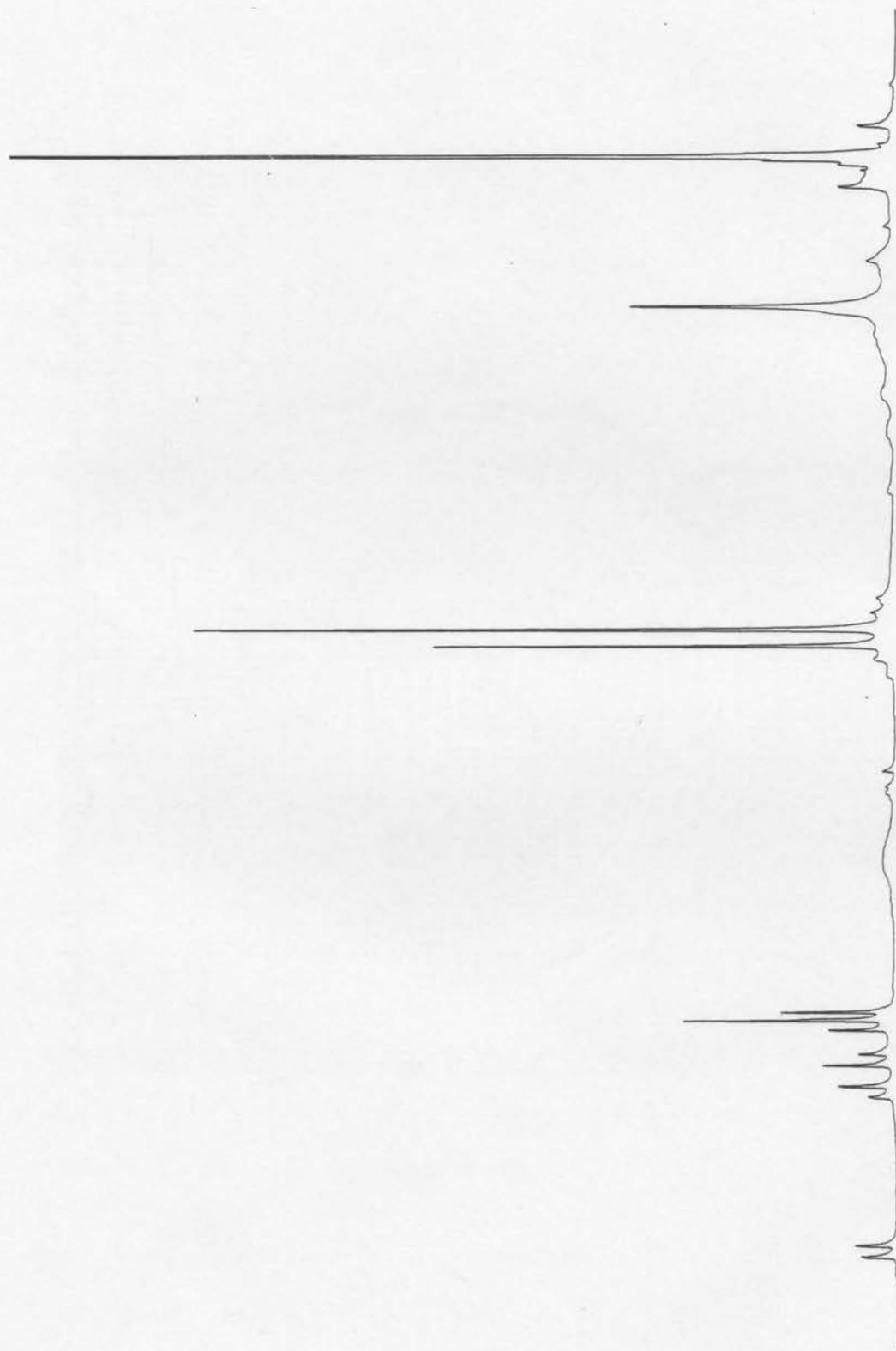
Figure 49   100 MHz NMR (CDCl$_3$) of TS-0-6-14
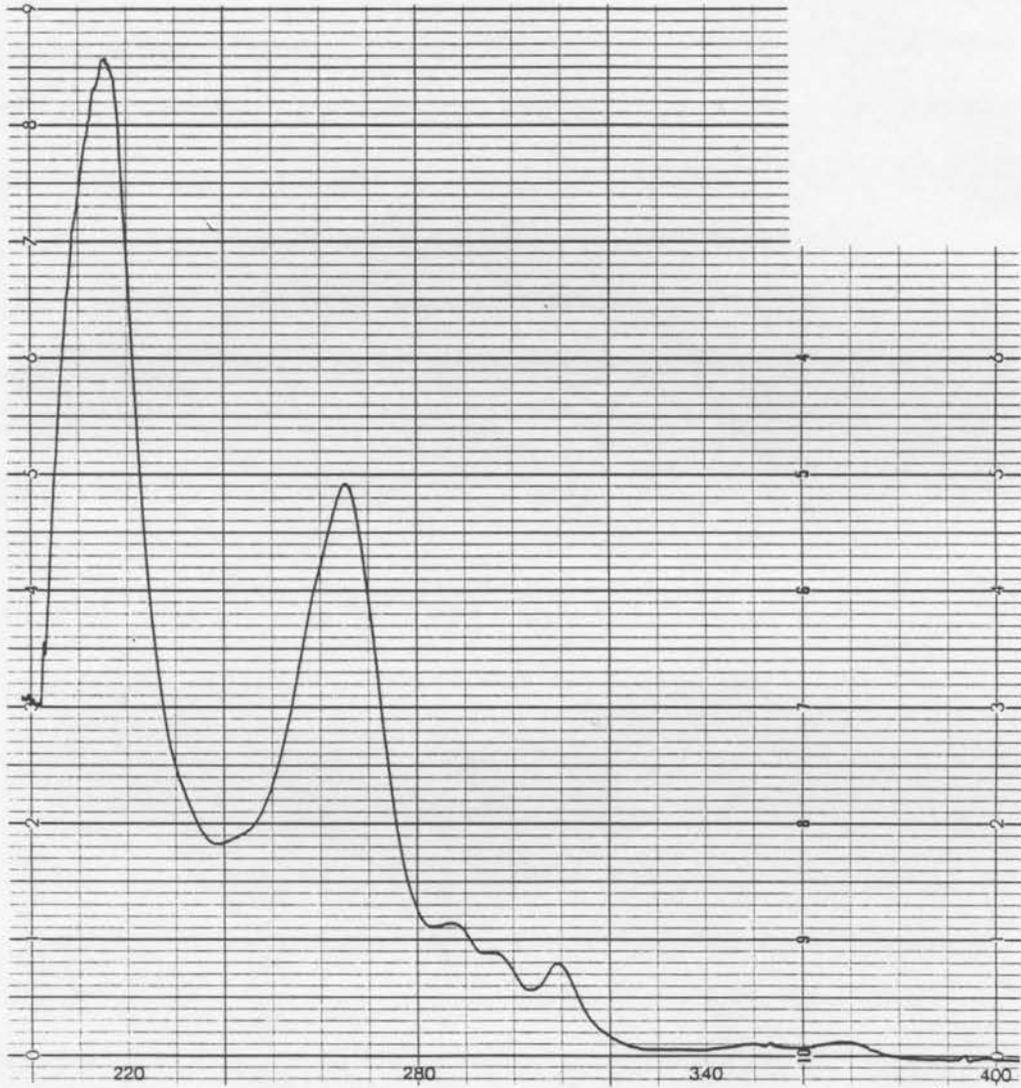
Figure 50 UV (EtOH) of TS-0-6-14

and 9.44 (d, 1H, J = 9 Hz). The NMR showed the presence of an H-5 proton at 9.18, which is <u>ortho</u> coupled, indicating the presence of a proton at H-6. The proton at H-5 did not shift upfield upon acetylation, indicating that C-4 is methoxy substituted. The presence of a pair of doublets at 7.58 and 7.85 indicated that H-9 and H-10 are not equivalent, and therefore TS-0-6-14 is a 2,3,4,7,8-dihydroxytrimethoxyphenanthrene with a methoxy substituent at C-4. There are six possible structures based on these data - 39-44 below. Structures 39 and 40 are known, and their NMR

$\underline{39}$ $R_3 = R_4 = CH_3$, $R_1 = R_2 = H$

$\underline{40}$ $R_1 = R_2 = CH_3$, $R_3 = R_4 = H$

$\underline{41}$ $R_2 = R_4 = CH_3$, $R_1 = R_3 = H$

$\underline{42}$ $R_1 = R_4 = CH_3$, $R_2 = R_3 = H$

$\underline{43}$ $R_1 = R_3 = CH_3$, $R_2 = R_4 = H$

$\underline{44}$ $R_2 = R_3 = CH_3$, $R_1 = R_4 = H$
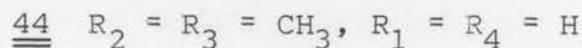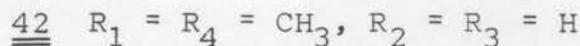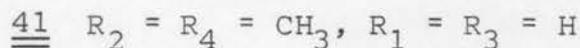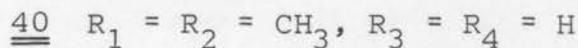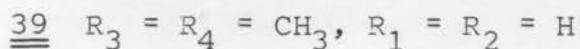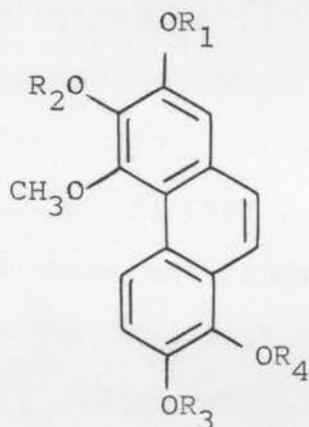
data do not correspond to TS-0-6-14. Structure 41 is again similar to 2,7-dihydroxyphenanthrene which does not show a color when sprayed with ferric chloride, whereas TS-0-6-14 shows a reddish-brown color when sprayed. This makes 41

an unlikely structure, but means that TS-0-6-14 may be <u>42</u> (3,7-dihydroxy-2,4,8-trimethoxyphenanthrene), <u>43</u> (3,8-dihydroxy-2,4,6-trimethoxyphenanthrene) or <u>44</u> (2,8-dihydroxy-3,4,7-trimethoxyphenanthrene).

An NMR ($CDCl_3$) (Figure 51) of TS-0-6-36 showed peaks at 2.63 (s, 4H), 3.73 (s, 3H, $-OCH_3$), 3.92 (s, 3H, $-OCH_3$), 3.97 (s, 3H, $-OCH_3$), 6.60 (s, 1H), 6.77 (s, 1H) and 7.90 (s, 1H). The UV (EtOH) (Figure 52) showed peaks at λ max 315, 304 (sh), 282 and 216 nm. Addition of base shifted the spectrum to λ max 331 (sh), 320 (sh), 308 and 223 nm. An IR ($CDCl_3$) showed a broad peak at 3400, along with peaks at 2920, 2820, 1590, 1510, 1280 and 1115 cm^{-1}. An MS showed M+ 302 (46%), 287 (4%), 255 (8%), 199 (37%), 184 (33%), 155 (59%), 91 (100%) and 83 (99%). Acetylation of TS-0-6-36 provided TS-0-6-36A whose NMR ($CDCl_3$) showed peaks at 2.35 (s, 6H, -OAc), 2.72 (s, 4H), 3.77 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$), 3.92 (s, 3H, $-OCH_3$), 6.70 (s, 1H), 6.87 (s, 1H) and 8.03 (s, 1H). The molecular formula, $C_{21}H_{22}O_7$, of TS-0-6-36A was confirmed by an exact mass peak at 386.1355. The UV spectrum and the four proton singlet at 2.63 ppm identified TS-0-6-36 as a 9,10-dihydrophenanthrene. The one proton singlet at 7.90 was indicative of the deshielded H-5 proton of dihydrophenanthrenes. The presence of the two other aromatic protons as singlets, along with the four proton singlet of the C-9 and C-10 protons, establishing this compound as a 2,3,4,6,7-dihydroxydimethoxy-9,10-dihydrophenanthrene. Since the H-5 proton did not
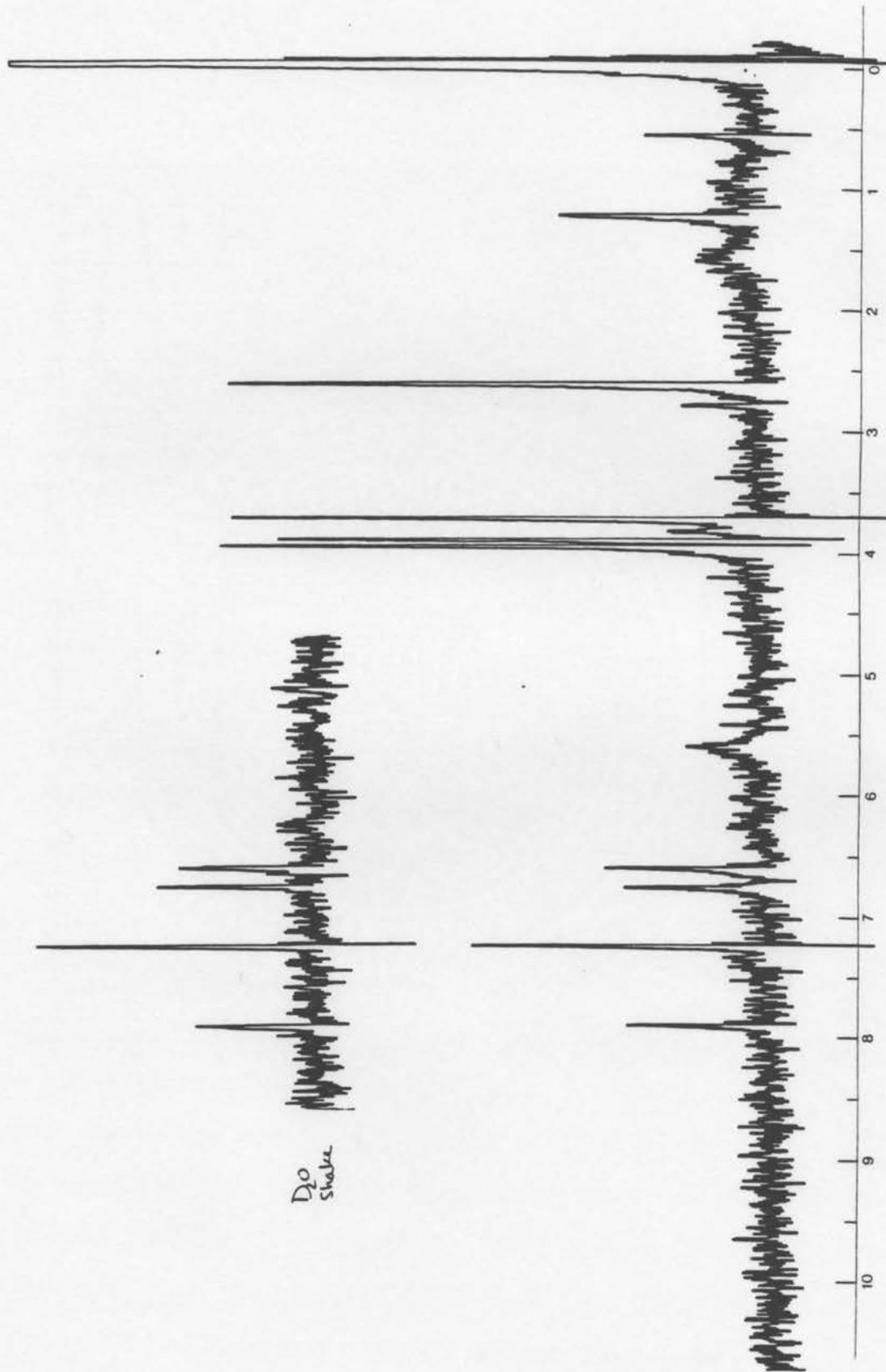
Figure 51 NMR (CDCl$_3$) of TS-0-6-35
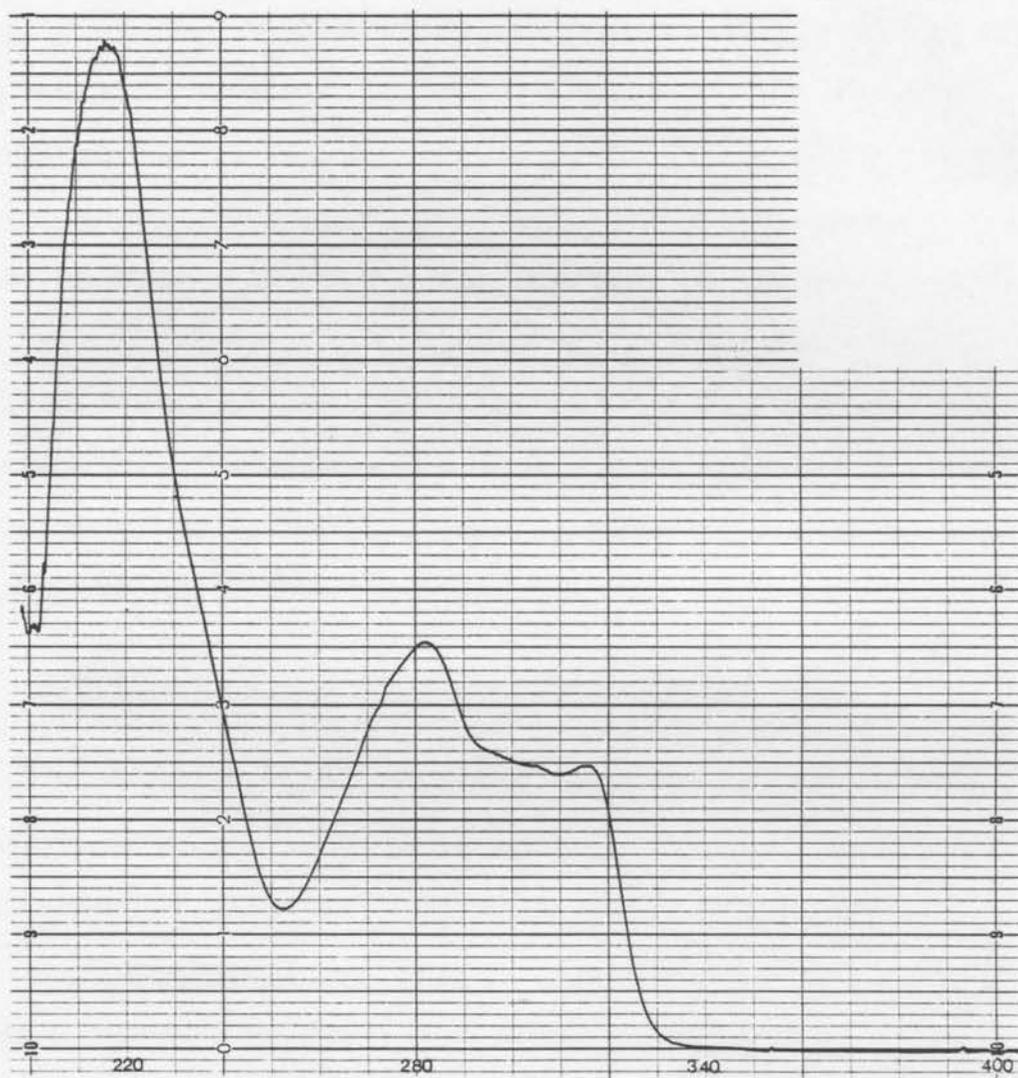
Figure 52   UV (EtOH) of TS-0-6-36

shift upfield when TS-0-6-36 was acetylated, the C-4 sub-
stituent is most likely a methoxy. This means that TS-0-9-36
has the same substituent pattern as does TS-0-9-13, except
that it is a 9,10-dihydrophenanthrene. As in the case of
TS-0-9-13, the 9,10-dihydro derivatives of <u>27</u>, <u>28</u>, <u>29</u> and
<u>30</u> are all known and do not correspond to the spectra
obtained for TS-0-6-36. Since TS-0-6-36 turned a reddish
color on Si gel in air, when exposed to iodine vapor, and
when sprayed with either Chloroamin-T or ferric chloride,
it is most likely 2,3-dihydroxy-9,10-dihydro-4,6,7-tri-
methoxyphenanthrene, the 9,10-dihydro derivative of
TS-0-9-13. The production of a phenanthrene along with the
corresponding 9,10-dihydrophenanthrene is common in many
<u>Combretum</u> <u>sp</u>.[92,94]

D. Synthesis of 2,7-Dihydroxyphenanthrene (<u>52</u>)

2,7-Dihydroxyphenanthrene, synthesized previously,[96]
was prepared in order to determine its color reactions with
Chloromine-T and ferric chloride spray reagents.

2-Isopropoxybenzaldehyde (<u>46</u>). 12.2 g (0.1 mol) of
2-hydroxybenzaldehyde (<u>45</u>) (Aldrich Chemical Co.), 40 g
of potassium carbonate and 40 ml of 2-bromopropane were
added to 40 ml of DMF and heated at reflux for 2.5 hr.[97]
The solution was then cooled, poured into 200 ml of water
and extracted with chloroform. Evaporation of the chloro-
form provided 14.5 g (94% yield) of <u>46</u>: NMR (CDCl$_3$) 1.35
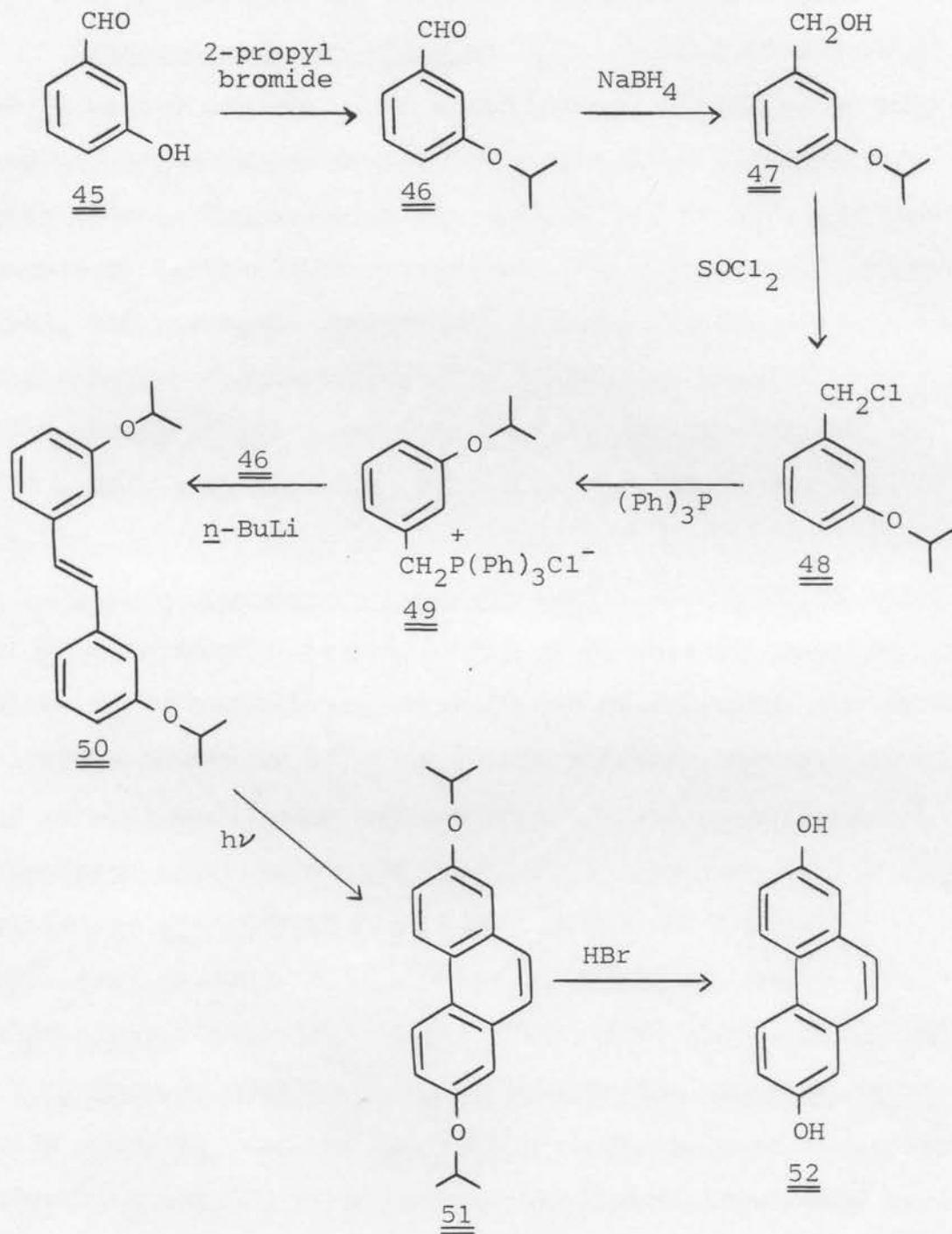
Figure 53   Synthetic Scheme for the Preparation
of 3,7-Dihydroxyphenanthrene

$\angle$d, 6H, -OCH(CH$_3$)$_2$, J = 6 Hz$\angle$, 4.62 $\angle$septet, 1H, -OCH(CH$_3$)$_2$, J = 6 Hz$\angle$, 7.03-7.5 (m, 4H, Ar), 10.03 (s, 1H, -CHO).

2-Isopropoxybenzyl alcohol (47). 10.0 g (0.061 mol) of 46 were dissolved in 75 ml of ethanol and 90 ml of 9% sodium hydroxide containing 9.0 g of sodium borohydride were added. The solution was stirred for 30 min, and then heated on a steam bath for 45 min. The ethanol was evaporated, and the basic aqueous solution was extracted with chloroform. The chloroform was evaporated to give 10.0 g (98% yield) of 47: NMR (CDCl$_3$) 1.32 $\angle$d, 6H, -OCH(CH$_3$)$_2$, J = 6 Hz$\angle$, 3.33 (bs, 1H, -OH), 4.57 (m, 3H), 6.76-7.43 (m, 4H, Ar).

2-Isopropoxybenzyl chloride (48). 10.0 g (0.06 mol) of 47 were added dropwise to 5.8 ml of thionyl chloride with ice bath cooling. After 30 min of stirring, the solution was heated to 50°C for 30 min and then cooled. 10 ml of ethanol were added and the solution was evaporated. Kugelrohr distillation (0.3 mm, 120°C) provided 6.34 g (56% yield) of 48: NMR (CDCl$_3$) 1.39 $\angle$d, 6H, -OCH(CH$_3$)$_2$, J = 6 Hz$\angle$, 4.47 (m, 3H), 6.60-7.30 (m, 4H, Ar); IR (neat) 2960, 2920, 1595, 1580, 1485, 1445, 1265, 1110, 1000 and 705 cm^{-1}.

2-Isopropoxybenzyltriphenylphosphonium chloride (49). 6.34 g (0.034 mol) of 48, 15.7 g (0.06 mol) of triphenylphosphine and 100 ml of xylenes were added to a round bottomed flask and the solution heated at reflux for 18 hr.[98] The solution was cooled to room temperature and filtered.

The filtrate was washed with xylenes and dried to give 9.8 g (69% yield) of 49.

3,3'-Diisopropoxystilbene (50). 20 ml of tetrahydrofuran and 4.11 g (9.2 mmol) of 49 were placed in a flame dried round bottomed flask with an argon atmosphere. The solution was cooled to $0^{\circ}C$ in an ice bath, and stirring was maintained while 11 mmol of n-butyllithium were added dropwise. The red solution was allowed to warm to room temperature and stirred for 30 min, at which time it was again cooled to $0^{\circ}C$ and 1.82 g (11 mmol) of 46 were added dropwise. After addition was complete, the solution was allowed to warm to room temperature and stirred overnight. The solution was quenched with 1 M hydrochloric acid, and partitioned between water and diethyl ether. The ether solution was evaporated, and the oil chromatographed with chloroform to provide 2.27 g (83% yield) of 50: NMR (CDCl$_3$) 1.22 (d, 3H, J = 6 Hz), 1.36 (d, 3H, J = 6 Hz), 4.13-4.83 (m, 2H), 6.60-7.45 (m, 8H, Ar).

2,7-Diisopropoxyphenanthrene (51). 0.5 g (1.7 mmol) of 50 and 0.02 g of iodine were dissolved in 340 ml of cyclohexane and irradiated for 6 hr in a quartz immersion well equipped with a 450 amp UV light source, a Corex filter and a water cooling jacket.[99] Evaporation of the cyclohexane and purification by flash chromatography with hexane: ethyl acetate (4:1) provided 51: NMR (CDCl$_3$) 1.42 $\angle$d, 12H, -OCH(CH$_3$)$_2$, J = 7 Hz$\angle$, 5.72 $\angle$septet, 2H, -OCH(CH$_3$)$_2$, J = 7 Hz$\angle$, 7.15-7.37 (m, 4H, H-1, H-3, H-6 and H-8), 7.63

(s, 2H, H-9 and H-10), 8.50 (d, 2H, H-4 and H-5, J = 9.5 Hz).

2,7-Dihydroxyphenanthrene (52). To a solution of 51 in 10 ml of acetic acid was added 1 ml of 48% hydrobromic acid.[97] The solution was heated for 3 hr, at which time it was diluted with water and extracted with diethyl ether. The ether solution was concentrated and chromatographed to give 65 mg (4% yield from the stilbene) of 2,7-dihydroxy-phenanthrene: NMR (d_6-acetone) 7.13 (m, 4H), 7.62 (s, 2H, H-9 and H-10), 8.53 (d, 2H, H-4 and H-5, J = 10 Hz).

E. Synthesis of 3,7-Dihydroxy-2,4-dimethoxyphenanthrene (57)

4-Isopropoxy-3,5-dimethoxybenzaldehyde (54). 2.5 g (13.7 mmol) of syringaldehyde (53) (Aldrich Chemical Co.), 5.7 g of potassium carbonate and 5.15 ml of 2-bromopropane were added to 20 ml of DMF. The stirred solution was heated at reflux for 2.5 hr. The solution was cooled and then partitioned between chloroform and water. Evaporation of the chloroform provided 2.81 g (91.5% yield) of 54: NMR (CDCl$_3$) 1.33 $\angle$d, 6H, -OCH(CH$_3$)$_2$, J = 6 Hz$\angle$, 3.92 (s, 6H, -OCH$_3$), 4.52 $\angle$septet, 1H, -OCH(CH$_3$)$_2$, J = 8 Hz$\angle$, 7.08 (s, 2H, Ar), 9.78 (s, 1H, -CHO).

3',4-Diisopropoxy-3,5-dimethoxystilbene (55). 4.18 g (9.3 mmol) of 49 and 20 ml of tetrahydrofuran were placed in a flame dried flask under an argon atmosphere. The solution was cooled to 0°C and 6.9 ml of n-butyllithium were added dropwise. The red solution was allowed to warm
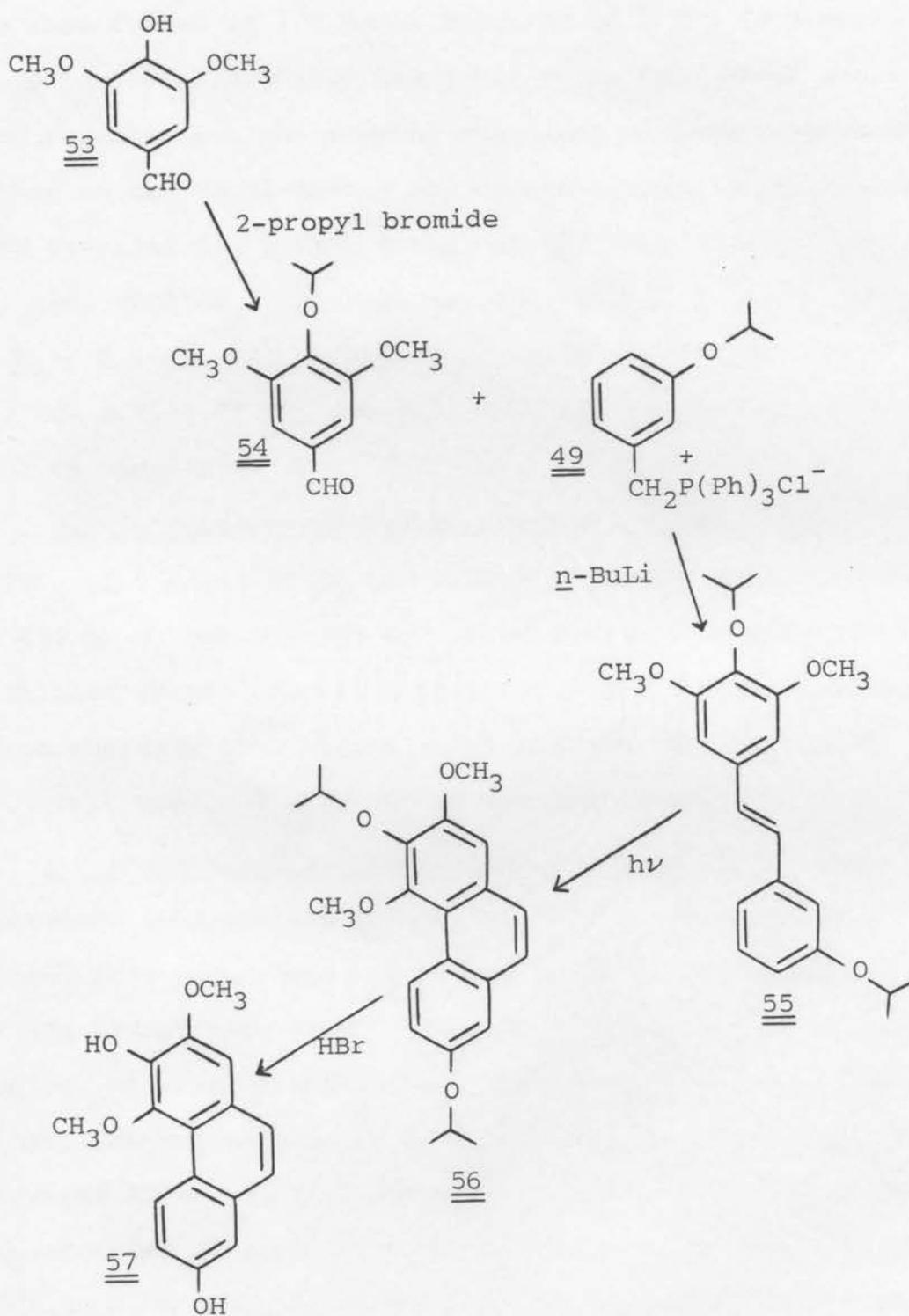
Figure 54   Synthetic Scheme for the Preparation

of 3,7-Dihydroxy-2,4-dimethoxyphenanthrene

to room temperature and stirred for 30 min. The solution
was then cooled to $0^{\circ}C$ and a solution of 2.0 g (8.9 mmol)
of <u>54</u> in tetrahydrofuran was added dropwise. After addi-
tion the solution was stirred overnight at room temperature.
Workup in the usual manner and chromatography with chloro-
form provided 2.2 g (70% yield) of <u>55</u>: NMR (CDCl$_3$) 1.30
$\angle$m, 12H, $-OCH(CH_3)_2\angle$, 3.66 (s, 3H, $-OCH_3$), 3.92 (s, 3H,
$-OCH_3$), 4.4 $\angle$m, 2H, $-OCH(CH_3)_2\angle$, 6.50 (s, 1H, Ar), 6.53
(s, 1H, Ar), 6.77 (s, 1H, Ar), 7.03 (s, 1H, Ar) and 6.77-
7.27 (m, 4H, Ar).

<u>3,7-Diisopropoxy-2,4-dimethoxyphenanthrene</u> (<u>56</u>).
0.71 g (2.0 mmol) of <u>55</u> and 0.02 g of iodine were dissolved
in 400 ml of cyclohexane and irradiated in the previously
mentioned quartz immersion cell for 4 hr. Evaporation and
chromatography with hexane:ethyl acetate (3:1) provided
<u>56</u>. This was used directly in the next step.

<u>3,7-Dihydroxy-2,4-dimethoxyphenanthrene</u> (<u>57</u>). The
protected phenanthrene <u>56</u> and 10 ml of acetic acid were
placed in a round bottomed flask, to which was added 1 ml
of 48% hydrobromic acid. The solution was heated for 3 hr,
cooled, diluted with water and then extracted with diethyl
ether. Chromatography on Si gel (chloroform:methanol, 25:1)
provided 30 mg (5.5% yield from the stilbene) of <u>57</u>: NMR
(d$_6$-acetone) (Figure 55) 3.97 (s, 3H, $-OCH_3$), 4.02 (s, 3H,
$-OCH_3$), 7.00 (bs, 1H, $-OH$), 7.08 (s, 1H, H-1), 7.09-7.28
(m, 2H), 7.48 (s, 2H, H-9 and H-10), 8.12 (bs, 1H, $-OH$) and
9.31 (d, 1H, H-5, J = 10 Hz); UV (EtOH) (Figure 56) showed
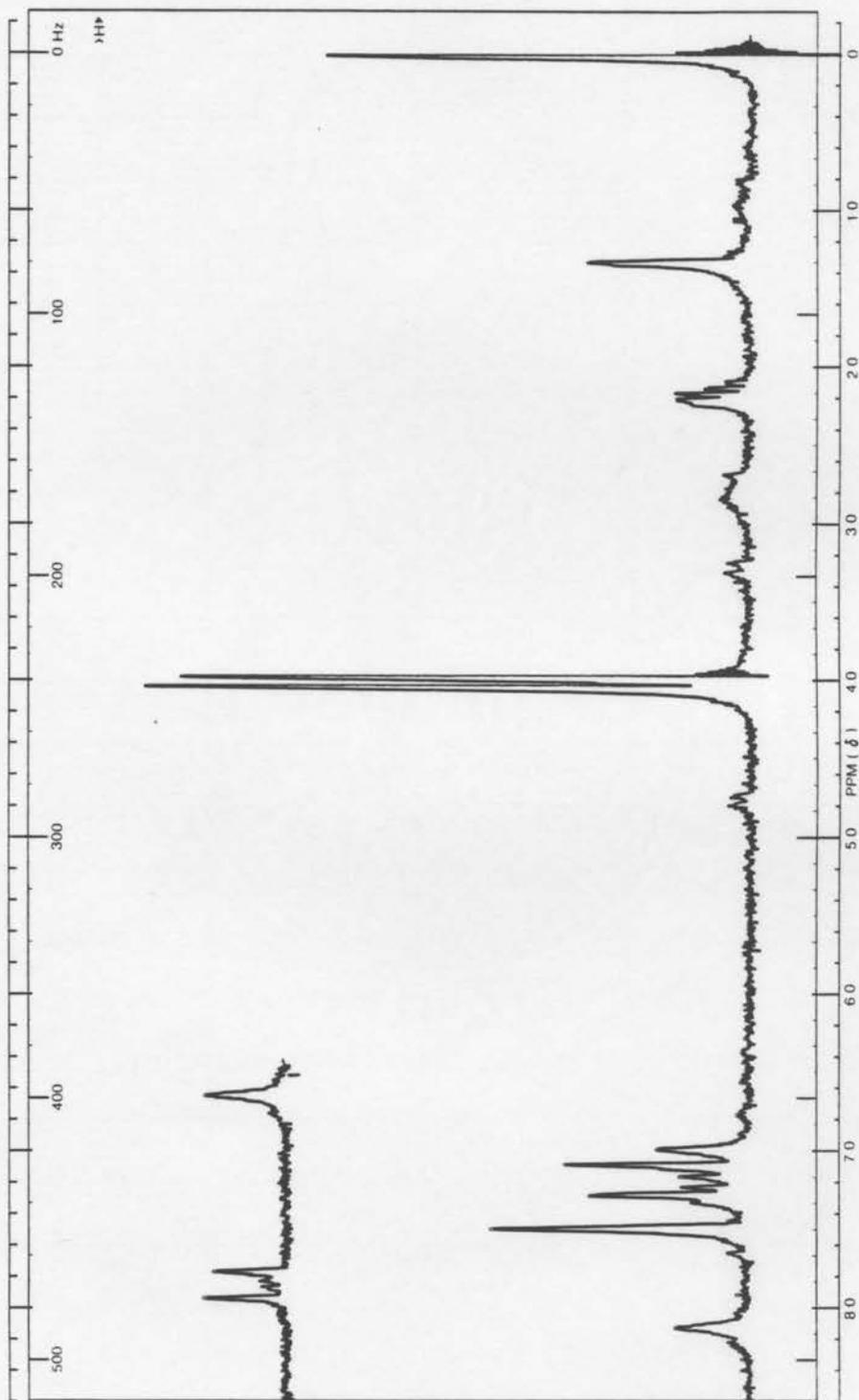
Figure 55 NMR (d_6-acetone) of 3,7-Dihydroxy-2,4-dimethoxyphenanthrene
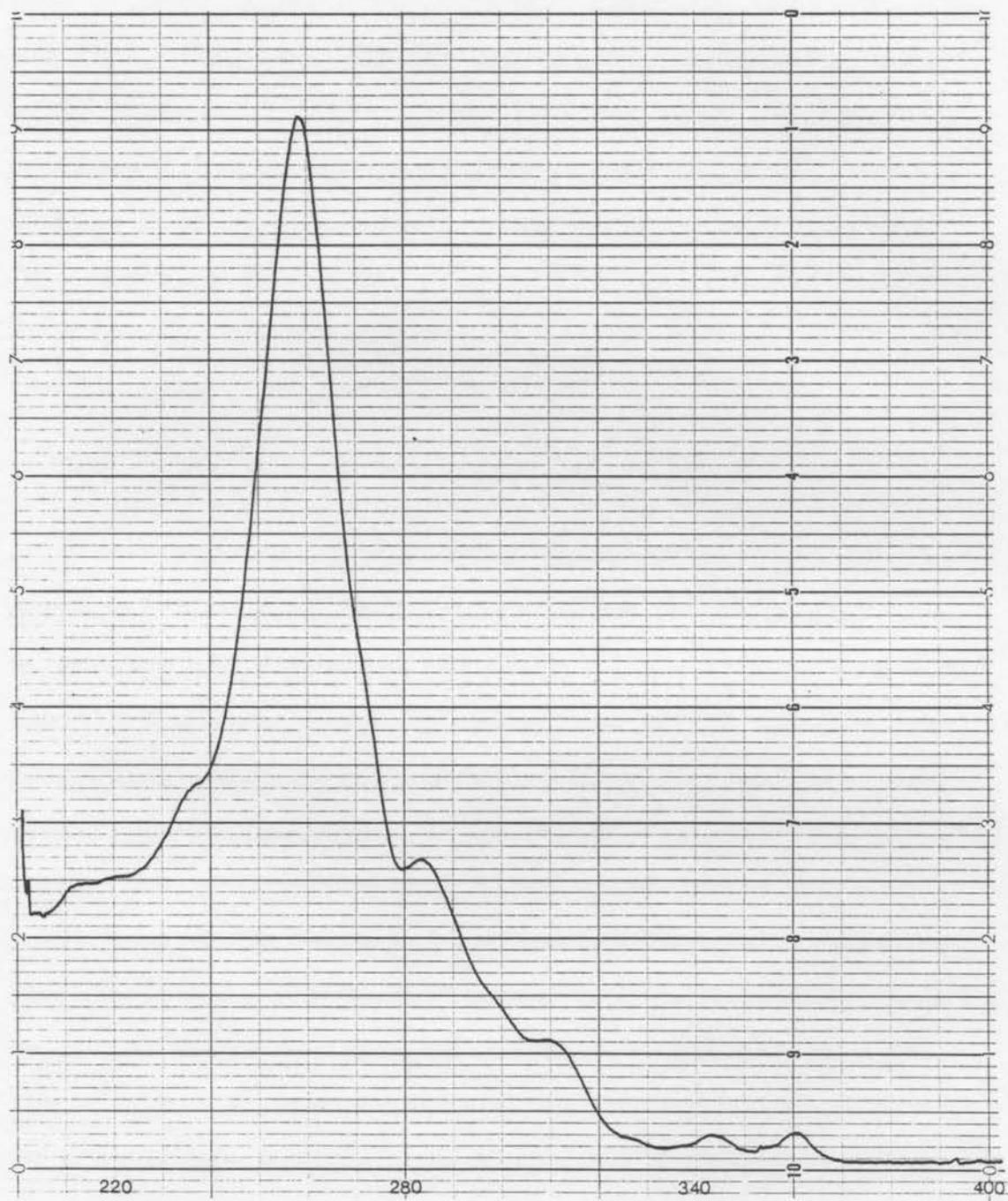
Figure 56

UV (EtOH) of 3,7-Dihydroxy-2,4-dimethoxyphenanthrene

λmax 360, 343, 310, 283, 258, 237 (sh) and 220 nm. Addi-
tion of base caused a bathochromic shift to λmax 376, 356,
334 (sh), 305, 284, 269 and 236 nm; IR (neat) 3400 (broad),
2920, 2825, 1610, 1500, 1470, 1285 and 1070. Acetylation
provided 3,7-diacetoxy-2,4-dimethoxyphenanthrene: NMR
($CDCl_3$) 2.37 (s, 3H, -OAc), 2.45 (s, 3H, -OAc), 3.95 (s,
3H, $-OCH_3$), 3.98 (s, 3H, $-OCH_3$), 7.17 (s, 1H, H-1), 7.20-
7.63 (m, 2H, H-6 and H-8), 7.67 (s, 2H, H-9 and H-10), 9.50
(d, 2H, H-5, J = 9.5 Hz).

III. Discussion

Four previously unreported phenanthrenes and one 9,10-
dihydrophenanthrene were isolated from O. cebolleta.
Structures are suggested for three of the phenanthrenes and
the dihydrophenanthrene by comparison of NMR spectra with
previously reported compounds and by color reaction with
several spray reagents. These identifications were aided by
comparison with synthesized 3,7-dihydroxyphenanthrene (a
previously known compound) and 3,7-dihydroxy-2,4-dimethoxy-
phenanthrene, a previously unreported phenanthrene. Because
we relied on color tests to distinguish between some of
the possible structures, the structural assignments must
be considered tentative. Each should be synthesized for
final proof.

Phenanthrenes and 9,10-dihydrophenanthrenes have been
isolated from eight plant families: Combretaceae, Dioscora-
ceae, Papaveraceae, Euphorbiaceae, Juncaceae, Moraceae,

Orchidaceae and Poaceae. These phenanthrenes and dihydro-
phenanthrenes can be divided into two distinct groups,
based on their substituents and their proposed biogenetic
pathways.

Juncusol[100] and micrandrol-C[101] (Figure 57), isolated
from the Juncaceae and Euphorbiaceae respectively, are
representative of those phenanthrenes and dihydrophenan-
threnes having alkyl substituents. In most cases, these
substituents are methyl groups, but compounds like juncusol,
with a vinyl substituent are known. The presence of alkyl
substituents has led to the theory that this class of
phenanthrenes is derived via terpene biogenesis. Of the
more than 45 phenanthrene derivatives isolated from the
other six plant families, none contain substituents other
than hydroxy or methoxy groups.

Four phenanthrene derivatives have previously been
isolated from orchids. Orchinol[102] (Figure 57) has been
found in six genera (11 different species) of orchids.[103]
Two similar 9,10-dihydrophenanthrenes, hircinol and loro-
glossol (Figure 57), have been isolated from a single
species, Loroglossum hircinum.[104] The only other previously
known phenanthrene derivative from orchids was the quinone,
cypripedin (Figure 57), that has been isolated from
Cypripedium calceolus.[105]

The biogenesis of phenanthrenes and dihydrophenan-
threnes is at present unknown, but there are several main
theories to explain their formation. Since stilbenes and
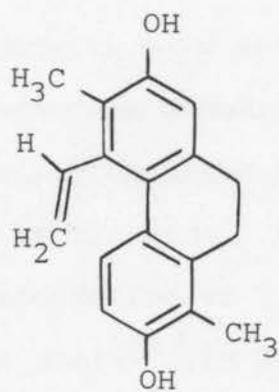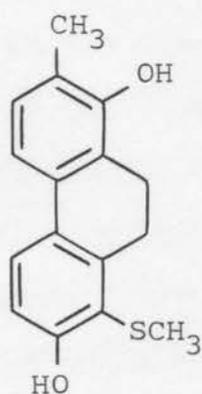
Juncusol   Micrandrol-C   Orchinol
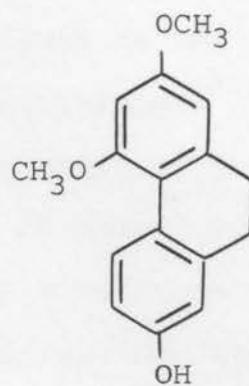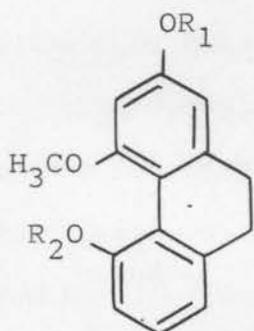
Hircinol $R_1 = R_2 = H$

Loroglossol $R_1 = H$,

$R_2 = CH_3$
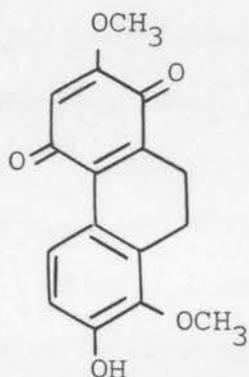
Cypripedin
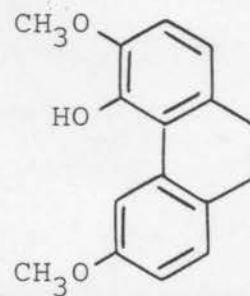
Thebaol
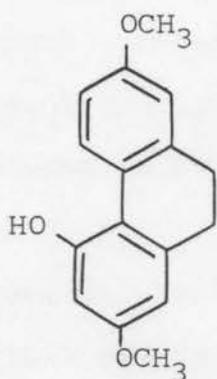
3,7-Dimethoxy-5-
hydroxyphenanthrene
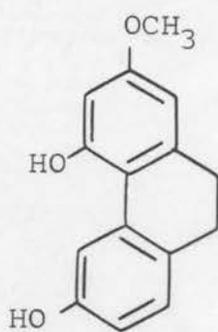
Cannabidihydro-
phenanthrene
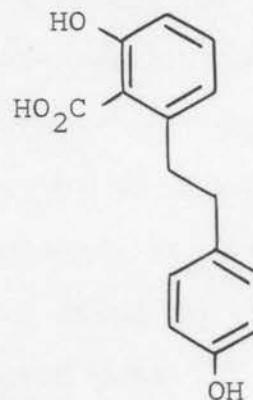
Lunularic
Acid

Figure 57   Phenanthrenes and 9,10-Dihydrophenanthrenes
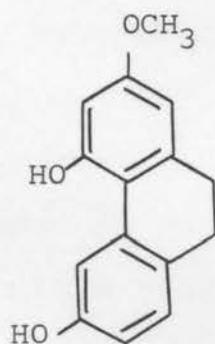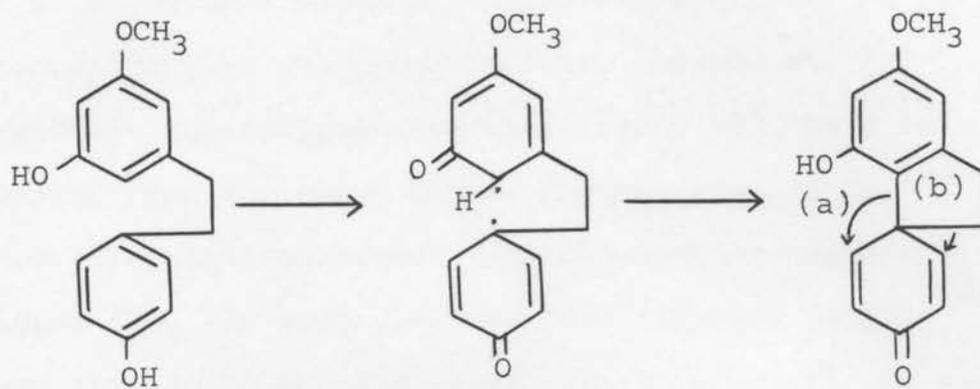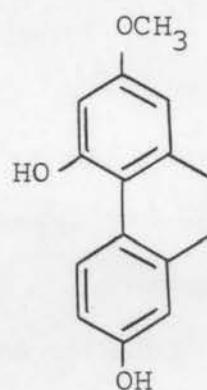
Figure 58 Proposed Biogenesis of Phenanthrenes

Although phenanthrenes and dihydrophenanthrenes are not known to have human physiological activity, it is interesting that two phenanthrenes, theabol and 3,7-dimethoxy-5-hydroxyphenanthrene (Figure 57), have been isolated from the opium poppy, Papaver somniferum,[103] and a dihydrophenanthrene, cannabidihydrophenanthrene (Figure 57), has been isolated from Cannabis sativa.[105] These two plants are well known for their physiological activity, but it has been attributed to other constituents. The possibility exists that phenanthrenes and dihydrophenanthrenes may have physiological activity, and therefore may be the components of O. cebolleta which give it the hallucinogenic activity. This possibility is reinforced by the close structural similarity of Δ^1-tetrahydrocannabinol (THC) (Figure 59) to dihydrophenanthrenes, and the recent report[107] of the analgesic activity of a 1,9-dihydroxyoctahydrophenanthrene (Figure 59) which has a closer structural link to the dihydrophenanthrenes. Biological testing of the phenanthrenes and dihydrophenanthrenes is necessary to determine if they do possess physiological activity.
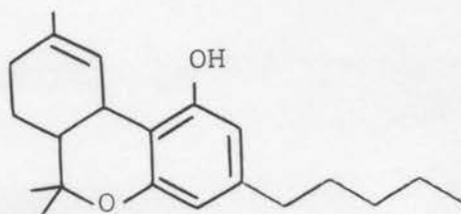
Δ^1-THC
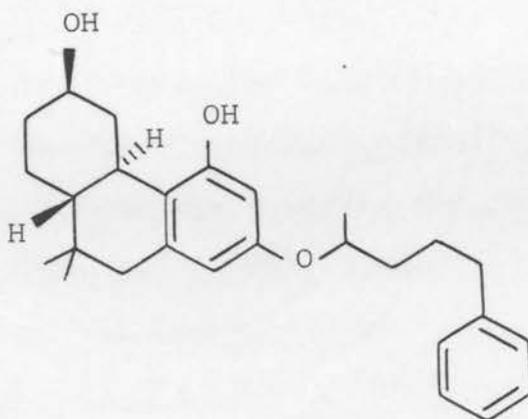
a 1,9-Dihydroxyoctahydrophenanthrene

Figure 59

Physiologically Active Compounds Similar to Phenanthrenes

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Appendix 1

Screening of Rocky Mountain Plants 1976

Collection Number	NCI Test Number	Family	Genus species	Alkaloid Test	KB Cell Test ED/50	P388[a] Test (T/C)%	Toxicity[b] mg/kg	Anti-feedant Activity
TRS-2	B664492	Liliaceae	Erythronium grandiflorum Persh.	3+	66	97_{100}	400^d	+
TRS-3	B664493	Polemoniaceae	Polemonium pulcherrimum ssp. delicatum (Rydb) Brand.	-	66	$105_{18.7}$	37.5	-
TRS-5	B664494	Ranunculaceae	Ranunculus alismaefolius Geyar Ex. Benth.	not tested	66	112_{200}	400^d	+
TRS-6	B664495	Ranunculaceae	Ranunculus adoneus A. Gray	1+	66	96_{200}	400^d	0
TRS-7	B664496	Boraginaceae	Mertensia ciliata (James) G. Don	-	110	102_{40}	400^d	not tested
TRS-8	B664497	Brassicaceae	Thalaspi montanum L.	not tested	100	105_{400}	400^d	0
TRS-9	B664498	Scrophulariaceae	Castilleja rhexifolia Rydb.	large +	100	110_{100}	400^d	not tested
TRS-10	B664499	Primulaceae	Primula parryi A. Gray	-	34	101_{25}	50	not tested
TRS-11	B664500	Ranunculaceae	Trollius laxus Salisb. var. albiflorus A. Gray	large +	100	112_{400}	400^d	-

Collection Number	NCI Test Number	Family	Genus species	Alkaloid Test	KB Cell Test ED/50	P388[a] Test (T/C)%	Toxicity[b] mg/kg	Anti-feedant[c] Activity
TRS-12	B664501	Rosaceae	Potentilla diversifolia Lehm.	−	100	112_{150}	300^{d}	0
TRS-13	B664502	Polygonaceae	Polygonum bistortoides Pursh.	−	100	114_{150}	300^{d}	0
TRS-14	B664503	Asteraceae	Senecio dimorphophyllus Greene	not tested	100	119_{400}	400^{d}	+
TRS-15	B664504	Caryophyllaceae	Cerastium arvense L.	1+	100	106_{400}	400^{d}	0
TRS-17	B664505	Hydrophyllaceae	Phacelia sericea (Graham) Gray	not tested	88	$118_{37.5}$	150^{d}	−
TRS-18	B664506	Brassicaceae	Arabis holbelii Hornem.	not tested	100	101_{400}	400^{d}	−
TRS-19	B664507	Brassicaceae	Cardamine cordifolia A. Gray	not tested	100	105_{100}	400^{d}	not tested
TRS-20	B664508	Scrophulariaceae	Penstemon whippleanus A. Gray	2+	100	105_{400}	400^{d}	not tested
TRS-21	B664509	Scrophulariaceae	Pedicularis bracteosa Benth. var. paysoniana (Pennell)	2+	100	105_{400}	400^{d}	not tested
TRS-22	B664510	Orchidaceae	Habenaria dilatata (Persh.) Hook	2+	100	104_{200}	400^{d}	0

Collection Number	NCI Test Number	Family	Genus species	Alkaloid Test	KB Cell Test ED/50	P388[a] Test (T/C)%	Toxicity[b] mg/kg	Anti-feedant[c] Activity
TRS-23	B664511	Scrophulariaceae	Veronica wormskjoldii R. & S.	1+	100	102_{100}	400^d	0
TRS-25	B664512	Scrophulariaceae	Pedicularis groenlandica Retz.	1+	100	105_{100}	400^d	not tested
TRS-26	B664513	Scrophulariaceae	Castilleja sulphurea Rydb.	-	100	108_{200}	400^d	0
TRS-27	B664514	Scrophulariaceae	Pedicularis racemosa Dougl. ex. Hook.	-	100	108_{300}	300^d	0
TRS-28	B664515	Crassulaceae	Sedum rhodanthum A. Gray	not tested	100	106_{100}	300^d	not tested
TRS-29	B664516	Polygonaceae	Oxyria digyma (L.) Hill	-	100	111_{75}	150^d	0
TRS-30	B664517	Onagraceae	Epilobium hornemanni Reichb.	-	59	$101_{12.5}$	100	-

(a) Highest (T/C)% reported. Subscript is dosage per injection.   (b) Lowest dose that showed toxicity.   (c) + denotes feeding stimulant, - denotes feeding deterrent, 0 denotes no effect.   (d) No toxicity shown by the highest dose tested.

Appendix 2

Screening of Rocky Mountain Plants[a]

Collection Number	Family	Genus species	Sample	NCI Test Number	Alkaloid Test	KB cell Test ED/50	P388[b] Test (T/C)%	Toxicity[c] mg/kg
TRS-37	Rosaceae	Potentilla fissa Nutt.	hexane		−			
			A	B838411	−	20, 40	105_{50}	200
			B	B838412	−	30, 53	115_{400}	400[d]
TRS-38	Leguminosae	Thermopsis montana Nutt. ex. T. & G.	hexane		3+			
			A	B838413	3+	100	111_{200}	400
			B	B838414	−	100	105_{200}	200
TRS-42	Boraginaceae	Cryptantha virgata (Porter) Payson	hexane		−			
			A	B838415	−	110	110_{50}	400
			B	B838416	−	65	114_{150}	300[d]
TRS-44	Loasaceae	Mentzelia decapetala (Pursh) Urb. & Gilg.	hexane		−			
			A	B838417	−	100	108_{200}	400[d]
			B	B838418		100	104_{200}	400
TRS-45	Polygonaceae	Eriogonum campanulatum Nutt.	hexane		−			
			A	B838419	−	18, 56	$118_{6.25}$	12.5
			B	B838420	−	18, 15	$117_{6.25}$	12.5

Collection Number	Family	Genus species	Sample	NCI Test Number	Alkaloid Test	KB Cell Test ED/50	P388[b] Test (T/C)%	Toxicity[c] mg/kg
TRS-46	Onagraceae	Epilobium angustifolium L.	hexane		-			
			A	B838421	-	20, 38	97/50	200
			B	B838422		30, 63	108/200	400
TRS-47	Compositae	Chrysothamnus viscidiflorus (Hook) Nutt.	hexane		-			
			A	B838423	-	98, 70	102/50	400
			B	B838424	-	20	96/100	200
TRS-48	Iridaceae	Iris missouriensis Nutt.	hexane		-			
			A	B838425	-	100	103/400	400[d]
			B	B838426	-	100	113/400	400[d]
TRS-49	Compositae	Senecio hydrophilus Nutt.	hexane		-			
			A	B838427	1+	100	107/400	400[d]
			B	B838428	-	100	109/400	400[d]
TRS-50	Gentianaceae	Frasera speciosa Griseb.	hexane		-			
			A	B838429	1+	100	109/100	400[d]
			B	B838430	-	110	107/100	400
TRS-53	Rosaceae	Potentilla fruticosa L.	hexane		-			
			A	B838431	-	29, 75	105/50	400
			B	B838432	-	38	109/200	400[d]

Collection Number	Family	Genus species	Sample	NCI Test Number	Aklaloid Test	KB Cell Test Test ED/50	P388[b] Test (T/C)%	Toxicity[c] mg/kg
FRS-67	Liliaceae	Allium brevistylum A. Wats	hexane		–			
			A	B838433	–	100	103_{100}	400^{d}
		Insufficient material to do this extract						
FRS-69	Polemoniaceae	Gilia aggregata (Pursh) Spreng	hexane		–			
			A	B838434	–	29, 62	103_{100}	200
			B	B838435	–	100	105_{200}	400^{d}
FRS-70	Primulaceae	Dodecatheon radicatum Greene	hexane		–			
			A	B838436	–	100	101_{50}	100
			B	B838437	–	100	104_{50}	100
FRS-71	Scrophulariaceae	Pedicularis crenulata Benth.	hexane		–			
			A	B838438	1+	100	108_{200}	400^{d}
			B	B838439	not tested	100	109_{400}	400^{d}
FRS-72	Scrophulariaceae	Castilleja flava Watson	hexane		–			
			A	B838440	–	100	112_{400}	400^{d}
			B	B838441	not tested	100	105_{400}	400^{d}
FRS-86	Polygonaceae	Eriogonum umbellatum Torr.	hexane		–			
			A	B838444	–	43	104_{400}	400^{d}
			B	B848445	–	31	102_{100}	400^{d}

Collection Number	Family	Genus species	Sample	NCI Test Number	Alkaloid Test	KB cell Test ED/50	P388[b] Test (T/C)%	Toxicity[c] mg/kg
FRS-105	Polygonaceae	Eriogonum alatum Torr.	hexane		−			
			A	B840752			98_{100}	100
			B	B840753			101_{50}	100
FRS-103	Polygonaceae	Eriogonum annum Nutt.	hexane		−			
			A	B840754			105_{50}	400[d]
			B	B840755			104_{100}	400[d]
FPS-102	Polygonaceae	Eriogonum flavum Nutt.	hexane		−			
			A	B840756			107_{400}	400[d]
			B	B840757			101_{100}	400[d]
FRS-98	Polygonaceae	Eriogonum inflatum Torr. & Frem.	hexane		−			
			A	B840758			106_{50}	400[d]
			B	B840759			101_{200}	400[d]
FRS-104	Polygonaceae	Eriogonum jamesii Benth var flavescens S. Watts	hexane		−			
			A	B840760			105_{100}	400[d]
			B	B840761			106_{400}	400[d]
FRS-101	Polygonaceae	Eriogonum pauciflorum Pursh var graphaloides	hexane		−			
			A	B840762			107_{200}	400[d]
			B	B840763			112_{200}	400[d]

Collection Number	Family	Genus species	Sample	NCI Test Number	Alkaloid Test	KB Cell Test ED/50	P388[b] Test (T/C)%	Toxicity[c] mg/kg
TRS-55	Scrophulariaceae	Penstemon virens Pennel	hexane		−			
			A	B840764			107_{200}	400^d
			B	B840765			108_{200}	400^d
TRS-56	Liliaceae	Zygadenus paniculatus (Nutt.) S. Watts	hexane		−			
			A	B840766				50
			B	B840767			115_{100}	200
TRS-57	Cruciferae	Physaria vitalifera Rydb.	hexane		−			
			A	B840768			106_{50}	400
			B	B840769			109_{100}	400^d
TRS-59	Compositae	Haplopappus armerioides (Nutt.) Gray	hexane		−			
			A	B840770			103_{50}	400^d
			B	B840771			103_{50}	200
TRS-61	Polemoniaceae	Phlox multiflora A. Nels.	hexane		−			
			·A	B840772			115_{200}	400^d
			B	B840773			109_{200}	400

(a) Blank spaces are tests not performed.   (b) Highest (T/C)% reported.
Subscript is dosage per injection.   (c) Lowest dose that showed toxicity.
(d) No toxicity shown by the highest dose tested.