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VAR. ADIANTIFOLIUM HORT.

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THE ISOLATION AND CHARACTERIZATION OF

THE ALKALOIDS OF THALICTRUM MINUS L. VAR. ADIANTIFOLIUM HORT.

DISSERTATION

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

By

Paul Luther Schiff, Jr., B.Sc., M.Sc.

The Ohio State University
1967

Approved by

[Signature]
Adviser
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FIELDS OF STUDY

Major Field: Pharmacognosy


CONTENTS

ACKNOWLEDGMENT ii
VITA AND FIELDS OF STUDY iv
TABLES xii
FIGURES xiii
CHARTS xv

I. INTRODUCTION AND HISTORICAL 1

A. Botanical and taxonomic description of the Thalictrum genus 1
B. Pharmacological significance of Thalictrum plants 2
C. Alkaloids of the Thalictrum genus 5
   1. Types of alkaloids 5
   2. Classical degradations and syntheses of the representative alkaloid types 12
D. Biosynthesis of the representative alkaloid types 31
E. Literature survey of Thalictrum alkaloids since 1965 36
F. Ultraviolet spectra of Thalictrum alkaloids 58
G. Nuclear magnetic resonance spectra of Thalictrum alkaloids 64
H. Mass spectra of Thalictrum alkaloids 69

II. EXPERIMENTAL 96

A. Materials 96
CONTENTS (Cont'd)

B. Methodology—Chemical and physical analysis
   1. Infrared spectrophotometric analysis 96
   2. Ultraviolet spectral analysis 96
   3. Nuclear magnetic resonance spectrometric analysis 97
   4. Melting point determinations 97
   5. Microanalysis 97
   6. Mass spectrometric analysis 97
   7. Thin-layer chromatographic analysis 97
   8. Adsorption chromatographic analysis 98
   9. Ion exchange analysis 98
  10. Optical rotation measurements 98

C. Preliminary investigations 99
   1. General extraction of the alkaloids 99
   2. Fractionation of the total extract into tertiary and quaternary alkaloid fractions
      a. Gradient pH separation of the tertiary alkaloids 99
      b. Thin-layer chromatography of fractions from the pH gradient extraction 100
   3. Separation and isolation of the tertiary alkaloids 102
      a. Column chromatography of the pH gradient fraction I
         i. The isolation of alkaloid A 105
      b. Column chromatography of the pH gradient fraction II
         i. The isolation of alkaloid B (O-methylthalicberine) 107
         ii. The isolation of alkaloid C (Adiantifoline) 107

vi
CONTENTS (Cont'd)

b.

iii. The isolation of alkaloid D (Adiantifoline)  
   108  
iv. The isolation of alkaloid E (Adiantifoline)  
   108

c. Column chromatography of the pH gradient fraction III  
   109

D. Extraction and fractionation of Thalictrum minus L. var. adiantifolium Hort. root alkaloids  
   110  
1. Extraction of total alkaloids  
   110  
2. Fractionation of the total extractive into a tertiary alkaloid fraction, a quaternary alkaloid fraction and a neutral-acid fraction  
   111  
a. Fractionation of the tertiary alkaloid fraction into tertiary phenolic and tertiary nonphenolic fractions  
   114  
i. The isolation of alkaloid F (Thalifendine iodide)  
   116  
3. Separation and isolation of tertiary nonphenolic alkaloids  
   117  
a. pH gradient separation of the tertiary nonphenolic alkaloid fractions  
   117  
i. The isolation of alkaloid G (Berberine iodide)  
   120  
b. Column chromatography of pH gradient fraction I  
   120  
i. The isolation of alkaloid H  
   121  
ii. The isolation of alkaloid J  
   122  
iii. The isolation of alkaloid K (Noroxyhydrastinine)  
   122  
   vii
## CONTENTS (Cont'd)

<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.</td>
<td>Column chromatography of pH gradient fraction II</td>
<td>123</td>
</tr>
<tr>
<td>d.</td>
<td>Column chromatography of pH gradient fraction III</td>
<td>124</td>
</tr>
<tr>
<td>i.</td>
<td>The isolation of alkaloid L</td>
<td>125</td>
</tr>
<tr>
<td>4.</td>
<td>Separation and isolation of the tertiary phenolic alkaloids</td>
<td>126</td>
</tr>
<tr>
<td>a.</td>
<td>Preliminary purification and fractionation of the crude tertiary phenolic alkaloid fraction</td>
<td>126</td>
</tr>
<tr>
<td>i.</td>
<td>The isolation of alkaloid M (Thalifendine iodide)</td>
<td>127</td>
</tr>
<tr>
<td>b.</td>
<td>Column chromatography of the tertiary phenolic alkaloid fraction</td>
<td>130</td>
</tr>
<tr>
<td>i.</td>
<td>The isolation of alkaloid N (Thalifoline)</td>
<td>132</td>
</tr>
<tr>
<td>5.</td>
<td>Separation and isolation of the quaternary alkaloids</td>
<td>132</td>
</tr>
<tr>
<td>a.</td>
<td>Fractionation of the crude quaternary alkaloid reineckates into 50 acetone-water soluble and insoluble fractions</td>
<td>132</td>
</tr>
<tr>
<td>i.</td>
<td>The isolation of alkaloid P (Magnoflorine iodide)</td>
<td>133</td>
</tr>
<tr>
<td>ii.</td>
<td>The isolation of alkaloid Q (Berberine iodide)</td>
<td>133</td>
</tr>
<tr>
<td>iii.</td>
<td>The synthesis of tetrahydro alkaloid Q (Tetrahydroberberine)</td>
<td>134</td>
</tr>
<tr>
<td>6.</td>
<td>Treatment of the acid-neutral fraction</td>
<td>136</td>
</tr>
<tr>
<td>a.</td>
<td>Column chromatography of an aliquot (10 gm.) of the acid-neutral fraction</td>
<td>136</td>
</tr>
</tbody>
</table>
CONTENTS (Cont'd)

6.

a. The isolation of neutral compound A (β-sitosterol) 136

ii. The preparation of the acetate of neutral compound A (β-sitosterol acetate) 137

E. General extraction and fractionation of Thalictrum minus L. var. adiantifolium Hort. top alkaloids 139

1. Extraction of the total alkaloids 139

2. Fractionation of the total alkaloid extract into an ether soluble tertiary alkaloid fraction, ether insoluble-chloroform soluble tertiary alkaloid fraction, quaternary alkaloid fraction and acid-neutral fraction 139

a. Fractionation of the ether soluble tertiary alkaloid fraction into phenolic and nonphenolic fractions 140

3. Separation and isolation of the ether soluble tertiary nonphenolic alkaloids 141

a. pH gradient separation of the ether soluble tertiary nonphenolic alkaloid fraction 141

b. Column chromatography of the ether soluble tertiary nonphenolic alkaloid fraction 145

i. The isolation of alkaloid R (Adiantifoline) 147

4. Separation and isolation of the ether insoluble-chloroform soluble tertiary alkaloids 147
CONTENTS (Cont'd)

4. Column chromatography of the ether insoluble-chloroform soluble tertiary alkaloid fraction Page
   i. The isolation of alkaloid S 147
   ii. The isolation of alkaloid T (Berberine iodide) 149
   iii. The isolation of alkaloid U (Thalifendine iodide) 150
   iv. The isolation of alkaloid V (Berberine chloride) 150

F. Degradation of alkaloid D (adiantifoline) and thalicarpine 151
   1. Sodium-liquid ammonia cleavage of alkaloid D (adiantifoline) 151
      a. The phenolic cleavage product 153
      b. The nonphenolic cleavage product 153
   2. Permanganate oxidation of alkaloid D (adiantifoline) 155
   3. Sodium-liquid ammonia cleavage of thalicarpine 156
      a. The phenolic cleavage product 157
      b. The nonphenolic cleavage products 157
   4. Permanganate oxidation of thalicarpine 159

G. Synthesis of alkaloid K (noroxyhydrastinine) and alkaloid N (thalifoline) 160
   1. The synthesis of alkaloid K (6,7-methylenedioxy-1-oxo-1,2,3,4-tetrahydroisoquinoline) (noroxyhydrastinine) 160

x
CONTENTS (Cont'd)

2. The synthesis of alkaloid N (2-methyl-6-methoxy-7-hydroxy-1-oxo-1,2,3,4-tetrahydroisoquinoline) (thalifoline) 162
   a. The synthesis of O-benzylvanillin 163
   b. The synthesis of 3-methoxy-4-benzyloxy-β-nitrostyrene 163
   c. The synthesis of 3-methoxy-4-benzyloxy-β-phenethylamine 164
   d. The synthesis of N-formyl-3-methoxy-4-benzyloxy-β-phenethylamine 165
   e. The synthesis of 6-methoxy-7-hydroxy-3,4-dihydroisoquinoline 166
   f. The synthesis of 6-methoxy-7-hydroxy-3,4-dihydroisoquinoline methiodide 168
   g. The synthesis of 2-methyl-6-methoxy-7-hydroxy-1-oxo-1,2,3,4-tetrahydroisoquinoline (thalifoline) 168

III. DISCUSSION 172

IV. SUMMARY OF FINDINGS 196
   Appendix A 198
   Bibliography 213
TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Physical and Chemical Properties of the <em>Thalictrum</em> Series of Alkaloids</td>
<td>76</td>
</tr>
<tr>
<td>2.</td>
<td>Results of the Gradient pH Separation</td>
<td>100</td>
</tr>
<tr>
<td>3.</td>
<td>Results of the Column Chromatography of the pH Gradient Fraction I</td>
<td>105</td>
</tr>
<tr>
<td>4.</td>
<td>Results of the Column Chromatography of the pH Gradient Fraction II</td>
<td>106</td>
</tr>
<tr>
<td>5.</td>
<td>Results of the Column Chromatography of the pH Gradient Fraction III</td>
<td>109</td>
</tr>
<tr>
<td>6.</td>
<td>Weights and <em>R</em>&lt;sub&gt;f&lt;/sub&gt; Values of the Extractives from the pH Gradient Separation of the Tertiary Nonphenolic Alkaloids</td>
<td>119</td>
</tr>
<tr>
<td>7.</td>
<td>Results of the Column Chromatography of the pH Gradient Fraction I</td>
<td>121</td>
</tr>
<tr>
<td>8.</td>
<td>Results of the Column Chromatography of the pH Gradient Fraction II</td>
<td>124</td>
</tr>
<tr>
<td>9.</td>
<td>Results of the Column Chromatography of the pH Gradient Fraction III</td>
<td>125</td>
</tr>
<tr>
<td>10.</td>
<td>Results of the Column Chromatography of the Tertiary Phenolic Alkaloid Fraction</td>
<td>131</td>
</tr>
<tr>
<td>11.</td>
<td>Results of the Thin-Layer Chromatography of the Ether Soluble Tertiary Nonphenolic pH Gradient Alkaloid Fractions</td>
<td>143</td>
</tr>
<tr>
<td>12.</td>
<td>Results of the Column Chromatography of the Combined pH Gradient Fractions</td>
<td>146</td>
</tr>
<tr>
<td>13.</td>
<td>Results of the Column Chromatography of the Ether Insoluble-Chloroform soluble Tertiary Alkaloid Fraction</td>
<td>148</td>
</tr>
</tbody>
</table>
FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>The Protoberberine Alkaloids of <em>Thalictrum</em> Species</td>
<td>89</td>
</tr>
<tr>
<td>2.</td>
<td>The Aporphine Alkaloids of <em>Thalictrum</em> Species</td>
<td>90</td>
</tr>
<tr>
<td>3.</td>
<td>The Benzylisoquinoline Alkaloids of <em>Thalictrum</em> Species</td>
<td>91</td>
</tr>
<tr>
<td>4.</td>
<td>The Bisbenzylisoquinoline Alkaloids of <em>Thalictrum</em> Species</td>
<td>94</td>
</tr>
<tr>
<td>5.</td>
<td>The Aporphine-Benzylisoquinoline Alkaloids of <em>Thalictrum</em> Species</td>
<td>95</td>
</tr>
<tr>
<td>6.</td>
<td>The Phenanthrene Alkaloid of <em>Thalictrum</em> Species</td>
<td>95</td>
</tr>
<tr>
<td>7.</td>
<td>Thin-Layer Chromatogram of the pH Gradient Fractions</td>
<td>101</td>
</tr>
<tr>
<td>8.</td>
<td>A Prepared Adsorption Column</td>
<td>103</td>
</tr>
<tr>
<td>9.</td>
<td>A Gradient Elution Apparatus after Parr</td>
<td>104</td>
</tr>
<tr>
<td>10.</td>
<td>Thin-Layer Chromatogram of the Tertiary Non-phenolic Alkaloids from the pH Gradient Separation</td>
<td>118</td>
</tr>
<tr>
<td>11.</td>
<td>Thin-Layer Chromatogram of the pH Gradient Alkaloid Fractions</td>
<td>144</td>
</tr>
<tr>
<td>12.</td>
<td>The Infrared Spectrum of Adiantifoline (CHCl₃)</td>
<td>198</td>
</tr>
<tr>
<td>13.</td>
<td>The Infrared Spectrum of Adiantifoline (KBr)</td>
<td>199</td>
</tr>
<tr>
<td>14.</td>
<td>The Infrared Spectrum of Alkaloid A (CHCl₃)</td>
<td>200</td>
</tr>
<tr>
<td>15.</td>
<td>The Infrared Spectrum of Alkaloid J (KBr)</td>
<td>201</td>
</tr>
<tr>
<td>16.</td>
<td>The Infrared Spectrum of Noroxyhydrastinine (KBr)</td>
<td>202</td>
</tr>
<tr>
<td>17.</td>
<td>The Infrared Spectrum of Synthetic Noroxyhydrastinine (KBr)</td>
<td>203</td>
</tr>
<tr>
<td>18.</td>
<td>The Infrared Spectrum of Alkaloid L (KBr)</td>
<td>204</td>
</tr>
<tr>
<td>19.</td>
<td>The Infrared Spectrum of Thalifoline (KBr)</td>
<td>205</td>
</tr>
<tr>
<td>20.</td>
<td>The Infrared Spectrum of Synthetic Thalifoline (KBr)</td>
<td>206</td>
</tr>
<tr>
<td>Figures</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>21.</td>
<td>The Infrared Spectrum of Alkaloid S (KBr)</td>
<td>207</td>
</tr>
<tr>
<td>22.</td>
<td>The Nuclear Magnetic Resonance Spectrum of Adiantifoline</td>
<td>208</td>
</tr>
<tr>
<td>23.</td>
<td>The Nuclear Magnetic Resonance Spectrum of Alkaloid A</td>
<td>209</td>
</tr>
<tr>
<td>24.</td>
<td>The Nuclear Magnetic Resonance Spectrum of Synthetic Noroxyhydrastinine</td>
<td>210</td>
</tr>
<tr>
<td>25.</td>
<td>The Nuclear Magnetic Resonance Spectrum of Alkaloid S</td>
<td>211</td>
</tr>
<tr>
<td>26.</td>
<td>The Nuclear Magnetic Resonance Spectrum of Alkaloid S</td>
<td>212</td>
</tr>
<tr>
<td>Chart</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>I.</td>
<td>Flow Sheet for the Extraction of the Total Alkaloid</td>
<td>113</td>
</tr>
<tr>
<td>II.</td>
<td>Flow Sheet for the Fractionation of the Tertiary Alkaloid Fraction</td>
<td>115</td>
</tr>
<tr>
<td>III.</td>
<td>Flow Sheet for the Purification and Fractionation of the Crude Tertiary Phenolic Alkaloid Fraction</td>
<td>129</td>
</tr>
<tr>
<td>IV.</td>
<td>Flow Sheet for the Separation and Isolation of the Quaternary Alkaloids</td>
<td>135</td>
</tr>
<tr>
<td>V.</td>
<td>Flow Sheet for the Treatment of the Acid-Neutral Fraction</td>
<td>138</td>
</tr>
</tbody>
</table>
I. INTRODUCTION AND HISTORICAL

A. Botanical and Taxonomic Description of the Genus Thalictrum

The purpose of this investigation was to isolate, characterize and identify as many alkaloids as possible from the roots and tops of *Thalictrum minus* L. var. *adiantifolium* Hort. In addition, several syntheses were undertaken in order to positively establish the structure of some of these alkaloids.

*Thalictrum* is an ancient name of doubtful origin; perhaps from the Greek *thallo* (to grow green). *Thalictrum* (Meadow Rue) a genus of plants of the *Ranunculaceae* family (Crowfoot), includes a wide range of herbs which are mostly dispersed in temperate and cold countries (1). *Ranunculus* is the latin word for a "little frog", referring to the habitat, in ponds and wet places, of many of the species(2). The *Ranunculaceae* include many alkaloid bearing genera, some of which are *Aconitum*, *Caltha*, *Cimicifuga*, *Clematis*, *Consolida*, *Coptis*, *Delphinium*, *Eranthis*, *Helleborus*, *Hydrastis*, *Isopyrum*, *Nigella*, *Paeonia*, *Thalictrum* and *Zanthorrhiza* (3, 4).

A monograph of the entire *Thalictrum* genus was published in 1885 by *Lecoyer* (5) in which he described 69 species. Since then, a number of treatises have been published concerning the botanical description of the genus *Thalictrum* (6, 7, 8, 9, 10, 11).

The members of the *Ranunculaceae* family, whose genera number 35 or more and species 1500 or more, are mainly annual or perennial herbs (sometimes little shrubs and woody climbers) found mostly in the temperate and artic regions of the northern hemisphere (12, 13, 14, 15, 16, 17).
The members of the Genus *Thalictrum*, whose species number 80-90, are erect and mostly tall perennial herbs, chiefly of the north temperate zone (18, 19, 20, 21, 22, 23, 24, 25).

*T. minus* L. is an exceedingly variable species (27, 28). It is widespread in Europe and temperate Asia and many of the more marked varieties have been distinguished as species (29). Thus, *T. minus* L. var. *adiantifolium* Hort. is sometimes called *T. adiantifolium*.

B. Pharmacological Significance of *Thalictrum* Plants

*Thalictrum* species have been used as household remedies throughout the world for many years.

*Thalictrum flavum* was used by the English, the Russians and the Americans in the 18th and 19th centuries as an aperient, stomachic, purgative, diuretic, febrifuge and anti-hydrophobic (30, 31).

*Thalictrum minus* fomentations were used by the Russians in treating snakebite and fevers (32). The Chinese used *Thalictrum siense* for pectoral complaints (33) while in India *Thalictrum foliolosum* and other species were used as stomachics, bitters, tonics, antiperiodics, alteratives, diaphoretics, laxatives, antipyretics, purgatives, blood purifiers and in the treatment of snake bite, jaundice, leprosy, vomiting of pregnancy, rheumatism, indolent ulcers, conjunctivitis, gastritis, duodenal ulcers and oriental sore (34, 35, 36). The Japanese also found use of *Thalictrum* as similar home remedies (37).

The American Indians of Nevada treated gonorrhea and the common cold with a tea brewed from *Thalictrum fendleri*, Engelm. (38) while other American Indians and natives of Canada used the roots of certain native *Thalictrum* species as snakebite remedies (39).
Most of the Thalictrum species contain the alkaloids berberine and magnoflorine to which some of the medicinal properties of these plants may be attributed. Berberine, a quaternary protoberberine alkaloid, causes a stimulant action on the movements of the gastrointestinal tract, a depression of the cardiovascular system, a sharp fall of blood pressure, a depression of both the auricles and ventricles and a distinct dilatation of the heart (40, 41). Magnoflorine, a quaternary aporphine alkaloid, exerts a ganglionic blocking type action in cats, rabbits and dogs which result in an induced hypotension (42, 43, 44).

Ovsepyan (45) investigated the total alkaloid hydrochlorides of Thalictrum minus and observed cardiotonic and pressor effects in frogs, cats and dogs.

Daleva and Sherkava (46), two Bulgarian workers, noted that alkaloid fractions from Bulgarian Thalictrum minus produced hypotensive effects, slight diuresis, excitation of the choline reactive system and a spasmolytic action on the intestine.

Patil et al. (47) observed that a fraction of tertiary alkaloid hydrochlorides from the roots of Thalictrum revolutum produced powerful and prolonged hypotensive effects in dogs, cats and rabbits. This fraction also produced an antispasmodic action on isolated rabbit intestine, an inhibition of the isolated rat uterus, a stimulation of the guinea pig uterus, head-drop in rabbits, generalized central depressant effects in rats, slowing of the isolated turtle heart and emesis in pigeons. Lethality was observed in mice, rats, pigeons, rabbits and dogs.

Kupchan and Yokoyama (48) reported the isolation of a tertiary nonphenolic alkaloid from Thalictrum dasycarpum, Fisch. and Lall roots which they called thalicarpine and which produced transient hypotensive effects in cats.
Malikov et al. (49) observed that thalizopine, an alkaloid isolated from *Thalictrum isopyroidum*, produced irregular hyperemia of the brain and liver, edema of the brain and white matter of the spine and protein dystrophy of parenchymal cells in rats and rabbits.

Thalsimine, a new alkaloid from *Thalictrum simplex*, produced a sedative effect in mice, decreased arterial pressure with simultaneous bradycardia, increased amplitude of cardiac contractions, and a spasmolytic action in cats according to the investigations of Sadritdinov and Kamilov (50).

In 1965, Patil et al. (51) tested the effects of extracts from eleven different *Thalictrum* species for their ability to affect blood pressure in dogs, relax the intestinal smooth muscle of the rabbit and depress the isolated rabbit heart. It was observed that the extracts of *Thalictrum minus* race B, *Thalictrum rochebrunianum* and *Thalictrum rugosum* showed considerable activity in all three pharmacological tests and seemed to be the most promising candidates for further investigation.

Hahn et al. (52) studied the effect of thalicarpine on the cardiovascular system of the anesthetized dog and on several smooth muscle preparations and found that a dose of 2 mg./Kg. produced in the dog moderate pressor activity of rather long duration which was sometimes accompanied by a mild tachycardia. In addition, doses of 10 mg./Kg. produced intense, long lasting, non-cholinergic hypotension. Direct depressant effects were seen on several smooth muscle preparations as well as reduction of drug induced spasmogenic effects.

In 1966, Zhelyazkov (53) observed the effects of the alkaloids of *Thalictrum minus* var. *flavum* upon animals treated with other drugs. Hypotensive effects of 5-hydroxytryptamine were changed to hypertensive effects by treatment with these alkaloids. In addition, the alkaloids also
changed the hypertensive effects of dihydroergotamine into hypotensive effects. Repeated introduction of dihydroxyphenylalanine (100 mg./Kg.) into animals treated with these alkaloids always produced a clearly expressed pressor reaction.

C. Alkaloids of the Thalictrum genus

1. Types of alkaloids. The Thalictrum alkaloids have been found to possess one or a combination of three basic nuclei; the benzyltetrahydroisoquinoline nucleus (I), the aporphine nucleus (II) and the protoberberine nucleus (III).
The protoberberines (V and VI) are a group of alkaloids which can theoretically be derived from the benzyltetrahydroisoquinolines (IV) by condensation with formaldehyde. In vitro, this condensation yields a mixture of (V and VI), when the substituents in the benzyl group are hydroxyls but only (VI) when the hydroxyls are fully alkylated.

![Structural diagrams of compounds IV, V, and VI](image)

The protoberberines occur in a wide variety of botanical families. They are distributed in many genera of the Papaveraceae, generally as the tetrahydro bases, while in the Berberidaceae, Menispermaceae, Ranunculaceae, Rutaceae and Anenaceae they occur mostly in the quaternary dehydro form (54, 55).

The aporphine alkaloids are derivable from the benzylisoquinolines by the abstraction of two hydrogens in such a manner that the two benzene nuclei now form part of a 9,10-dihydrophenanthrene. Aporphines usually have substituents only in the 1,2,9,10 and 11 positions.

![Structural diagram of aporphine VII](image)
The majority of aporphines have 4 oxygen atoms. The aporphines occur most abundantly in the Papaveraceae, in which case the nitrogen atom is methylated, but they are also widespread in the Anonaceae, Lauraceae, Monimiaceae and Ranunculaceae in which families they frequently occur as secondary amines (56, 57).

The bisbenzyltetrahydroisoquinoline alkaloids occur in nature and are formed by the linkage of two molecules of a simple benzyltetrahydroisoquinoline alkaloid, the junction being one or more ether linkages between two or more aromatic nuclei (58, 59).

Faltis (60) suggested that the alkaloids are formed by intermolecular dehydrogenation of two molecules of 1-(4-hydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline or of its methylation products; in this process new C-O bonds are formed, phenolic coupling occurring at nuclear positions ortho to hydroxyl groups. Three such modes of dehydrogenation, A, B and C, are indicated by arrows below.
Dehydrogenation by Path A leads to a structure (IX) containing one diphenyl ether group (dauricine class). Reaction by Routes B and C and by Routes A and B results in cyclic structures (X), oxyacanthine class) and (XI), berbamine class), respectively, each containing two diaryl ether bridges. Other reactions resulting in the linking of a benzyl group and an isoquinoline residue are also possible; two examples of this alternative mode are illustrated below. Reaction by paths D and E furnishes a bisbenzylisoquinoline (XII) (isochondodendrine class) that is structurally symmetrical. Dehydrogenation by routes E and F leads to an unsymmetrical structure (XIV) (Curine class).
Bisbenzylisoquinoline alkaloids are widely distributed in plants of the *Menispermaceae* family growing mainly in South America and the Far East, but have also been found in genera belonging to other families, e.g. *Daphnandra*, *Berberis*, *Magnolia*, *Nectandra* and *Thalictrum* (60).

The benzylisoquinoline-aporphine dimer (XV) is a relatively new and novel type of alkaloid formed by the linkage of a benzylisoquinoline fragment and an aporphine fragment through an ether linkage between the aromatic nuclei.

![Diagram](XV)

To date, only three alkaloids, thalicarpine (61), its naturally occurring oxidation product dehydrothalicarpine (62) and thalmelatine (63) comprise this group.
2. Classical degradations and syntheses of the representative alkaloid types. Berberine (XVI) has the composition \( C_{20}H_{18}O_{4}N^+X^- \) and contains one methylenedioxy group and two methoxy groups. It can be reduced to tetrahydroberberine (XVII), \( C_{20}H_{20}O_{4}N \). The principal information of structural significance comes from the oxidation with alkaline permanganate, in which berberine yields oxyberberine (XVIII), berberilic acid (XIX) and berberal (XX), all of which retain the 20 carbon atoms of the alkaloid.

Berberilic acid (XIX), \( C_{20}H_{19}O_{9}N \), contains two carboxyl groups and is readily converted into anhydroberberilic acid, \( C_{20}H_{17}O_{8}N \), which contains one carboxyl group. Hydrolysis with sulfuric acid converts it into hemipinic acid (XXII) and the amino acid (XXI). Berberilic acid must therefore have the structure (XIX).

Berberal (XX), \( C_{20}H_{17}O_{7}N \), is hydrolyzed by sulfuric acid to noroxyhydrastinine (XXIII) and pseudo-opianic acid (XXIV). Pseudo-opianic acid may be reduced to pseudo-meconine (XXV) and may be oxidized to hemipinic acid (XXVI).

Oxyberberine, the carbonyl compound (XVIII), has been synthesized by the route (XXVII) \( \rightarrow \) (XXVIII) \( \rightarrow \) (XXIX), and it has been oxidized to berberine (XVI), thus constituting a total synthesis of the alkaloid (64).
The simplest aporphine alkaloids are apomorphine (XXXII) and morphothebaine (XXXIII), which do not occur naturally, but are obtained by acid rearrangement of morphine (XXX) and thebaine (XXXI) respectively. The methods used for the elucidation of their structures and for their syntheses are, however, generally applicable to the naturally occurring alkaloids of this group.

Apomorphine (XXXII) is formed when morphine (XXX) is heated with concentrated hydrochloric acid at 140 - 150 °C. The dimethyl ether (XXXIV) undergoes Hofmann degradation giving a mixture of two methine bases (XXXV) and (XXXVI). The nitrogen free product of exhaustive methylation is 3,4-dimethoxy-8-vinylphenanthrene (XXXVII), which can be converted through the acid (XXXVIII) and subsequently through the amide (XXXIX), the amine (XL), and the phenol (XLI) into 3,4,8-trimethoxyphenanthrene (XLII). The structure for apomorphine was confirmed by the total synthesis of the dimethyl ether (XXXIV) using a Pschorr phenanthrene cyclization process (XLV \rightarrow XXXIV) (65).
In 1966, a fundamentally new aporphine synthesis was reported by Cava et al. (66). In this synthesis, a structurally simpler aporphine precursor was used and the key synthetic step was a special application of the stilbene-phenanthrene photocyclization. The reaction of 1-benzyl-6,7-dimethoxy-3,4-dihydroisoquinoline (XLVIII) with ethyl chloroformate gave 1-benzylidene-2-carbethoxy-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (XLIX) in 67% yield, which was then irradiated in ethanol solution in the presence of iodine to yield N-carbethoxy-6a,7-dehydroornuciferine (L) in 15% yield. This carbethoxy derivative was then reduced with lithium aluminum hydride in ether to yield 6a,7-dehydroornuciferine (LI) in 70% yield. A subsequent catalytic reduction of the dehydro compound in acetic acid using platinum oxide catalyst afforded dl-nuciferine (LII) in 70% yield.
In the same year, Kupchan and Kanojia (67) reported a new application of the photolysis of iodoaromatic compounds to effect photocyclization of aporphines. Condensation of a phenethylamine (LIII) with the iodo-acid chloride (LIV) gave the amide (LV) which underwent a Bischler-Napieralski ring closure with polyphosphate ester to a 1-benzyl-3, 4-dihydroisoquinoline derivative (LVI). The tetrahydroisoquinoline (LVIII) derivative was prepared by reducing the methiodide of the 3, 4-dihydroisoquinoline (LVII) with sodium borohydride in methanol. An aqueous solution of the hydrochloride salt of the resulting tetrahydroisoquinoline (LVIII) was irradiated, whereupon photocyclization to the aporphine hydrochloride (LIX) was effected. An alternative approach consisted of irradiating an acetylated or benzoylated tetrahydroisoquinoline (LXI) in cyclohexane solution to yield the corresponding acylnoraporphine (LXII).

\[ \text{LIV} \rightarrow \text{LV} \rightarrow \text{LVI} \rightarrow \text{LVII} \rightarrow \text{LVIII} \rightarrow \text{LIX} \]
Papaverine (LXIII), a tertiary benzylisoquinoline alkaloid, is one of the principal alkaloids of opium. When papaverine is fused with potassium hydroxide, two substances $C_{11}H_{11}O_2N$ and $C_9H_{12}O_2$, are formed, and these two account for the twenty carbon atoms of the alkaloid. The substance $C_{11}H_{11}O_2N$ is basic and on demethylation and distillation with zinc dust is converted into isoquinoline (LXVI). On oxidation it affords m-hempinic acid (LXVII). The base $C_{11}H_{11}O_2N$ was thus found to be 6,7-dimethoxyisoquinoline (LXIV) and this identity was confirmed by synthesis. The compound $C_9H_{12}O_2$ was easily oxidized to veratric acid (LXVIII) by permanganate, and was thus found to be 3,4-dimethoxy-toluene (LXV). The mode of linkage of these two units in papaverine was shown by the oxidation of the alkaloid to 6,7-dimethoxy-isoquinoline-1-carboxylic acid (LXIX), in which the position of the hydroxyl group is shown by further oxidation to pyridine-1,2,3-tricarboxylic acid (LXX).
Synthesis of papaverine was accomplished by subjecting the amide (LXXVI) (synthesized by the route (LXI) \(\rightarrow\) (LXXVI)) to Bischler-Napieralski isoquinoline ring closure. The resulting 3,4-dihydropapaverine (LXXVII) may be then dehydrogenated to papaverine (LXIII). Reduction of papaverine (LXIII) with tin and hydrochloric acid yields a tetrahydrobenzylisoquinoline derivative (LXXVIII). Thus, a general synthesis of benzylisoquinolines and tetrahydrobenzylisoquinolines has been effected (68).
Several methods of degradation have been applied to the study of the bisbenzylisoquinoline alkaloids. The two most important are the fission of the molecule into two fragments by oxidation, before or especially after Hofmann degradation and fission at the diphenyl ether linkage only, by sodium and liquid ammonia, to give two simple benzylisoquinolines.

Hofmann degradation of the methyl ether of oxyacanthine (LXXIX) gave a methine (LXXX), which on oxidation afforded an acid (LXXXI), which showed that the phenolic hydroxyl group in oxyacanthine was ortho to the ether linkage. The methine base on ozonolysis yielded the aldehydes (LXXXII and LXXXIII); of these, (LXXXII) on exhaustive methylation afforded nitrogen free product (LXXXIV), which when subjected to reduction, catalytically (LXXXV) and by Clemmensen's method, yielded (LXXXVI), identical with a synthetic specimen.
The reduction of O-methyloxyacanthine (LXXXVII) with sodium in liquid ammonia yielded two phenolic cleavage products (LXXXVIII) and (LXXXIX) that were structural isomers. Identification of these fragments provided evidence for the structure of the alkaloid, as well as for steric configurations of the asymmetric centers. On compound was identified as the (+)-phenol (LXXXVIII), the enantiomer of the alkaloid armepavine. The other product (LXXXIX) was levorotatory and with diazomethane afforded the trimethoxy compound (XC). This, then, established the constitution of oxyacanthine.
Theoretically, hydrogenolysis of the diphenyl ether linkage can take place in two ways, depending on which "side" of the oxygen atom the fission occurs. In actual practice, it has been found that the fission of bisbenzylisoquinoline alkaloids occurred predominantly in one way to form fragments of the coclaurine (XCI) type. This was especially true when the cleavage was carried out in benzene or toluene as a solvent.

Two different methods have been employed in attempts to synthesize bisbenzylisoquinoline alkaloids (XCIV). In the first, two 1-benzyl-2-methyl-1,2,3,4-tetrahydroisoquinoline units (XCII and XCIII) are joined together with ether bridges by the Ullman reaction.
In the second method, two appropriately substituted diphenyl ethers are first synthesized, one containing two \(-\text{CH}_2\text{CH}_2\text{NH}_2\) groups (XCV) and the other two \(-\text{CH}_2\text{COOH}\) groups (XCVI). These two ethers are then united through amide linkages (XCVII) and subsequently converted to isoquinoline rings (XCVIII and XCIX) (69, 70, 71, 72).
The benzylisoquinoline-aporphine dimer thalicarpine (C) underwent a Hofmann degradation to a Hofmann methine (CI), which underwent a second Hofmann degradation to yield a des-N-methine (CII). Sodium and liquid ammonia cleavage split the molecule into three smaller moieties (CIII + CIV + CV), two of which were non-phenolic bases (CIII and CIV) and the third a phenolic base (CV). The yield of (CV) was the same regardless of the reaction conditions but the ratio of the yields of (CIV) and (CIII) varied according to the conditions used for the reaction. The stronger the conditions used, the more compound (CIII) was obtained. This fact suggested that (CIV) and (CIII) were initially formed in the reaction and that (CIV) was further transformed into (CIII)(73).
Mollov and Dutchevska (74) were the first to use permanganate oxidation on benzylisoquinoline-aporphine dimers. Treatment of thalicarpine (C) with potassium permanganate in acetone yielded two products, an isoquinolone derivative (CVI) and a highly conjugated aromatic aldehyde (CVII).

Thalicarpine (C) was synthesized from L-(+)-6'-bromolaudanosine (CVIII) and L-(+)-N-methyllaurotetanine (CIX) (75).
D. Biosynthesis of the Representative Alkaloid Types

There are several simple 1, 2, 3, 4-tetrahydroisoquinolines which are plausibly derived by a Mannich reaction involving a hydroxylated phenylalanine or phenethylamine (CX) and an appropriate aldehyde (CXI). Salsolidine (CXII) is an example of this type of reaction.

A large number of alkaloids may be hypothetically derived from norlaudanosoline (CXV) which is plausibly formed from 3, 4-dihydroxyphenylalanine (CXIII) and 3, 4-dihydroxyphenylacetaldehyde (CXIV). Oxidation and methylation of norlaudanosoline (CXV) yields papaverine (CXVI). Oxidative coupling of the two phenolic rings of norlaudanosoline yields the aporphine alkaloids which can have two possible hydroxylation patterns represented by the alkaloids corydine (CXVII) and dicentrine (CXVIII). All of these biosynthetic precursors mentioned for our purpose are the simplest substances necessary to yield the final product and are not necessarily the actual biological precursors.
Many of the bisbenzylisoquinoline alkaloids, such as magnoline (CXXII) and berbamine (CXXIII), are plausibly formed by the oxidative coupling of two molecules of the trihydroxyisoquinoline (CXXI) which could be formed from 3, 4-dihydroxyphenylalanine (CXIX) and tyrosine (CXX).

\[(\text{CXIX}) \rightarrow (\text{CXX}) \rightarrow (\text{CXXI}) \rightarrow (\text{CXXII}) \rightarrow (\text{CXXIII})\]
Reaction of norlaudanosoline (CXXIV) with formaldehyde, or its biological equivalent, in a Mannich reaction leads to isomers (CXXV) and (CXXVI) which may be regarded as precursors of the alkaloids canadine (CXXVII) and coreximine (CXXVIII), respectively. However, the majority of alkaloids of this type are derived from the isomer (CXXV).
Oxidation of the isomer (CXXV) yields compound (CXXIX) which on alkylation affords berberine (CXXX). Oxidative cleavage of a C-N bond in the protoberberine (CXXV) affords another structural type, represented by the alkaloid protopine (CXXXI). Almost all the benzylisoquinoline and related alkaloids have a hydroxylation pattern which is consistent with their formation from 3,4-dihydroxy-phenylalanine or tyrosine (76).
E. Literature survey of *Thalictrum* alkaloids since 1965

The literature prior to 1965 has been reviewed by Fong (184) and Schiff (185), all of the alkaloids reported to date are listed in Table 1.

In late 1964, the reinvestigation of the structures of thalicrine (CXXXII) and homothalicrine (CXXXIII) by Fujita et al. (77) resulted in the revision of their structures to aromoline (CXXXIV) and O-methyl-aromoline (homoaromoline) (CXXXV) respectively.

![Chemical structures](image)

R=H=thalicrine (CXXXII)  
R=CH₃=homothalicrine (CXXXIII)

R=H=aromoline (CXXXIV)  
R=CH₃=homoaromoline (CXXXV)

From Moldavian *Thalictrum minus* in the U.S.S.R., Kuchkova et al. (78) obtained thalictrime. In addition, it was observed that the distribution of the alkaloids in the plant varied during various phases of vegetation. In the above ground portion of the plant the alkaloid content was maximum during June (flowering period) and in the roots during August (fruited period).
Telezhenetskaya and Yunusov (79) cleaved the alkaloid thalmine (CXXXVI) from *Thalictrum minus*, with sodium and liquid ammonia to 1-(4-methoxybenzyl)-2-methyl-6-methoxy-tetrahydroisoquinoline (CXXXVII). Similar cleavage of O-ethylthalmine (CXXXVIII) gave 1-(4-hydroxybenzyl)-6-ethoxy-7-methoxy-N-methyltetrahydroisoquinoline (CXXXIX) as the oxalate salt and 1-(4-methoxybenzyl)-6-methoxy-7-hydroxy-N-methyltetrahydroisoquinoline (CXL). On these results, structure (CXXXVI) was suggested for thalmine.
Thalmidine (CXL) was cleaved by sodium in liquid ammonia into O-methylarmepavine (CXLI) and isococlaurine (CXLI) by the same workers (79) and it was thus suggested that thalmidine and O-methylthalicberine (CXLIV) are identical.
Gheorghiu et al. (80) extracted separately the underground parts, stems and leaves of *Thalictrum minus var. elatum* with 1 formic acid and after suitable workup, which included paper chromatography, obtained aconitine and berberine from the underground parts but only aconitine from the leaves. No alkaloids were reported isolated from the stems.

Ciezynski and Borkowski (81), using ascending paper chromatography, observed the presence of eight alkaloids in extracts of *Thalictrum rugosum* roots. After appropriate fractionations and chromatography on a silica gel column, a tertiary nonphenolic base named rugosine was obtained. In addition, berberine iodide (CXLV), jatrorrhizine iodide (CXLVI) and columbamine iodide (CXLVII) were later found to be present (82).

Two new alkaloids, thalmethine (CXLVIII) and O-methylthalmethine (CXLIX), were isolated from the above ground parts of a Bulgarian variety of *Thalictrum minus* by Mollov and workers (83). O-methylthalmethine (CXLIX), upon treatment with hydrogen and platinum oxide or sodium borohydride gave a dihydro derivative (CL), which on treatment with formaldehyde-formic acid yielded quantitatively the known O-methylthalicberine (CLI).
Reduction of O-methylthalmethine (CXLIX) with sodium in liquid ammonia gave first dihydrothalmethine (CLII), then d-O, O, N-trimethyl coclaurine (CLIII) and dl-isococlaurine (CLIV). Thalmethine (CXLVIII) and diazomethane yielded O-methylthalmethine (CXLIX). Thalmethine (CXLVIII) and sodium borohydride yielded a dihydro derivative (CLV) which upon treatment with formaldehyde-formic acid yielded thalicberine (CLVI).
Shamma et al. (84) isolated two new alkaloids, thalifendlerine (CLVII) and thalifendine (CLVIII), from Thalictrum fendleri in 1965. Thalifendlerine, a benzyltetrahydroisoquinoline, upon treatment with diazomethane gave an O-methyl derivative, identical with the known tetrahydrotakatonine (CLIX). NMR spectrum, mass spectrum and negative Gibbs test showed that the -OH group was in the benzyl moiety. Thalifendlerine (CLVII) was thus shown to be 1-(4-hydroxybenzyl)-2-methyl-5,6,7-trimethoxytetrahydroisoquinoline.

\[
\begin{align*}
\text{m/e 236 (base peak) (CLX)} & \quad \text{m/e 107 (CLXI)}
\end{align*}
\]

Separation of the quaternary, water soluble alkaloid fraction gave, in addition to berberine, jatrorrhizine and magnoflorine, the optically inactive yellow crystalline alkaloid chloride, thalifendine (CLVIII). Hydrogenation of this phenolic protoberberine over platinum oxide gave tetrahydrothalifendine (CLXII), which, upon O-methylation with diazomethane gave tetrahydroberberine (CLXIII). The phenolic function of thalifendine was found to be at C-10 since the alkaloid chloride was clearly different from berberrubine chloride (CLXV), prepared by heating berberine chloride (CLXIV) in an inert atmosphere.
Shamma and Dudock (85) also isolated a 5-hydroxylated berberine which they named thalidastine (CLXVI). Thalidastine chloride (CLXVI), upon heating with 2N hydrochloric acid at 100°C for 15 minutes, or at 85°C in vacuo, or at 20°C in a few weeks, gave deoxythalidastine chloride (CLXVII). In addition, deoxythalidastine bromide was found to be clearly different from dehydroberberrubinium bromide (CLXVIII).

Hydrogenation of thalidastine gave tetrahydrothalidastine (CLXIX) while hydrogenation of deoxythalidastine gave tetrahydrothalifendine (CLXX). Deoxythalidastine (CLXVII), the dehydration product of thalidastine (CLXVI) might be more properly named dehydrothalidastine.

Tomimatsu et al. (171) isolated thalicarpine from T. dasycarpum Fisch. and Lall. var hypoglaucum (Rydb.) Boivin via a petroleum ether extraction of the premoistened ammoniacal roots. In addition, thalicarpine was also isolated from a four year old sample of an evaporated petroleum ether extract of T. revolutum roots.
In 1965, Mollov et al. (86) isolated thalmethine, O-methyl-thalmethine and berberine from a Bulgarian species of Thalictrum minus.

In 1966, Shamma et al. (87) revised the structures of hernandezine (CLXXI) and thalsimine (CLXXII) using nuclear magnetic resonance data, mass spectral data and the identities of sodium and liquid ammonia cleavage products. Sodium in liquid ammonia cleavage of hernandezine (CLXXI) and O-methylthalifendlerine (CLXXIII) gave the same trimethoxy derivative (CLXXIV) whose structure was determined from its nuclear magnetic resonance spectrum. In addition, a sublimable crystalline phenol, N-methyl coclaurine, was obtained (CLXXV).
Thalsimine (CLXXII) has been shown to be closely related to hernandezine (CLXXI), in which one of the C-N-CH$_3$ functions of hernandezine is replaced by the imino-function -C=N-. When thalsimine or 1',2'-dihydrothalsimine (CLXXVI) was cleaved with sodium in liquid ammonia, coclaurine (CLXXVII) was formed.
Tomimatsu and Beal (88) isolated the tertiary phenolic base obamegine from Thalictrum rugosum roots. The alkaloid was characterized via the O, O-dimethyl and O, O-diethyl ethers. Obamegine (CLXXVIII) belongs to the berbamine group of bisbenzylisoquinoline alkaloids.
The Polish workers Borkowski, Frencel and Michniewska (89) studied the comparative chromatography of the complex of quaternary bases from 17 Thalictrum species. Using descending paper chromatography and four different solvent systems, they observed that all 17 species showed almost identical alkaloid composition with magnoflorine and berberine as the principal constituents.

In addition, investigation of phenolic and nonphenolic tertiary alkaloid fractions by Borkowski's group (90) using paper chromatography showed that all 17 species had similar composition.

In 1966, Fong, Beal and Cava (91) reported the isolation of berberine, jatrorrhizine, magnoflorine, hernandezine and a new tertiary base, named thalibrunine from the roots of Thalictrum rochebrunianum. Additionally, a small amount of an incompletely characterized tertiary base was isolated and designated alkaloid A.

The Russian workers, Yunusov and Maekh (92), isolated the alkaloid thalsimine from the leaves and seeds of Thalictrum simplex. This was the second time that this alkaloid had been isolated from this plant.

The Bulgarian workers Mollov and Duchevska (93) studied the permanganate oxidation products of thalicarpine (CLXXIX) and thalmelatine. Oxidation of thalicarpine (CLXXIX) and O-ethylthalmelatine (CLXXXIII) with potassium permanganate in acetone gave, in both cases, an optically inactive aromatic aldehyde (CLXXX) as the main product. Under these conditions, 1-oxo-6,7-dimethoxy-N-methyltetrahydroisoquinoline (CLXXXI) was isolated from the reaction mixture of the former while 1-oxo-6-methoxy-7-ethoxy-N-methyltetrahydroisoquinoline (CLXXXII) was isolated from the latter. Hydrogenation of the aldehyde using Adams
catalyst in acetic acid converted it to a tetrahydro derivative (CLXXXIV) which had no carbonyl band in the infrared spectra but showed characteristic -OH absorption. Treatment of this tetrahydro derivative with sodium in liquid ammonia resulted in 2,10-dimethoxyaporphine (CLXXXV), which also resulted from the cleavage of thalicarpine (CLXXIX) with sodium and liquid ammonia.
Dutschewska and Mollov (94) were the first to demonstrate the presence of dehydrothalicarpine (CLXXXVI), an oxidation product of thalicarpine, in *Thalictrum minus* var. *elatum*. On hydrogenation in acetic acid, dehydrothalicarpine consumed one mole of hydrogen and was converted to thalicarpine (CLXXXIX). The diastereoisomer of thalicarpine expected from this reaction was not mentioned. Sodium in liquid ammonia cleavage of dehydrothalicarpine gave (−)-6'-hydroxy-laudanosine and (+)-2,10-dimethoxyaporphine (CLXXXVIII), both of which were also obtained by sodium in liquid ammonia cleavage of thalicarpine. Oxidation of dehydrothalicarpine with potassium permanganate in acetone gave the same aldehyde (CLXXX) as was obtained in oxidation of thalicarpine and O-ethylthalmeletine. Dehydrothalicarpine is secondarily formed in the alkaloid mixture by aerial oxidation of thalicarpine. Solutions of pure thalicarpine in benzene, chloroform or methanol were observed to darken when left for long periods of time at room conditions.
and finally the presence of dehydrothalicarpine was detected. Dehydrothalicarpine was, however, present in the fresh isolated alkaloid mixture, obtained by treatment of the plant under mild conditions.
Andrew and Bradsher (95) reported the synthesis of deoxythalidastine (CLXXXIX) the deoxy product of the Thalictrum fendleri alkaloid thalidastine. 6, 7-Methylenedioxyisoquinoline-1-carboxaldehyde oxime (CXC) in dimethylformamide at 100°C and 2-methoxy-3-benzyloxybenzyl bromide (CXCl) gave a product which was cyclized directly and simultaneously debenzylated by heating with 48% hydrobromic acid for 10 minutes at 100°C giving a 68% yield of deoxythalidastine bromide (CLXXXIX), identical with the product obtained from natural thalidastine.

In 1966, the Russian workers Telezhenskaya, Ismailov and Yununsov (96) reported that the sodium in liquid ammonia cleavage products of thalmidine were identical to those of O-methylthalicberine. Thus, thalmidine was identified as O-methylthalicberine, as was suspected at an earlier date (79). Additionally, the revised structure of the alkaloid thalmine was elucidated using sodium in liquid ammonia cleavages, Hofmann degradations, ozonolysis and other reactions. In the revised structure (CXCII), the ether bridge was placed at position 5 of the left hand ring instead of position 8, as had been postulated in an earlier structure (CXCII) (79).
The first reference to the alkaloidal components of *Thalictrum tuberiferum* appeared in 1965, when Chi (97) isolated berberine as its chloride salt and reduced it to tetrahydroberberine by the use of zinc powder in acetic acid.

Mollov and Georgiev (98) reported the isolation of thalifoetidine (CXCIV), a new bisbenzylisoquinoline alkaloid from *Thalictrum foetidum* in 1966. Methylation of thalifoetidine with diazomethane and subsequent sodium in liquid ammonia cleavage resulted in d-O, O, N-trimethylcoclaurine (CXCV) and a diphenolic base (CXCVI), which on methylation with diazomethane gave methylthalifendlerine (CXCVII). On the basis of these studies Mollov and Georgiev proposed a tentative structure for thalifoetidine (CXCIV).
Mollov et al. (99) isolated thalsimine (CXCVIII) and hernandezine (CXCIX) from the alkaloid mixture of Thalictrum simplex using alumina chromatography. Hydrogenation of thalsimine (CXCVIII) over platinum and subsequent methylation with formaldehyde-formic acid yielded hernandezine (CXCIX). Cleavage of thalsimine with sodium in liquid ammonia gave, in addition to coclaurine (CC), the same nonphenolic compound (CCI) obtained by cleavage of hernandezine.
In the most recent study concerning the alkaloids of *Thalictrum fendleri*, Shamma et al. (186) reported the isolation and characterization of three new tertiary phenolic alkaloids from extracts of the whole plant. The tertiary alkaloids, as their hydrochloride salts, were subjected to partition chromatography on a cellulose column, using methyl ethyl ketone-water as a solvent system.

Thalidezine (CCII), a new bisbenzyl tetrahydroisoquinoline alkaloid, upon treatment with ethereal diazomethane was converted to hernandezine (CCIII). Cleavage of O-ethylthalidezine (CCIV) with sodium in liquid ammonia yielded a major and a minor nonphenolic product. Mass spectral and nuclear magnetic resonance studies of these products showed that the major product was 1-(4-methoxybenzyl)-2-methyl-5-ethoxy-6,7-dimethoxytetrahydroisoquinoline (CCV) and the minor product was 1-(4-methoxy benzyl)-2-methyl-5-ethoxy-7-methoxy-tetrahydroisoquinoline (CCVI).

![Chemical structure of Thalidezine (CCII) and Hernandezine (CCIII)]
The mass spectrum of the major nonphenolic product (CCV) exhibited a molecular ion at m/e 371, in accordance with the formula $C_{22}H_{29}O_4N$, a base peak at m/e 250 (CCVII) and an important peak at m/e 121 (CCVIII).
The mass spectrum of the minor nonphenolic product (CCVI) exhibited a molecular ion at m/e 341, corresponding to the formula C_{21}H_{27}O_{3}N, a base peak at m/e 220 (CCIX) and an important peak at m/e 121 (CCVIII).

The mass spectrum of thalidezine itself showed a molecular ion at m/e 639, corresponding to the formula C_{38}H_{42}O_{7}N_{2} and two intense peaks at m/e 411 (CCX) and m/e 192 (CCXI).

The other two new phenolic alkaloids, thaliporphine (CCXII) and preocoteine (CCXIII), were aporphines and were characterized by their nuclear magnetic resonance spectra, mass spectra and their O-methylation products.
In addition, the known tertiary alkaloids hernandezine (CCXII) and thalicarpine were isolated.

In a most recent communication, Kuchkova et al. (187) (CCXIV) reported the isolation of L-canadine-β-chloromethylate (CCXIV) from *Thalictrum minus*.

![Chemical structures](image-url)
F. Ultraviolet Spectra of Thalictrum Alkaloids

A review of the ultraviolet absorption spectra in general, including the ones reviewed here was recently presented by Sangster and Stuart (100).

The term isoquinoline, when applied to alkaloids, is often loosely used to denote the tetrahydroisoquinoline ring system. With the exception of papaverine, all the alkaloids of these types are tetrahydroisoquinolines.

The ultraviolet spectra of most tetrahydroisoquinoline alkaloids are characteristic of one or more nonconjugated aromatic rings with maxima at approximately 285mu (log $\varepsilon$ 3.7). Where the aromatic rings are joined through an oxygen atom, the spectra are not very different from nonconjugated type spectra, indicating that the benzene rings are not significantly coupled. Papaverine with an isoquinoline ring and a nonconjugated benzene ring is more complex, and the spectrum is similar to that of isoquinoline itself. The three main regions of absorption are at 238mu (log $\varepsilon$ 4.8), 279 (3.8) and 313 - 317 (3.6) (100).

There are a large number of types of bisbenzylisoquinoline alkaloids depending on the points of attachment of the various aromatic rings but their spectra are quite similar with maxima at 283mu (log $\varepsilon$ 3.8) (100).

The aporphine alkaloids can be subdivided by chemical types into the aporphines and noraporphines, the aporphine-benzyltetrahydroisoquinolines, the oxyaporphines and the proaporphines.
It is convenient to classify the simple aporphines into three main ultraviolet types, the spectra being more markedly dependent on the position of the substituent rather than the nature of the substituent. The spectra may be regarded as derived from the basic biphenyl system with the added influence of several auxochromes. Biphenyl has a maximum at 264\textmu m and 2-methylbiphenyl has a maxima at 245\textmu m and 282\textmu m. When positions 10 and 11 are unsubstituted (aporphine type, R = CH$_3$) (CCXV), the spectra show one peak at 270-280\textmu m (log € 4.3) and a shoulder or smaller peak at 310-320\textmu m (log € 3.3) (100).

Most of the aporphine alkaloids are substituted at positions 10 or 11, and all the known alkaloids are invariably substituted at positions 1 and 2. It was recognized that the spectra of the other aporphines fall into two characteristic types: those with position 11 free (boldine type) (CCXVI) and those with position 11 substituted (corydine type) (CCXVII). These spectra show maxima at 220\textmu m and two in the region 270-310\textmu m. The shapes of the curve and the intensity of the latter two maxima depend on the substitution in ring D.
Alkaloids with position 11 free (boldine type) show maxima at 282\(\text{mu}\) and 303-310\(\text{mu}\) (log \(\varepsilon\) 4.2).

Alkaloids with position 11 substituted (corydine type) show maxima at 268-272\(\text{mu}\) (log \(\varepsilon\) 4.2) and another maximum with lowered intensity at 303-310\(\text{mu}\) (log \(\varepsilon\) 3.8) (100).

These differences have been related to the degree of strain in the biphenyl system (176). X-ray analyses of aporphine alkaloids show that the biphenyl ring is appreciably strained (176). The alkaloids can exist in two conformational forms as shown by structures (CCXVIII) and (CCXIX).

Optical rotatory dispersion studies (176) have also been used to confirm the substitution pattern and the assumption that a positive specific rotation at the D-line (589\(\text{mu}\)) is associated with the \(\alpha\)-H (S-series) configuration. The spectra of the aporphines are unchanged in acid, but in basic solution the phenolic aporphines undergo a significant bathochromic shift.

The aporphine-benzyltetrahydroisoquinoline alkaloids (such as thalicarpine and thalmelatine) show maxima at 282\(\text{mu}\) (log \(\varepsilon\) 4.2) and 302\(\text{mu}\) (log \(\varepsilon\) 4.1). If, however, the C ring of the aporphine moiety shows some unsaturation (as in dehydrothalicarpine), the maxima become 268\(\text{mu}\) (log \(\varepsilon\) 4.8) and 331\(\text{mu}\) (log \(\varepsilon\) 4.3) (100).
The oxyaporphine alkaloids, which are yellowish colored, possess a highly unsaturated chromophoric system with extended absorption in the ultraviolet and visible. Liriodenine (CCXX) shows three main absorption bands at 245-270μ (log ε 4.1), 309 (3.6) and 413 (3.8). On acidification, the spectrum is shifted to longer wavelengths with a series of undulating maxima between 325μ and 460μ (log ε 3.5) (100).

\[ \text{(CCXX)} \]

The alkaloids of the proaporphine group show two main maxima at 230μ (log ε 4.4) and 290μ (log ε 3.5), with occasional splitting of the peaks. The spectra of the proaporphine system (CCXXI) have been shown to be consistent with the addition of the spectra of homoveratrylamine (CCXXII) and 4-methyl-3-allylcyclohexa-2,5-dien-1-one (CCXXIII). The 290μ band shows a bathochromic shift in alkali. The known proaporphines rearrange to aporphines on acid treatment, the latter having the characteristic 11-unsubstituted aporphine spectra (100).

\[ \text{(CCXXI)} \]

\[ \text{(CCXXIII)} \]
The protoberberines can be subdivided into four subgroups: the protoberberines, the tetrahydroprotoberberines, the oxyprotoberberines and the dihydroprotoberberines.

The yellow alkaloid berberine (CCXXIV) is typical of the alkaloids of the protoberberine group. Berberine chloride (CCXXIV) shows spectra with three maxima at 267mu, 347 and 426 (log ε 4.45, 4.42 and 3.75), the band at 426mu is in keeping with the fact that the alkaloid is yellow in the visible. In alcoholic solution, low concentrations of alkali change the spectrum, and at a concentration of $2 \times 10^{-3}$N or stronger, berberinol is believed to have formed. Berberinol (CCXXV) has maxima at 280mu and 362mu, and in 0.25N alcoholic potassium hydroxide, berberine shows maxima at 272mu and 353mu which represent a 1:3 mixture of berberine and berberinol (100).

The limited reduction of berberine yields tetrahydroberberine (CCXXVI), which shows maxima at 209mu and 284 (log ε 4.45, 3.71) and a shoulder at 230mu (log ε 4.07). This spectrum is characteristic for members of this group (100).
The only member so far known in the oxyprotoberberine group is berlambine, isolated from the *Berberis* genus and is identical with oxyberberine (CCXX.VII) (100).

\[ \text{(CCXXVII)} \]

Lambertine (CCXXVIII) is the only representative of the dihydroprotoberberine group, and the spectrum in ethanol shows only a single band at 285mu (log $\varepsilon$ 4.45) (100).

\[ \text{(CCXXVIII)} \]
G. Nuclear Magnetic Resonance Spectra of *Thalictrum* Alkaloids

The following chemical shifts are most often observed for the functional groups of the *Thalictrum* alkaloids:

- N-methyl groups: 2.0 - 3.0 δ
- O-methyl groups: 3.0 - 4.0 δ
- Methylene dioxy groups: 6.0 δ
- Aromatic protons: 6.0 - 9.0 δ

In the aporphine systems (CCXXIX), the chemical shifts of methoxyl groups at C-1 appear at higher fields (3.42 - 3.63 δ) than those at C-2, C-9 or C-10 (3.72 - 3.89 δ). Methoxyl groups at C-11 have intermediate chemical shifts (3.65 - 3.72 δ) (101).

The higher chemical shifts of methoxyl groups at positions 1 and 11 of the aporphine nucleus compared to those at positions 2, 3, 9 and 10 have been attributed to the hindrance associated with positions 1 and 11 in the nonplanar diphenyl system (102, 103).

With good resolution, the methylene dioxy function when at C-1 and C-2 of the aporphine system gives rise to two doublets corresponding to a small difference in chemical shifts between the nonequivalent protons due to the twisted biphenyl system. The two doublets are usually centered near 5.87 δ and 6.02 δ (101).
The aromatic hydrogen at C-11 in the aporphine system is found downfield between 7.57 $\delta$ and 8.05 $\delta$, while the other hydrogens at C-3, C-8 and C-9 are located relatively upfield between 6.38 $\delta$ and 7.05 $\delta$ and cannot be easily differentiated from one another (101).

Bick et al. (104) showed that in the NMR spectra of bisbenzyl-losquinoline alkaloids (CCXXX), methoxyl groups at 7-positions of the isoquinoline moiety have consistently higher chemical shifts (3.02 $\delta$ - 3.20 $\delta$) than those at other positions.

In comparison with the N-methyl groups of thalicarpine (CCXXXI) at 2.48 $\delta$ and 2.50 $\delta$, the absorption of thalicarpine's aldehydic oxidation product (CCXXII) is shifted to lower frequencies (2.99 $\delta$) on account of the influence of the adjacent double bond. The absorption of the proton at position 11 is shifted from 8.28 $\delta$ in thalicarpine to 9.23 $\delta$ in the aldehydic oxidation product. This shift is due to the already established dependence of the shielding of o-protons in diphenyls on the dihedral angle between the planes of the benzene rings. In the case when the dihedral angle is zero, the frequency shift is the largest (105).
As a result of the change of the dihedral angle between the planes of the two aromatic rings of the apropine part and the formation of a planar phenanthrene system in the alkaloid dehydrothalicarpine (CCXXXIII), the proton at C-11, which in thalicarpine (CCXXXI) absorbs at 8.28 δ, is shifted to much lower frequency at 9.38 δ (106).
Examination of the NMR spectrum of (CCXXXIV), indicated that ring C exists, preferentially, in that conformation which permits minimum steric interaction with ring A. Thus, the aromatic hydrogens of ring A appear as a broad singlet at $6.61 \pm 0.02 \delta$ whereas the two methoxyl groups are found at almost equivalent positions of $3.78\delta$ and $3.84\delta$. The spectrum of the corresponding N-methyl derivative (CCXXXV), however, shows that the 2-methyl group exerts a steric repulsion on ring C which is sufficient to force ring C close to ring A. Thus, while the hydrogen at C-5 appears in the normal position of $6.57\delta$, that at C-8 is shifted upfield as a result of shielding by ring C to $5.99\delta$. The methoxyl group at C-7 is similarly affected, appearing at $3.52\delta$ (107).

Tomita et al. (108) demonstrated that in 1-benzyl-1,2,3,4-tetrahydroisoquinoline system, the necessary requirements for being in the conformational state (CCXXXVI) (i.e., for diamagnetic ring current of the benzyl benzene to affect C-8 protons positively) are the presence of the methyl amine and 7-alkoxyl groups. Influence of 6 and 4' substituents is negligible, and in case of 7-hydroxyls the effect is to a smaller extent, although detectable. Further, introduction of the substituents at C-8 position of the system, results in the appearance of a
diamagnetic effect of the benzyl-benzene ring on the methyl amine protons, and the direction of the effect reverses as shown in (CCXXXVII).

(CCXXXVI)   (CCXXXVII)
H. Mass Spectra of *Thalictrum* Alkaloids

The potential importance of mass spectrometry as a technique for structure determination in the 1-benzyl-1,2,3,4-tetrahydroisoquinoline alkaloid field has recently been revealed (109, 110, 111). It is known that benzyltetrahydroisoquinoline alkaloids (CCXXXVIII) show a very characteristic fragmentation pattern (CCXXXVIII → CCXL + CCXL) in which cleavage occurs readily between the benzyl and the isoquinoline portions of the molecule. This fragmentation is associated with the ease of formation by fission of a bond which is at the same time doubly benzylic and beta to a basic nitrogen atom.

![Chemical Structures](attachment:chemical_structures.png)

The mass spectrum of dauricine (CCXLII), a bisbenzyltetrahydroisoquinoline tail to tail dimer, shows a fragmentation similar to that presented above i.e., cleavage of the carbon-carbon bond which is both beta to nitrogen and beta to two aromatic systems. The base peak in the mass spectrum of dauricine is found at m/e 206 (CCXLIII). Loss of a methyl radical from (CCXLIII) gives an ion m/e 191, and successive loss of a hydrogen gives a fragment ion at m/e 190.
Attachment of two tetrahydroisoquinoline rings by an ether linkage and the benzylic rings by another ether linkage results in a cyclic, head to head bisbenzyltetrahydroisoquinoline base, of which oxyacanthine (CCXLIV) is an example. A greatly increased intensity of the molecular ion peak in the mass spectrum of oxyacanthine compared with dauricin results because oxyacanthine must cleave two bonds, except for carbon-hydrogen, in order to form an abundant fragment with mass less than the molecular weight. The most intense peak in the mass spectrum of oxyacanthine is found at m/e 198 (CCXLV) with an isotope peak at m/e 198.5. This fragment is formed by two cleavages similar to the formation of m/e 206 from dauricin, with two groups retained on the tetrahydroisoquinoline groups.
Two peaks of low relative intensity which are found in the high-mass region of the spectrum are of structural significance. A fragment a m/e 501, loss of 107 mass units, corresponds to loss of one benzylic group plus a hydrogen atom (CCXLVI) and/or (CCXLVII).
A fragment at m/e 416 (M-192) corresponds to loss of one tetrahydroisoquinoline unit plus a hydrogen atom (CCXLVIII).

Obamegine (CCXLIX) differs from oxyacanthine by the reversal of the ether linkage between the two benzylic groups. The mass spectrum of obamegine shows the same major fragmentations as described for oxyacanthine (109, 110).

A typical member of the berbamine group of bisbenzyltetrahydroisoquinoline alkaloids is isotetrandrine (CCL). Characteristic fragmentations for both the berbamine and oxyacanthine group of bisbenzyltetrahydroisoquinoline alkaloids may be seen using isotetrandrine as an example (111).
A peak common to all oxyacanthine-berbamine type alkaloids appears at m/e 174, but the genesis and composition are not apparent.

Another example of the head to head diphenyl ether linkage is the alkaloid O-methylthaliciberine (CCLI), which differs from oxyacanthine and obamegine in the position of the ether linkage between the two tetrahydroisoquinoline units (6 to 8 instead of 7 to 8). By comparing the mass spectrum of O-methylthaliciberine with oxyacanthine, it is possible to recognize from the peaks that the tetrahydroisoquinoline half of O-methylthaliciberine is similar to oxyacanthine. The similarity and the difference in molecular weights (622 vs 608, 14 mass units) are characteristic of the fact O-methylthaliciberine has a methoxyl group in the benzyl half of the molecule rather than a hydroxyl group as in oxyacanthine.
In summary, mass spectroscopy can be used to recognize different types of bisbenzyltetrahydroisoquinoline alkaloids and to determine whether substituents are in the tetrahydroisoquinoline part of the molecule or in the benzyl part. Mass spectrometry is less sensitive to the differences in attachment such as exist in oxyacanthine, obamegine and O-methylthalicberine (109).
### TABLE 1

Physical and Chemical Properties of the Thalictrum Series of Alkaloids

<table>
<thead>
<tr>
<th>Alkaloid</th>
<th>Formula</th>
<th>Thalictrum species</th>
<th>Plant part</th>
<th>Melting Point</th>
<th>Specific rotation</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Aconitine</td>
<td></td>
<td>T. minus elatum</td>
<td>l</td>
<td></td>
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<td>80</td>
</tr>
<tr>
<td>Aromoline</td>
<td>$C_{36}H_{39}O_6N_2$</td>
<td>T. thunbergii</td>
<td>r</td>
<td>221-222°C</td>
<td>$[\alpha]^{23}_D +341.2$</td>
<td>77, 133, 134, 135, 136</td>
</tr>
<tr>
<td>Berbamine</td>
<td>$C_{37}H_{40}O_6N_2$</td>
<td>T. pedunculatum</td>
<td>wp</td>
<td>157°C</td>
<td></td>
<td>153</td>
</tr>
<tr>
<td>Berberine</td>
<td>$C_{20}H_{18}O_4N_1$</td>
<td>T. dasycarpum Fisch. and Lall. var. hypoglaucum Rydb. Boivin</td>
<td>r</td>
<td>258°C</td>
<td></td>
<td>139</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T. fendleri</td>
<td></td>
<td></td>
<td></td>
<td>84</td>
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<td></td>
<td></td>
<td>T. flavum</td>
<td>r</td>
<td></td>
<td></td>
<td>140, 141</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T. foliolosum</td>
<td>rh</td>
<td>260°C</td>
<td></td>
<td>142</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T. minus var. adiantifolium</td>
<td>r</td>
<td>262°C</td>
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<td>145</td>
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<td></td>
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<td>T. minus elatum</td>
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<td></td>
<td></td>
<td>T. rochebrunianum</td>
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<td>261-262°C d.</td>
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<td>T. rugosum</td>
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<td></td>
<td>$C_{20}H_{18}O_4NCl$</td>
<td>T. foliolosum</td>
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<td>T. tuberiferum</td>
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<td>Compound</td>
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<tr>
<td>Tetrahydroberberine C$<em>{20}$H$</em>{18}$O$_4$N</td>
<td>T. pedunculatum wp Edgew.</td>
<td>145°C</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>T. revolutum r</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T. minus</td>
<td>239-240°C</td>
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<tr>
<td>Tetrahydroberberine (not isolated-synthetic sample) C$<em>{20}$H$</em>{21}$O$_4$N</td>
<td>T. acteaefolium r</td>
<td>169-170°C</td>
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<tr>
<td>Columbamine C$<em>{20}$H$</em>{20}$O$_4$NI</td>
<td>T. rugosum r</td>
<td></td>
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<tr>
<td>Dehydrothalicarpine C$<em>{41}$H$</em>{46}$N$_2$O$_8$</td>
<td>T. minus var. elatum</td>
<td>180-182°C</td>
<td>[a]$^{22}_D + 54°$ (MeOH-Et$_2$O) (c 1, CHCl$_3$)</td>
<td></td>
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<tr>
<td>Elatrine C$<em>{40}$H$</em>{56}$O$_6$N$_3$</td>
<td>T. minus var. elatum wp</td>
<td>180-183°C D.</td>
<td>[a]$^{+228, 94}_D$ (EtOH)</td>
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<tr>
<td>picrate</td>
<td>T. minus var. elatum wp</td>
<td>174-175°C</td>
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<tr>
<td>picrolopaneate</td>
<td>T. minus var. elatum wp</td>
<td>178-182°C</td>
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<tr>
<td>Fetidine C$<em>{41}$H$</em>{50}$O$_8$N$_2$</td>
<td>T. foetidum ag</td>
<td>132-135°C (EtOAc)</td>
<td>[a]$^{15}_D + 121.4°$ (c 2.57, MeOH)</td>
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<tr>
<td>hydrochloride</td>
<td>T. foetidum ag</td>
<td>228-230°C D.</td>
<td>[a]$^{20}_D -30.9°$</td>
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<td>Melting Point (°C)</td>
<td>Optical Rotation ([α]D)</td>
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<td>Nitrate</td>
<td>T. foetidum</td>
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<td>Hydrobromide</td>
<td>T. foetidum</td>
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<td>Sulfate</td>
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<td>Methiodide</td>
<td>T. foetidum</td>
<td>210-215°C</td>
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<tr>
<td>Hernandezine C39H44O7N2</td>
<td>T. hernandezii</td>
<td>192-193°C (hexane)</td>
<td>[α]20+250</td>
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<td>157-158°C (MeOH)</td>
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<td>158-159°C (Et₂O)</td>
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<td>122-124°C (Me₂CO)</td>
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<td>T. fendleri</td>
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<td>162-163.5°C (MeOH)</td>
<td>[α]20+228°</td>
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<td>T. rochebrunianum</td>
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<td>138-140°C</td>
<td>[α]20+220°</td>
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<td>[α]21+425.3°</td>
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<td>Homoaromoline C37H40O₆N₂</td>
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<td>Jatro-rrhizine C20H20NO₄I</td>
<td>T. fendleri</td>
<td>228°C</td>
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**References:** 149, 87, 91, 87, 91, 99, 77, 133, 134, 135, 84,
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<tr>
<td>C$<em>{20}$H$</em>{20}$NO$_4$Cl</td>
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<td>T. revolutum r</td>
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<td>T. rochebruiumum r</td>
<td>248-249°C</td>
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<tr>
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<td>T. rugosum r</td>
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<td>T. rochebruiumum r</td>
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<td>T. dasycarpum var. Fisch. Lall. r</td>
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<td>hypoglaucum Rydb. Boivin</td>
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<td>248-249°C d.</td>
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<td>T. foliolosum rh</td>
<td>208°C</td>
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Temperature values in °C, d. = decomposition.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Species</th>
<th>Form</th>
<th>mp or dmp (°C)</th>
<th>Optical Rotation (°)</th>
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<tr>
<td>C_{20}H_{24}O_{4}NOH · 3H_{2}O</td>
<td>T. foliolosum</td>
<td>rh</td>
<td>[a]<em>{D}^{25} +308 (c 1, H</em>{2}O)</td>
<td>142</td>
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<tr>
<td>C_{20}H_{24}O_{4}N picrate</td>
<td>T. thunbergii</td>
<td>r</td>
<td>230-231°C d.</td>
<td>155, 156</td>
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<tr>
<td>Obamegine C_{36}H_{38}N_{2}O_{2}</td>
<td>T. rugosum</td>
<td>r</td>
<td>[a]<em>{D}^{241} (CHCl</em>{3})</td>
<td>88</td>
</tr>
<tr>
<td>O-methylthalicberine</td>
<td>T. thunbergii</td>
<td>r</td>
<td>186-187°C d.</td>
<td>79, 158, 159, 161, 162, 160, 163, 164</td>
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<tr>
<td>(also see thalmidine)</td>
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<tr>
<td>O-methylthalmethine C_{37}H_{38}N_{2}O_{6}</td>
<td>T. minus</td>
<td>ag</td>
<td>245-246°C (C_{6}H_{6})</td>
<td>[a]<em>{D}^{21} +237 (c 1, CHCl</em>{3})</td>
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<tr>
<td>Dihydro-O-methylthalmethine</td>
<td>T. minus</td>
<td>ag</td>
<td>278-280°C (C_{6}H_{6})</td>
<td>83</td>
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<td>(synthetic sample)</td>
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<tr>
<td>Tetrahydropalmatine C_{21}H_{25}O_{4}N</td>
<td>T. foliolosum</td>
<td>rh</td>
<td>143°C (MeOH)</td>
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</tr>
<tr>
<td>Preocoteine C_{21}H_{25}O_{5}N</td>
<td>T. fendleri</td>
<td>wp</td>
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<tr>
<td>Rugosine C_{20}H_{27}NO_{5}</td>
<td>T. rugosum</td>
<td>r</td>
<td>110-115°C</td>
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<tr>
<td>picrate</td>
<td>T. rugosum</td>
<td>r</td>
<td>168-170°C d (EtOH)</td>
<td>81</td>
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<tr>
<td>perchlorate</td>
<td>T. rugosum</td>
<td>r</td>
<td>217-219°C d (dil. MeOH)</td>
<td>[a]_{D}^{23} 0° (c 0.2, MeOH)</td>
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<td>M.p.</td>
<td>[a] D (c, solvent)</td>
<td>Ref.</td>
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<td>methiodide</td>
<td>T. rugosum</td>
<td>214-216°C</td>
<td>[a] D +135° (c. 0.2, MeOH)</td>
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<td>Takatone C_{21}H_{24}O_{4}Ni</td>
<td>T. thunbergii</td>
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<td>T. minus</td>
<td>192-193°C (EtOH-Pet ether)</td>
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<tr>
<td>Tetrahydro-takatone C_{21}H_{27}O_{4}N</td>
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<td>192-193°C</td>
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<tr>
<td>(not isolated-synthetic sample)</td>
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<tr>
<td>hydrobromide</td>
<td>T. thunbergii</td>
<td>184-185°C</td>
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<td>picrate</td>
<td>T. thunbergii</td>
<td>141.5-143°C</td>
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<tr>
<td>Thalibrunine C_{39}H_{46}O_{8}N_{2}</td>
<td>T. rochebrunianum</td>
<td>172-173°C</td>
<td>[a] D +160° (c 0.9, MeOH)</td>
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<td>[a] D +119.4° (c 0.68, CHCl_3)</td>
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<td>Thalicarpine C_{41}H_{48}O_{8}N_{2}</td>
<td>T. dasycarpum Fisch. Lall.</td>
<td>160-161°C</td>
<td>[a] D +89° (c 0.88, CHCl_3)</td>
<td>166, 167</td>
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<td>[a] D +133° (c 0.83, MeOH)</td>
<td>168, 75</td>
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<td>T. dasycarpum Fisch. Lall. var.</td>
<td>129-130°C</td>
<td>[a] D +83° (c 0.88, CHCl_3)</td>
<td>171</td>
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<td>hypoglaucum Rydb. Boivin</td>
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<td>159-160°C</td>
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<td>Species</td>
<td>Melting Point</td>
<td>[a]_D</td>
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<tr>
<td>Thalicberine C_{37}H_{40}O_{6}N_{2}</td>
<td>T. minus var. elatum</td>
<td>129-130°C (Me_2CO-Et_2O) 159-160°C (Et_2O)</td>
<td>83°</td>
<td>169, 170</td>
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<td>Thalicididine C_{20}H_{23}O_{4}N</td>
<td>T. thunbergii</td>
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<td>Thalicididine C_{21}H_{25}O_{5}N</td>
<td>T. minus</td>
<td>192-193°C (EtOH)</td>
<td>-84°</td>
<td>147, 173, 175, 176</td>
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<td>Thalicididine C_{21}H_{25}O_{5}N</td>
<td>T. minus</td>
<td>239-240°C d. (sealed tube)</td>
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<td>Thalicididine C_{21}H_{25}O_{5}N</td>
<td>T. minus</td>
<td>222-226°C d. (sealed tube)</td>
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<td>Thalicididine C_{21}H_{25}O_{5}N</td>
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<td>217-217.5°C (EtOH)</td>
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<tr>
<td>Thalicididine C_{21}H_{25}O_{5}N</td>
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<td>137-138°C (MeOH)</td>
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<td>147, 173, 177, 178</td>
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<tr>
<td>Thalicididine C_{21}H_{25}O_{5}N</td>
<td>T. minus</td>
<td>268-270°C</td>
<td>255.3°</td>
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<td>Thalicididine C_{21}H_{25}O_{5}N</td>
<td>T. minus</td>
<td>223-224°C d. (sealed tube)</td>
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<td>Thalicididine C_{21}H_{25}O_{5}N</td>
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<td>258-260°C</td>
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<td>Characteristic</td>
<td>Physical Properties</td>
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<tr>
<td>Methiodide</td>
<td>T. minus</td>
<td>r</td>
<td>236-237°C (sealed tube)</td>
<td>147</td>
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<td>Acetylated product</td>
<td>T. minus</td>
<td>r</td>
<td>191-192°C</td>
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<td>Thalic-opinine</td>
<td>C₂₁H₂₅O₅N</td>
<td>T. isopyroides</td>
<td>wp</td>
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<td>Thalic-thuberine</td>
<td>C₂₁H₂₃O₄N</td>
<td>T. thunbergii</td>
<td>r</td>
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<td>Thalic-trimine</td>
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<td>T. minus</td>
<td>wp</td>
<td>78, 137</td>
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<td>Sulfate</td>
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<td>wp</td>
<td>208-210°C</td>
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<td>Hydrochloride</td>
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<td>177-179°C d.</td>
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<td>Methiodide</td>
<td>T. minus</td>
<td>wp</td>
<td>182-183°C</td>
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<tr>
<td>Nitrate</td>
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<td>wp</td>
<td>178-179°C d.</td>
<td>137</td>
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<td>Thalic-trinine</td>
<td>C₂₁H₂₅O₄N</td>
<td>T. simplex</td>
<td>ag</td>
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<tr>
<td></td>
<td>C₃₈H₄₆O₇N₂</td>
<td>T. simplex</td>
<td>l</td>
<td>182</td>
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<tr>
<td>Thalic-trucarpine</td>
<td>C₄₁H₄₆O₈N₂</td>
<td>T. dasycarpum</td>
<td>r</td>
<td>183</td>
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</tbody>
</table>

**References:**

1. [a] 20D -65.0° (c 3.08, CHCl₃) (150)
2. [a] D +80.9° (CHCl₃) (182)
3. [a] 26D +72° (c 0.89, CHCl₃) (183)
<table>
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<tr>
<th>Compound</th>
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<th>Form</th>
<th>Physical Properties</th>
<th>Optical Data</th>
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<tr>
<td>Thalidase C$<em>{39}$H$</em>{44}$O$_7$N$_2$</td>
<td>T. dasycarpum</td>
<td>r</td>
<td>base resisted crystallization</td>
<td>[α]$_D^{27.5}$ -70° (EtOH)</td>
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<td>oxalate</td>
<td>T. dasycarpum</td>
<td>r</td>
<td>173-174°C</td>
<td>[α]$_D^{26}$ -53° (EtOH)</td>
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<td>picrate</td>
<td>T. dasycarpum</td>
<td>r</td>
<td>175-177°C</td>
<td>[α]$_D^{23}$ -41° (MeOH)</td>
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<td>methiodide</td>
<td>T. dasycarpum</td>
<td>r</td>
<td>182-184°C</td>
<td>[α]$_D^{25}$ +138° (MeOH)</td>
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<tr>
<td>Thalidastine C$<em>{19}$H$</em>{16}$O$_5$NCl</td>
<td>T. fendleri</td>
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<td>above 230°C d.</td>
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<tr>
<td>Deoxythalidastine (not isolated-synthetic sample) C$<em>{19}$H$</em>{14}$O$_4$NCl</td>
<td>T. fendleri</td>
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<td>above 210°C d.</td>
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<td>Tetrahydrothalidastine (not isolated-synthetic sample) C$<em>{19}$H$</em>{19}$O$_5$N</td>
<td>T. fendleri</td>
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<td>201-202°C</td>
<td>85</td>
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<tr>
<td>Thalidezine C$<em>{38}$H$</em>{42}$O$_7$N$_2$</td>
<td>T. fendleri</td>
<td>wp</td>
<td>158-159°C</td>
<td>[α]$_D^{25}$ +235° (CHCl$_3$)</td>
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<tr>
<td>Thalifendine C$<em>{19}$H$</em>{16}$O$_4$NCl</td>
<td>T. fendleri</td>
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<td>above 230°C sinters</td>
<td>optically inactive 84</td>
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<td>Molecular Formula</td>
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<td>Melting Point (°C)</td>
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<tr>
<td>Tetrahydrothalifendine (non isolated synthetic sample)</td>
<td>C_{19}H_{19}O_{4}N</td>
<td>T. fendleri</td>
<td>209-211°C</td>
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<tr>
<td>Thalifenderine</td>
<td>C_{20}H_{22}O_{4}N</td>
<td>T. fendleri</td>
<td>177-178°C</td>
<td>[α]_{D}^{25} -108° (MeOH)</td>
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<tr>
<td>O-methylthalifenderine (not isolated synthetic sample)</td>
<td>C_{21}H_{27}O_{4}N</td>
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<td>195-197°C</td>
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<td>Thalifoetidine</td>
<td>C_{38}H_{42}O_{7}N_{2}</td>
<td>T. foetidum</td>
<td>168-170°C</td>
<td>[α]<em>{D}^{21} -88.6° (C 1, CHCl</em>{3})</td>
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<td>O-methylthalifoetidine (not isolated synthetic sample)</td>
<td>C_{39}H_{44}O_{7}N_{2}</td>
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<td>108-109°C</td>
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<td>C_{20}H_{23}O_{4}N</td>
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<td>170-172°C (MeOH)</td>
<td>Dextrorotatory 186</td>
</tr>
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<td>Formula</td>
<td>Temperature</td>
<td>Specific Rotation</td>
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<td>151-153°C</td>
<td>[a]&lt;sub&gt;D&lt;/sub&gt; -71.02°</td>
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<td>H&lt;sub&gt;2&lt;/sub&gt;O-MeOH 1:3</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O-MeOH 1:3</td>
<td>235-237°C</td>
<td>[a]&lt;sub&gt;D&lt;/sub&gt; +110°</td>
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<td>Thalmelatine</td>
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<td>131-135°C</td>
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<td>EtOH</td>
<td>120-123°C</td>
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<td>Thalmethine</td>
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<td>275-277°C</td>
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<td>Dihydro-thalmethine</td>
<td>C&lt;sub&gt;38&lt;/sub&gt;H&lt;sub&gt;42&lt;/sub&gt;O&lt;sub&gt;6&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;</td>
<td>196-197°C</td>
<td>[a]&lt;sub&gt;D&lt;/sub&gt; +252.2°</td>
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<td>(CHCl&lt;sub&gt;3&lt;/sub&gt;)</td>
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<td>192-193°C</td>
<td>[a]&lt;sub&gt;D&lt;/sub&gt; +252.2°</td>
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<td>O-methyl-thalicberine</td>
<td>(EtOH)</td>
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<td>(CHCl&lt;sub&gt;3&lt;/sub&gt;)</td>
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<td>254-255°C</td>
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<td>147-157°C d.</td>
<td>(EtOH)</td>
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<td>(EtOH)</td>
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<td>233-235°C d.</td>
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<td>209-211°C d.</td>
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<td>T. simplex</td>
<td>se, l</td>
<td>198-218°C d.</td>
<td>92</td>
</tr>
</tbody>
</table>

superscript a: r, roots; ag, above ground; rh, rhizomes; wp, whole plant; t, tops; l, leaves; se, seeds; up, underground parts.
Berberine

Jatrorrhizine

Columbamine

Thalifendine

Palmatine

Tetrahydroberberine
FIGURE 1

The Protoberberine Alkaloids of Thalictrum Species
FIGURE 2

The Aporphine Alkaloids of *Thalictrum* Species
FIGURE 3

The Benzylisoquinoline Alkaloids of Thalictrum Species
R=H=Aromoline (Formerly thalicrine)
R=CH₃=Homoaromoline (Formerly homothalicrine)

Berbamine

Fetidine

Obamegine
Hernandezine

Thalidezine

R=H=Thalicberine       R=CH₃=O-methylthalicberine

R=H=Thalmethine        R=CH₃=O-methylthalmethine
FIGURE 4
The Bisbenzylisoquinoline Alkaloids of *Thalictrum* Species
FIGURE 5

The Aporphine-Benzylisoquinoline Alkaloids of *Thalictrum* Species

**R**=H=Thalmelatine  **R**=CH₃=Thalicarpine

**Dehydrothalicarpine**

FIGURE 6

The Phenanthrene Alkaloid of *Thalictrum* Species

**Thalicthuberine**
II. EXPERIMENTAL

A. Materials

The plant material used in this investigation was the roots and tops of *Thalictrum minus* L. var. *adiantifolium* Hort. (*Ranunculaceae*), which were cultivated and harvested at the College of Pharmacy Drug Garden, The Ohio State University, Columbus, Ohio. The identification of the plant has been verified by Dr. Bernard Boivin. After harvesting, the roots and tops were separated. The roots were washed with water, air dried, oven dried at 60°C and ground to a fine particle size (80-100 mesh) by means of a Wiley mill. The tops were air dried, oven dried at 60°C and ground to a fine particle size (80-100 mesh) by means of a Wiley mill.

B. Methodology—Chemical and Physical Analysis

1. Infrared spectrophotometric analysis. The infrared spectra were taken in either a chloroform solution (8-10%) or a potassium bromide pellet using a Perkin-Elmer Infracord Spectrophotometer, model 237.

2. Ultraviolet spectral analysis. The ultraviolet absorption spectra were determined in methanol or ethanol on a Cary Model 15 recording spectrophotometer. With the exception of alkaloid D, which

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1Botanist, Central Experimental Farm, Plant Research Institute, Department of Agriculture, Ottawa, Ontario, Canada. Herbarium specimens are on file in the College of Pharmacy, The Ohio State University, Columbus, O.
2Perkin-Elmer Corporation, Norwalk, Connecticut
3Allied Physics Corporation, Monrovia, California
was determined in ethanol, all compounds were analyzed also in 0.01N hydrochloric acid and 0.01N methanolic potassium hydroxide.

3. Nuclear magnetic resonance spectrometric analysis. The nuclear magnetic resonance spectra were measured in deuterochloroform on a Varian A-60 Nuclear Magnetic Resonance Spectrometer with tetramethylsilane as an internal standard unless otherwise specified. Where noted, a C-1024 time averaging computer was used.

4. Melting point determinations. Melting points were determined with a Thomas-Hoover Uni-Melt capillary melting point apparatus.

5. Microanalysis. Microanalyses were carried out by Mr. J. Alicine, Metuchen, New Jersey.

6. Mass spectrometric analysis. The mass spectra were taken with MS-9 mass spectrometer.

7. Thin layer chromatographic analysis. Thin layer chromatography was carried out on silica gel G using either 20 x 20 cm. or 5 x 20 cm. glass plates with a thickness of 250μ of adsorbent. The alkaloid spots were revealed by spraying the developed chromatogram with Munier and Machebouef's Modified Dragendorff's spray reagent (112). The alkaloids appeared as orange spots on a yellow background. Spraying of the plates with 1% acetic acid solution decolorized the yellow background but did not affect the orange spots. Unless otherwise specified, the

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4Varian Associates, Paloalto, California
5Arthur H. Thomas Company, Philadelphia, Pennsylvania
6Allied Electrical Industries
7E. Merck Ag., Darmstadt, Germany; Distributor: Brinkman Instruments Inc., Cantiague Road, Westbury, Long Island, New York
solvent system used was benzene-acetone-concentrated ammonium hydroxide (16:16:1).

8. Adsorption chromatographic analysis. The adsorbents used for column chromatography were 100 mesh silicic acid\(^8\) + celite 545\(^{11}\) (4:1) and neutral or basic alumina\(^9\).

9. Ion exchange analysis. The resin used for ion exchange was Amberlite IRA-410(C1), 20-50 mesh\(^{10}\).

10. Optical rotation measurements. The optical rotation of each compound was measured in a Carl-Zeiss polarimeter using a 2 decimeter sample tube. The solvents employed were either chloroform or methanol.

\(^8\)Mallincrockdt Chemical Works, St. Louis, Missouri
\(^9\)M. Woelm, Germany; Distributors: Alupham Chemicals, P.O. Box 30628, New Orleans, Louisiana, 70130
\(^{10}\)Rohm and Haas, Philadelphia; Distributors: Mallincrockdt Chemicals
\(^{11}\)Johns Manville Co.
C. Preliminary Investigations

1. General extraction of the alkaloids. *Thalictrum minus* L. var. *adiantifolium* roots (4.27 kg.) were moistened with a 20% ammonia solution and extracted with petroleum ether (30-60°C) until the extract gave negative tests with Valser's and Dragendorff's reagents. The petroleum ether was evaporated *in vacuo* at 40°C to leave an oily yellow residue. This residue was dissolved in chloroform (500 ml.) and the chloroform evaporated *in vacuo* at 40°C to leave a crude extract (33.0 gm.)

2. Fractionation of the total extract into tertiary and quaternary alkaloid fractions. The crude extract (33.0 gm.) was dissolved in benzene (200 ml.), filtered, and the filtrate shaken with 2% citric acid solution (200 ml.). The citric acid solution was drawn off and the benzene layer shaken again with 2% citric acid (100 ml.). The benzene layer was tested with Valser's and Dragendorff's reagents and found to contain no more alkaloids.

a. Gradient pH separation of the tertiary alkaloids (113)

The citric acid solutions were combined, filtered and the pH of the resulting solution (pH 2.6) was adjusted to pH 2.8 by the drop-wise addition of concentrated ammonium hydroxide solution. The resulting solution was shaken with benzene (300 ml.), the benzene layer removed, dried over anhydrous sodium sulfate, filtered and the solvent removed *in vacuo* at 40°C to leave a residue (0.124 gm.). The pH of the remaining aqueous solution was adjusted to 3.4 as before, and the solution shaken with benzene (300 ml.), and the benzene extract treated as before. This process of pH gradient extraction was repeated at 0.5 pH unit intervals, i.e., 3.4, 3.9, 4.4, 4.9, 5.4, 5.9, 6.4 and 6.9. In addition, an extraction was made at pH 8, following the previous extraction at pH 6.9.
Results are given in table 2. The pH 8 alkaline solution was then adjusted to pH 4.5 with citric acid and a 2% ammonium reineckate solution (filtered and adjusted to pH 4) added with vigorous stirring until precipitation was complete. The mixture was refrigerated overnight and the precipitate filtered by suction, washed with ether and air dried to yield a crude alkaloid reineckate.

**TABLE 2**

*Results of the Gradient pH Separation*

<table>
<thead>
<tr>
<th>pH</th>
<th>Weight (gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.8</td>
<td>0.12</td>
</tr>
<tr>
<td>3.4</td>
<td>0.19</td>
</tr>
<tr>
<td>3.9</td>
<td>0.33</td>
</tr>
<tr>
<td>4.4</td>
<td>0.95</td>
</tr>
<tr>
<td>4.9</td>
<td>2.99</td>
</tr>
<tr>
<td>5.4</td>
<td>3.75</td>
</tr>
<tr>
<td>5.9</td>
<td>2.39</td>
</tr>
<tr>
<td>6.4</td>
<td>0.74</td>
</tr>
<tr>
<td>6.9</td>
<td>0.18</td>
</tr>
<tr>
<td>8.0</td>
<td>0.08</td>
</tr>
</tbody>
</table>

b. Thin layer chromatography fractions from the pH gradient extraction

Thin layer chromatography of the pH gradient fractions revealed the presence of nine alkaloids (Fig. 7) with the following R_f values: 0.93, 0.88, 0.82, 0.71, 0.60, 0.52, 0.45, 0.35, and 0.05.
pH of Fraction

<table>
<thead>
<tr>
<th>pH</th>
<th>2.8</th>
<th>3.4</th>
<th>3.9</th>
<th>4.4</th>
<th>4.9</th>
<th>5.4</th>
<th>5.9</th>
<th>6.4</th>
<th>6.9</th>
<th>8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Front

Solvent system:
benzene-acetone-concentrated ammonium hydroxide
(16:16:1)

The numbers represent the intensity of the stain:
+3 heavy
+2 medium
+1 light

Origin

FIGURE 7
Thin layer chromatogram of the pH gradient fractions
On the basis of the thin layer chromatographic results, the pH gradient fractions were combined into three major fractions: pH 2.8 through 3.9 (fraction I), pH 4.4 through 5.4 (fraction II) and pH 5.9 through 6.9 (fraction III).

3. Separation and isolation of the tertiary alkaloids.

a. Column chromatography of the pH gradient fraction I.

A column (3.8 x 69.5 cm.) was packed in chloroform with a slurry of 100 mesh silicic acid-celite 545 (4:1) (200gm) to a height of 44 cm. in the following manner. The column was mounted on a ring stand via two Liebig clamps and was "leveled" (both front/back and left/right) with the aid of a small carpenters level. The column was one-fourth filled with chloroform. A small glass wool plug was moistened with chloroform, dropped into the column and firmly tamped into the neck above the stopcock with the aid of a long glass rod. Sea sand was added to the point where the neck of the column broadened out and a filter paper disc (Whatman 3 mm.) was tamped into place using a glass rod with a cork (of slightly less diameter than the column) on the end. The silicic acid-celite 545 mixture was slurried in chloroform and poured all at once, in a steady stream, at such an angle that the slurry hit the inside of the column about one-fourth of the way from the top. The adsorbent was allowed to settle for several hours and the stopcock was then opened fully to permit a firm packing. After the adsorbent had settled, the cork tamper was used to apply light manual pressure to the top of the column. A filter paper disc (Whatman 3 mm.) was placed on top of the column, followed by a layer of sea sand (ca. 1 cm.) and finally, a second filter paper disc (Whatman 3 mm.). This method of packing column was followed throughout the course of this investigation. The prepared column is pictured in Figure 8.
Fraction I (640 mg.) was dissolved in chloroform (20 ml.) and the solution was added to the column via a pipette. The stopcock was opened and the flow rate adjusted to approximately one drop every two to three seconds. Just as the last of the solution ran into the column, the walls of the column and the filter paper disc were rinsed with chloroform and elution begun with a gradient elution apparatus (114), using chloroform (2 L.) as the initial solvent and 12% methanol in chloroform (2 L.) as the diluting solvent (Figure 9). Effluent fractions of 20 ml. were collected via an automatic fraction collector.
FIGURE 9

A gradient elution apparatus after Parr
The results of the column chromatography are shown in Table 3.

**TABLE 3**

Results of the column chromatography of the pH gradient Fraction I

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Alkaloid R\textsubscript{f} values</th>
<th>Weight (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 40</td>
<td>NONE</td>
<td>79</td>
</tr>
<tr>
<td>42 - 43</td>
<td>0.84, 0.81, 0.67</td>
<td>20</td>
</tr>
<tr>
<td>43</td>
<td>0.84, 0.81, 0.71, 0.66</td>
<td>13</td>
</tr>
<tr>
<td>44</td>
<td>0.81, 0.71, 0.61</td>
<td>20</td>
</tr>
<tr>
<td>45</td>
<td>0.81, 0.71, 0.61</td>
<td>53</td>
</tr>
<tr>
<td>46 - 60</td>
<td>0.81, 0.61</td>
<td>20</td>
</tr>
<tr>
<td>61 - 62</td>
<td>0.81, 0.72</td>
<td>16</td>
</tr>
<tr>
<td>63 - 66</td>
<td>0.72, 0.56</td>
<td>31</td>
</tr>
<tr>
<td>67 - 92</td>
<td>0.56</td>
<td>109</td>
</tr>
<tr>
<td>93 - 150</td>
<td>0.25</td>
<td>123</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>641</strong></td>
</tr>
</tbody>
</table>

i. The isolation of alkaloid A.

The crude alkaloid (3.6 mg.) crystallized on plates from a methanolic solution of column fraction 45 (33 mg.). This alkaloid was designated alkaloid A. m.p. 150-151°C; $\lambda_{\text{max.}}^\text{MeOH} 349 \text{ m}\mu \left( \log \varepsilon 3.80 \right)$, $\lambda_{\text{max.}}^\text{0.01N Methanolic HCl} 284 \text{ m}\mu \left( \log \varepsilon 4.44 \right)$, $\lambda_{\text{max.}}^\text{0.01N Methanolic KOH} 349 \text{ m}\mu \left( \log \varepsilon 3.80 \right)$, 262 (4.49). The infrared spectrum in a potassium bromide pellet indicated the possibility of an imino function (C = N) at 1639 cm.\(^{-1}\). No other crystalline material was isolated.
b. Column chromatography of the pH gradient Fraction II.

A column (5.0 cm. x 82 cm.) was packed in chloroform with a silicic acid-celite 545 mixture (400 gm.) to a height of 48.5 cm. as previously described. The pH gradient Fraction II (7.69 gm.) was dissolved in chloroform (125 ml.), added to the top of the column and washed into the column with chloroform. A gradient elution apparatus, previously described, was employed using chloroform (2 L.) as the initial solvent and 50% methanol in chloroform (2 L.) as the diluting solvent. 20 ml. fractions were continuously collected via a fraction collector. Thin layer chromatography was performed and the results shown in Table 4.

TABLE 4

Results of the column chromatography of the pH gradient Fraction II

<table>
<thead>
<tr>
<th>Fractions (20 ml.)</th>
<th>Alkaloid Rf values</th>
<th>Weight (gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 96</td>
<td>NONE</td>
<td>0.329</td>
</tr>
<tr>
<td>97</td>
<td>0.81, 0.72, 0.64, 0.47</td>
<td>0.044</td>
</tr>
<tr>
<td>98 - 109</td>
<td>0.72, 0.64, 0.47</td>
<td>0.300</td>
</tr>
<tr>
<td>109 - 112</td>
<td>0.72, 0.64</td>
<td>0.036</td>
</tr>
<tr>
<td>113 - 130</td>
<td>0.72, 0.64, 0.58</td>
<td>0.204</td>
</tr>
<tr>
<td>131 - 156</td>
<td>0.72, 0.58</td>
<td>0.143</td>
</tr>
<tr>
<td>157 - 172</td>
<td>0.72, 0.75</td>
<td>0.113</td>
</tr>
<tr>
<td>173</td>
<td>0.88</td>
<td>0.369</td>
</tr>
<tr>
<td>174 - 178</td>
<td>0.88, 0.81</td>
<td>2.434</td>
</tr>
<tr>
<td>179</td>
<td>0.81, 0.72</td>
<td>0.413</td>
</tr>
<tr>
<td>180 - 186</td>
<td>0.86, 0.81, 0.72</td>
<td>2.347</td>
</tr>
<tr>
<td>187 - 192</td>
<td>0.86, 0.81, 0.72, 0.59</td>
<td>0.463</td>
</tr>
</tbody>
</table>

Total 7.400 gm.
After observing the thin layer chromatographic results, the column fractions were pooled as follows: 1-96, 97-172, 173, 174, 175-178, 179 and 180-192.

i. The isolation of alkaloid B (O-methylthalicberine)

Crude alkaloidal rosettes crystallized from an ethanolic solution of fractions 97-172 after standing at room temperature for three weeks. This alkaloid was designated alkaloid B. Several recrystallizations from methanol yielded white plates (55 mg.). m.p. 187.5-189°C; \( \lambda_{\text{max}} \text{MeOH} = 278 \text{m} \mu (\log \varepsilon = 3.65), \lambda_{\text{max}} \text{MeOH} = 261 \text{m} \mu \), the ultraviolet spectrum remained unchanged in 0.01N methanolic KOH and 0.01N methanolic HCl. The NMR spectrum showed four methoxy groups at 3.61\( \delta \) (3H), 3.75\( \delta \) (3H) and 3.87\( \delta \) (6H), two N-methyl groups at 2.11\( \delta \) (3H) and 2.83\( \delta \) (3H), and nine aromatic protons at 5.94\( \delta \) (1H), 6.15\( \delta \) (1H), 6.51\( \delta \) (1H), 6.59\( \delta \) (2H), 6.83\( \delta \) (2H) and 7.22\( \delta \) (2H).

The mass spectrum showed a molecular ion peak at \( m/e 622 \), a base peak at \( m/e 174 \) and other intense peaks at \( m/e 396 \) and \( m/e 198 \).

ii. The isolation of alkaloid C

Crude alkaloidal rosettes (less than 1 mg.) crystallized from a methanol-ether solution of fraction 173 after standing at room temperature for several weeks. This alkaloid was designated alkaloid C. m.p. 142-142°C, after darkening at 127-129°C. The infrared spectrum in a potassium bromide pellet indicated the possibility of an imino function (-C = N-) at 1640 cm.\(^{-1}\). Lack of compound prevented the determination of any further data.
iii. The isolation of alkaloid D (Adiantifoline)

Long yellow needles precipitated from an ethanol-ether solution of fractions 175-178 and were recrystallized twice from hot absolute ethanol to yield long yellow needles (316 mg.). This alkaloid was designated alkaloid D. m.p. 143.5-144°C; [α]D27.7 +90.0° (c 0.11, MeOH); λmax EtOH 312 μm (log ε 4.34), 302 (4.39) and 283 (4.51) with a shoulder at 292 μm; λmin EtOH 256 μm; λmax 0.01N Ethanolic HCl 312 μm (log ε 4.32), 301 (4.38) and 282 (4.49) with shoulders at 292 μm and 223 μm; λmax 0.01N Ethanolic NaOH 312 μm (log ε 4.28), 302 (4.41) and 283 (4.53) with a shoulder at 292 μm. The NMR spectrum indicated the possibility of eight methoxy groups at 3.59δ (3H), 3.78δ (9H), 3.82δ (3H), 3.89δ (3H), 3.94δ (3H) and 3.96δ (3H), two N-methyl groups at 2.44δ (3H) and 2.47δ (3H) and six aromatic protons at 6.24δ (1H), 6.55δ (2H), 6.60δ (2H) and 8.08δ (1H). The mass spectrum showed an apparent molecular ion peak at m/e 521, a base peak at m/e 30 and an intense peak at m/e 206.

Anal. Calcd. for C42H50N2O9: C, 69.41; H, 6.88; N, 3.85
Found: C, 70.03; H, 7.01; N, 3.96

iv. The isolation of alkaloid E (Adiantifoline)

Yellow rosette crystals (1.7 mg.) precipitated from a methanolic solution of fraction 174 after standing undisturbed in the freezer for several months. This alkaloid was designated alkaloid E. m.p. 123-125°C after darkening at 122-123°C. The infrared spectrum in a potassium bromide pellet was superimposable with that of alkaloid D.
c. Column chromatography of the pH gradient Fraction III

A column (2.6 cm. x 74.5 cm.) was packed in benzene with a grade V basic alumina (Woelm) (240 gm. slurry) to a height of 50 cm. as previously described. The pH gradient Fraction III (3.13 gm.) was dissolved in benzene (65 ml.), added to the top of the column and rinsed into the column with benzene. Elution was begun using benzene as an eluent and collecting 20 ml. fractions via a fraction collector. Thin layer chromatography was employed and the results shown in Table 5.

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Eluent composition</th>
<th>Alkaloid Rf values</th>
<th>Weight (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 17</td>
<td>benzene</td>
<td>NONE</td>
<td>39</td>
</tr>
<tr>
<td>18 - 29</td>
<td>benzene</td>
<td>0.81, 0.68</td>
<td>158</td>
</tr>
<tr>
<td>30 - 34</td>
<td>benzene</td>
<td>0.81, 0.73, 0.68</td>
<td>78</td>
</tr>
<tr>
<td>35 - 53</td>
<td>benzene</td>
<td>0.81, 0.73, 0.59</td>
<td>548</td>
</tr>
<tr>
<td>54 - 119</td>
<td>benzene</td>
<td>0.59</td>
<td>214</td>
</tr>
<tr>
<td>120 - 129</td>
<td>benzene</td>
<td>0.68, 0.59</td>
<td>25</td>
</tr>
<tr>
<td>130 - 139</td>
<td>benzene</td>
<td>0.73, 0.68, 0.59</td>
<td>16</td>
</tr>
<tr>
<td>140 - 229</td>
<td>benzene 20% chloroform</td>
<td>0.73, 0.59</td>
<td>150</td>
</tr>
<tr>
<td>230 - 244</td>
<td>benzene 50% chloroform</td>
<td>0.73</td>
<td>59</td>
</tr>
<tr>
<td>245 - 263</td>
<td>benzene 50% chloroform</td>
<td>0.73, 0.67</td>
<td>412</td>
</tr>
<tr>
<td>264 - 299</td>
<td>benzene 80% chloroform</td>
<td>0.73, 0.67, 0.45</td>
<td>408</td>
</tr>
<tr>
<td>300 - 320</td>
<td>benzene 100% chloroform</td>
<td>0.67, 0.45</td>
<td>81</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>2.148 gm.</strong></td>
</tr>
</tbody>
</table>

TABLE 5
Results of the column chromatography of the pH gradient Fraction III
After observing the thin layer chromatographic results, the column fractions were pooled as follows: 1-17, 18-39, 40-46, 47-52, 53-100, 101-180, 181-229, 230-250, 251-264, 265-280, 281-310 and 311-on. No crystalline products were obtained.

D. Extraction and fractionation of Thalictrum minus L. var. adiantifolium Hort. root alkaloids

1. Extraction of total alkaloids. Dried Thalictrum minus var. adiantifolium roots (34.09 kg) were extracted with 95% ethanol in percolators and in a continuous extraction apparatus via "cyclic percolation". In this process, the percolate is pumped back into the plant material as menstruum for additional percolation. Collection is made when the percolate is considerably more concentrated than the initial percolate.

For this purpose, an apparatus was used which consisted of a commercial earthen sanitary crock which was converted into a percolator for large scale extraction. The crock had an internal diameter of 36 cm, a height of 47 cm at the outer perimeter where it tapered at the bottom to the center at a thirty degree angle and where an opening with a diameter of 4.5 cm was located. The distance from this opening to the top was 56 cm. A mechanical valve for the regulation of solvent flow was fitted onto the small center opening in the bottom. Extending from this valve was a copper pipe elbow and a piece of Tygon tubing, which extended to the inlet of a liquid solvent pump. Another piece of Tygon tubing extended from the outlet of the pump over the top of the crock. With the valve open, and the pump turned on, the solvent

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could be recycled as often as desired. A layer of glass wool was placed at the bottom of the apparatus, which was covered with the plant material and then the menstruum. Ethanol (10 gal.) was added to the plant material and the percolate was recycled for 24 hours. The percolate was then collected and fresh solvent added. The percolate was concentrated to a sirupy consistency in a Precision Tubular Concentrator\textsuperscript{13}. The recovered solvent was reintroduced into the crock for another 24 hour period of "cyclic percolation" and subsequent concentration. This process was repeated eight times in all and all of the concentrated percolates were combined to yield a dark viscous sirup (2.78 kg.).

2. Fractionation of the total extractive into a tertiary alkaloid fraction, a quaternary alkaloid fraction and a neutral-acid fraction. The crude sirupy ethanolic extract (2.78 kg.) was dissolved in chloroform (21) and shaken with an equal volume of 2\% citric acid solution. The acid solution was drawn off and the chloroform was again shaken with an equal volume of 2\% citric acid solution. This process was repeated a third time before the chloroform solutions were dried over anhydrous sodium sulfate, filtered and the solvent removed \textit{in vacuo} at 40°C to leave a dark sirupy extract (383 gm.). This extract was designated as the acid-neutral fraction.

The citric acid solutions were pooled, cooled in the refrigerator overnight, filtered, basified with concentrated ammonium hydroxide to pH 8-9 and subsequently shaken with an equal volume of chloroform. The chloroform was drawn off and the remaining basic solution re-extracted a second time with a fresh, equal volume of chloroform. This

\textsuperscript{13}Precision Scientific Instruments, St. Louis, Missouri.
The extraction process was repeated twice more and the chloroform solutions were pooled, dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to yield a dark yellow sirup (110 gm.). This fraction was designated the tertiary alkaloid fraction. The remaining alkaline solution was acidified with citric acid to pH 5 and this solution added to a 2% ammonium reineckate solution with vigorous stirring until precipitation was complete. The mixture was refrigerated overnight, the precipitate filtered by suction, washed with ether and air dried to yield a crude, dark pink solid (1.02 kg.). This fraction was designated as the quaternary alkaloid reineckate fraction. Chart I illustrates the above described fractionations.
CHART I

Flow Sheet for the Extraction of the Total Alkaloids

Plant material

\[ \text{Ethanol} \]

Ethanol extract

\[ \text{Citrate solution} \quad \text{Chloroform} \]

\[ \text{NH}_4\text{OH to pH 8-9} \quad \text{Chloroform} \]

Basic solution

\[ \text{Chloroform} \quad \text{Na}_2\text{SO}_4 \]

Filter

Evaporate in vacuo 40°C

Acid-neutral fraction

(383 gm.)

\[ \text{Citric acid to pH 5} \]

\[ \text{Na}_2\text{SO}_4 \quad \text{Filter} \]

Evaporate in vacuo 40°C

Acid solution

Tertiary alkaloid fraction

(110 gm.)

\[ 2\% \text{ammonium reineckate solution} \]

Filtrate

Precipitate

\[ \text{Discard} \quad \text{Wash with ether} \]

\[ \text{Air dry} \quad \text{Quaternary alkaloid reineckate fraction} \]

(1.02 kg.)
a. Fractionation of the tertiary alkaloid fraction into tertiary phenolic and tertiary nonphenolic fractions.

The tertiary alkaloid fraction (110 gm.) was dissolved in chloroform and shaken with an equal volume of 5% sodium hydroxide solution. The chloroform was drawn off and subsequently re-extracted with an equal volume of base. This process was repeated twice more before the basic solutions were pooled and refrigerated. The chloroform solution was shaken with an equal volume of water, the water discarded, and the chloroform dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a crude alkaloidal residue (80 gm.). This residue was designated the tertiary nonphenolic alkaloid fraction.

The cold aqueous sodium hydroxide solution was acidified with citric acid to pH 5 and then basified with concentrated ammonium hydroxide to pH 8-9. The ammoniacal solution was shaken with an equal volume of chloroform, the chloroform drawn off and the basic solution re-extracted with a fresh equal volume of chloroform. This process was repeated twice more before the chloroform solutions were pooled, dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a yellow glassy residue (13 gm.). This residue was designated the tertiary phenolic alkaloid fraction.

The remaining ammoniacal solution was acidified with citric acid to pH 4, a solution of 2% ammonium reineckate added until precipitation was complete. The mixture was refrigerated overnight, the precipitate filtered by suction, washed with ether and air dried to yield a crude pink solid (2.8 gm.). See Chart II.
CHART II

Flow Sheet for the Fractionation of the Tertiary Alkaloid Fraction

Tertiary alkaloid fraction
(110 gm.)

5 NaOH

CHCl₃

Basic Solution

Citric acid to pH 5

NH₄OH to pH 8

CHCl₃

Water

Chloroform

Discard

Na₂SO₄

Filter

Evaporate in vacuo

40°C

Tertiary phenolic alkaloid fraction
(80 gm.)

Crude reindeckate
(2.8 gm.)

Filtrate

Precipitate

Filtrate

Air dry

Wash with Et₂O

Tertiary nonphenolic alkaloid fraction
(13 gm.)
i. The isolation of alkaloid F (Thalifendine iodide)

The crude pink reineckate (2.8 gm.) was dissolved in a 50% acetone-water solution and passed through an anion exchange resin column (IRA-410 (Cl)) (50 gm.) at a rate of approximately one drop per five seconds to convert the reineckate to the chloride. The eluate was evaporated in vacuo at 40°C to leave a dark brown residue. Treatment of a methanolic solution of this residue with a saturated aqueous solution of potassium iodide resulted in the formation of dark needles. Recrystallization of this alkaloidal iodide from methanol yielded dark yellow needles. This alkaloid was designated alkaloid F. m.p. 233-234°C decom., 431 mµ (log ε 3.67), 349 (4.29 mµ, 266 (4.35), 226 (4.53); MeOH 307 mµ, 249 mµ, with shoulders at 337 mµ, 276 mµ; 0.01N Methanolic HCl 348 mµ (log ε 4.33), 265 (4.39), 226 (4.53); 0.01N Methanolic HCl 380 mµ, 302 mµ, 249 mµ, with shoulders at 338 mµ and 275 mµ; 0.01N Methanolic KOH 374 mµ (log ε 4.35), 288 (4.46); 0.01N Methanolic KOH 325 mµ, 258 mµ, with shoulders at 245 mµ and 220 mµ. The NMR spectrum (in trifluoroacetic acid with tetramethylsilane as internal standard) of the chloride salt showed one methoxy group at 4.24δ (3H), one methylenedioxy group at 6.12δ (2H), and five aromatic protons at 6.92δ (1H), 7.48δ (1H), 7.92δ (2H) and 8.46δ (1H). An additional proton was suspected farther downfield (around 9.5δ) as the ultraviolet spectrum suggested a protoberberine alkaloid but the spectrum was only run from 0 - 9δ. The mass spectrum showed a molecular ion peak at m/e 321 (measured 321.0997 and calculated 321.1001 for C15H15NO4), a base peak at m/e 307 and other intense peaks at m/e 278, m/e 142 and m/e 127.
3. Separation and isolation of tertiary nonphenolic alkaloids.

a. pH gradient separation of the tertiary nonphenolic alkaloid fractions.

The crude tertiary nonphenolic residue (80 gm.) was dissolved in benzene (500 ml.), 0.1M citric acid solution (2 L.) was added to the benzene and the mixture transferred to a three liter round bottom flask. The benzene was removed in vacuo at 40°C to leave an aqueous acidic solution and a small amount of insoluble residue. The solution was filtered and its pH (2.5) was adjusted to 2.9 via a dropwise addition of concentrated ammonium hydroxide. The solution was subsequently extracted with four separate aliquots of benzene (1 L. each). The benzene extracts were pooled, dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a yellowish residue (1.805 gm.). The pH 2.9 solution was treated with concentrated ammonium hydroxide to pH 3.4 and subsequently extracted with four separate portions of benzene (1 L. each), which were treated as before. These pH gradient extractions were repeated at 0.5 pH increments up to pH 7.4 and then at pH 8.5. Thin layer chromatography using benzene-acetone-concentrated ammonium hydroxide (16:16:1) as the developing solvent revealed the presence of nine alkaloids. The results are summarized in Table 6 and a drawing of the thin layer chromatogram shown in Figure 10.
Solvent system:
Benzene-acetone-concentrated ammonium hydroxide
(16:16:1)

The numbers represent the intensity of the stain:

3 heavy
2 medium
1 light

FIGURE 10
Thin layer Chromatogram of the Tertiary Nonphenolic Alkaloids
from the pH Gradient Separation
### TABLE 6
Weights and \( R_f \) Values of the Extractives from the pH Gradient Separation of the Tertiary Nonphenolic Alkaloids

<table>
<thead>
<tr>
<th>pH Fraction</th>
<th>Weight (gm.)</th>
<th>( R_f ) Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.9</td>
<td>1.805</td>
<td>0.91, 0.83</td>
</tr>
<tr>
<td>3.4</td>
<td>0.765</td>
<td>0.91, 0.83</td>
</tr>
<tr>
<td>3.9</td>
<td>1.959</td>
<td>0.96, 0.91, 0.83, 0.70</td>
</tr>
<tr>
<td>4.4</td>
<td>1.274</td>
<td>0.96, 0.91, 0.83, 0.70</td>
</tr>
<tr>
<td>4.9</td>
<td>5.171</td>
<td>0.91, 0.83, 0.70</td>
</tr>
<tr>
<td>5.4</td>
<td>5.465</td>
<td>0.91, 0.83, 0.77, 0.70</td>
</tr>
<tr>
<td>5.9</td>
<td>2.262</td>
<td>0.83, 0.77, 0.70, 0.57, 0.44</td>
</tr>
<tr>
<td>6.4</td>
<td>5.655</td>
<td>0.83, 0.77, 0.70, 0.65, 0.57, 0.44</td>
</tr>
<tr>
<td>6.9</td>
<td>1.016</td>
<td>0.70, 0.65, 0.57, 0.44, 0.08</td>
</tr>
<tr>
<td>7.4</td>
<td>0.824</td>
<td>0.08</td>
</tr>
<tr>
<td>8.5</td>
<td>0.260</td>
<td>0</td>
</tr>
</tbody>
</table>

On the basis of the thin layer chromatographic results, the pH gradient fractions were combined into three major fractions: pH 2.9 through 4.4 (Fraction I), pH 4.9 and 5.4 (Fraction II) and pH 5.9 through 6.9 (Fraction III).
i. The isolation of alkaloid G (Berberine iodide)

The citric acid insoluble residue (at the beginning of the pH gradient separation) was treated with acetone and filtered. Crystalline matter deposited from the filtrate after a period of several days. This compound was suspected of being a salt of berberine, probably berberine citrate, so the compound was dissolved in hot acetone and treated with a saturated solution of potassium iodide in acetone. Long yellow needles of iodide salt formed on standing (30 minutes). This compound was designated alkaloid G. m.p. 258°C decomp.; with infrared spectra superimposable with that of authentic berberine iodide. In addition, there was no mixed melting point depression nor any difference in thin layer behavior in two different solvent systems.

b. Column chromatography of the pH gradient fraction I.

A column (4 cm, x 70 cm.) was packed in benzene with grade V neutral alumina (Woelm) (400 gm.) to a height of 39 cm. as previously described. Fraction I (5.8 gm.) was dissolved in benzene (100 ml.) and applied to the column. Elution was begun using benzene as an eluent and collecting 20 ml. fractions via a fraction collector. Thin layer chromatography was employed in analysis of the fractions and results of the column chromatography are shown in Table 7.


### TABLE 7

Results of the column chromatography of the pH gradient Fraction I

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Eluent Composition</th>
<th>Alkaloid R_f values</th>
<th>Weight (mg.)</th>
<th>Weight of crystalline alkaloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 23</td>
<td>benzene</td>
<td>None</td>
<td>258</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>benzene</td>
<td>0.90</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>25 - 35</td>
<td>benzene</td>
<td>0.94, 0.90, 0.86, 0.74</td>
<td>158</td>
<td></td>
</tr>
<tr>
<td>36 - 42</td>
<td>benzene</td>
<td>0.99, 0.97, 0.83</td>
<td>172</td>
<td></td>
</tr>
<tr>
<td>43 - 45</td>
<td>benzene</td>
<td>0.99, 0.97</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>46 - 96</td>
<td>benzene</td>
<td>0.97</td>
<td>98, 108</td>
<td></td>
</tr>
<tr>
<td>97 - 103</td>
<td>20% chloroform</td>
<td>0.97, 0.88</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>104 - 142</td>
<td>20% chloroform</td>
<td>0.97, 0.88, 0.80</td>
<td>483</td>
<td></td>
</tr>
<tr>
<td>143 - 166</td>
<td>50% chloroform</td>
<td>0.97, 0.88, 0.80, 0.61</td>
<td>1193</td>
<td>5</td>
</tr>
<tr>
<td>167 - 175</td>
<td>50% chloroform</td>
<td>0.85, 0.61</td>
<td>178</td>
<td></td>
</tr>
<tr>
<td>176 - 206</td>
<td>80% chloroform</td>
<td>0.85</td>
<td>443</td>
<td></td>
</tr>
<tr>
<td>207 - 228</td>
<td>100% chloroform</td>
<td>0.85, 0.65</td>
<td>173</td>
<td></td>
</tr>
<tr>
<td>228 - 355</td>
<td>1 chloroform</td>
<td>0.28</td>
<td>605, 3.7</td>
<td></td>
</tr>
</tbody>
</table>

Total 3955

After observing the thin layer chromatographic results, the fractions were pooled as follows: 1-43, 46-96, 97-113, 114-124, 125-143, 144-158, 159-170, 171-206, 207-290, 291-320 and 321-350.

i. The isolation of alkaloid H (Alkaloid A)

Crude orange alkaloidal plates (108 mg.) crystallized from a cold ethanolic solution of fractions 46-96. The product was recrystallized
from ethanol as light orange plates. This alkaloid was designated as
alkaloid H. m.p. 151.5-152.5°C; [\(\alpha\)]\textsubscript{D} +47.69° (c 0.933, MeOH);
\(\lambda_{\text{max}}\) MeOH 347 m\(\mu\) (log\(\varepsilon\) 3.85), 260 m\(\mu\) (4.53), \(\lambda_{\text{min.}}\) MeOH 249 m\(\mu\);
\(\lambda_{\text{max.}}\) 0.01N Methanolic HCl 413 m\(\mu\) (log\(\varepsilon\) 3.74), 338 (3.57), 284 (4.49),
\(\lambda_{\text{min.}}\) 0.01N Methanolic HCl 264 m\(\mu\) \(\lambda_{\text{max.}}\) 0.01N Methanolic KOH 348 m\(\mu\) (log\(\varepsilon\)
3.88), 261 (4.55), \(\lambda_{\text{min.}}\) 0.01N Methanolic KOH 250 m\(\mu\). In addition, the
methanolic spectrum shows a shoulder at 238 m\(\mu\). The infrared spectrum
in chloroform solution indicated the possibility of an imino function
(C = N) at 1640 cm\(^{-1}\). The NMR spectrum (55 scans, C-1024 Time
Averaging Computer) indicated the possibility of one N-methyl group at
2.25\(\delta\) (3H), four methoxy groups at 3.41\(\delta\) (3H), 3.50\(\delta\) (3H), 3.65\(\delta\)
(3H) and 3.77\(\delta\) (3H), one methylene dioxy group at 6.01\(\delta\) (2H) and at
least five aromatic protons (by integration). The spectrum was not
run further downfield than 8.0\(\delta\), thus any low field protons were missed.
The mass spectrum showed a molecular ion at m/e 648, corresponding
to an empirical formula of C\textsubscript{34}H\textsubscript{36}N\textsubscript{2}O\textsubscript{8}, a base peak at m/e 58 and other
intense peaks at m/e 633, m/e 618, m/e 442, m/e 422, m/e 403, m/e
324, m/e 220, m/e 204 and m/e 190.

ii. The isolation of alkaloid J

A very small amount (5 mg.) of brown microcrystalline
material crystallized from a methanolic solution of fractions 144-158.
This alkaloid was designated alkaloid J. m.p. 182-183°C; \(\lambda_{\text{max.}}\) MeOH 296m\(\mu\),
231 m\(\mu\) \(\lambda_{\text{min.}}\) MeOH 259 m\(\mu\), with a shoulder at 333 m\(\mu\); \(\lambda_{\text{max.}}\)
0.01N Methanolic HCl 306 m\(\mu\), 237 m\(\mu\) \(\lambda_{\text{max.}}\) 0.01N Methanolic KOH 300 m\(\mu\), 231 m\(\mu\).

iii. The isolation of alkaloid K (Noroxyhydrastinine)

Small light yellow alkaloidal rosettes (3.7 mg.) crystallized
from a methanolic solution of fractions 291-320. This material was
designated alkaloid K. m.p. 182-183°C; \(\lambda_{\text{MeOH max.}}\) 304 m\(\mu\) (log\(\varepsilon\) 3.67), 261 (3.58), 222.5 (4.31), \(\lambda_{\text{MeOH min.}}\) 280 m\(\mu\); no change in 0.01N methanolic HCl; no change in 0.01N methanolic KOH except for a greatly enhanced end absorption (below 223 m\(\mu\)). The infrared spectrum in a KBr pellet indicated the possibility of a N-H stretch of a cyclic lactam at 3175 cm\(^{-1}\) and 3040 cm\(^{-1}\) and the C = O stretch of a six membered lactam at 1670 cm\(^{-1}\). The mass spectrum showed a molecular ion peak at m/e 191 (measured 191.0578 and calculated 191.0582 for \(\text{C}_{10}\text{H}_{9}\text{NO}_3\)), a base peak at m/e 134, and other intense peaks at m/e 162 and m/e 104.

c. Column chromatography of the pH gradient Fraction II

A column (4 cm. x 70 cm.) was packed in benzene with grade V neutral alumina (Woelm) (500 gm.) to a height of 55 cm. as previously described. Fraction II (10.63 gm.) was dissolved in benzene (120 ml.) and applied to the column. Elution was begun using benzene as an eluent and collecting 20 ml. fractions via a fraction collector. Thin layer chromatography was employed for analysis of the fractions. The results of the column chromatography are shown in Table 8.
After observing the thin layer chromatography results, the fractions were pooled as follows: 1-32, 33-92, 93-146, 147-196, 197-243 and 244-on. No crystalline alkaloids, however, were isolated.

d. Column chromatography of the pH gradient Fraction III

A column (4 cm. x 73 cm.) was packed in benzene with grade V neutral alumina (Woelm) (500 gm.) to a height of 47 cm. as previously described. Fraction III (8.93 gm.) was dissolved in benzene (75 ml.) and applied to the column. Elution was begun using benzene as an eluent and collecting 20 ml. fractions via a fraction collector. Thin layer chromatography was employed using benzene-acetone-concentrated ammonium hydroxide (16:16:1) as a developing solvent and the results of the column chromatography are shown in Table 9.

### TABLE 8

Results of the column chromatography of the pH gradient Fraction II

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Eluent composition</th>
<th>Alkaloid R_f values</th>
<th>Weight (gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - .25</td>
<td>benzene</td>
<td>None</td>
<td>0.271</td>
</tr>
<tr>
<td>26 - 33</td>
<td>benzene</td>
<td>0.88, 0.78, 0.61</td>
<td>1.153</td>
</tr>
<tr>
<td>34 - 55</td>
<td>20% chloroform</td>
<td>0.88, 0.78</td>
<td>1.559</td>
</tr>
<tr>
<td>56 - 72</td>
<td>20% chloroform</td>
<td>0.88, 0.78, 0.41</td>
<td>0.709</td>
</tr>
<tr>
<td>73 - 97</td>
<td>50% chloroform</td>
<td>0.78, 0.41</td>
<td>0.542</td>
</tr>
<tr>
<td>98 - 142</td>
<td>50% chloroform</td>
<td>0.78, 0.41, 0.23</td>
<td>2.282</td>
</tr>
<tr>
<td>143 - 190</td>
<td>80% chloroform</td>
<td>0.78, 0.41</td>
<td>2.051</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>8.567</strong></td>
</tr>
</tbody>
</table>
### TABLE 9

Results of the column chromatography of the pH gradient Fraction III

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Eluent composition</th>
<th>Alkaloid R_f values</th>
<th>Weight (gm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 21</td>
<td>benzene</td>
<td>None</td>
<td>0.250</td>
</tr>
<tr>
<td>22 - 33</td>
<td>20% chloroform</td>
<td>0.97, 0.90, 0.82</td>
<td>0.220</td>
</tr>
<tr>
<td>34 - 60</td>
<td>20% chloroform</td>
<td>0.97, 0.90, 0.82, 0.71</td>
<td>1.258</td>
</tr>
<tr>
<td>61 - 90</td>
<td>20% chloroform</td>
<td>0.90, 0.82, 0.71</td>
<td>1.452</td>
</tr>
<tr>
<td>91 - 104</td>
<td>50% chloroform</td>
<td>0.82, 0.71, 0.61</td>
<td>0.233</td>
</tr>
<tr>
<td>105 - 133</td>
<td>80% chloroform</td>
<td>0.90, 0.82, 0.71</td>
<td>0.862</td>
</tr>
<tr>
<td>134 - 135</td>
<td>80% chloroform</td>
<td>0.90, 0.82, 0.71, 0.17</td>
<td>0.140</td>
</tr>
<tr>
<td>136 - 154</td>
<td>100% chloroform</td>
<td>0.90, 0.82, 0.71, 0.28, 0.17</td>
<td>0.880</td>
</tr>
<tr>
<td>155 - 156</td>
<td>100% chloroform</td>
<td>0.82, 0.71, 0.28, 0.17</td>
<td>0.076</td>
</tr>
<tr>
<td>157 - 162</td>
<td>100% chloroform</td>
<td>0.71, 0.28, 0.17</td>
<td>0.218</td>
</tr>
<tr>
<td>163 - 168</td>
<td>100% chloroform</td>
<td>0.28, 0.17</td>
<td>0.175</td>
</tr>
<tr>
<td>169 - 178</td>
<td>100% chloroform</td>
<td>0.28</td>
<td>0.203</td>
</tr>
<tr>
<td>179 - 320</td>
<td>10% methanol</td>
<td>------</td>
<td>1.673</td>
</tr>
</tbody>
</table>

**Total** 7.640

After observing the thin layer results, the fractions were pooled as follows: 21-30, 31-80, 81-90, 91-108, 109-129, 130-180, 181-230, 231-250, 251-263, 264-294 and 295-310.

### i. The isolation of alkaloid L

A yellow crystalline residue was obtained from an ethereal solution of fractions 31-80 upon standing. This residue was recrystallized from methanol to yield large orange rosettes (2.2 mg.). This
alkaloid was designated alkaloid L. m.p. 190-191°C, after partially liquefying and then resolidifying at 115°C; \( \lambda_{\text{max.}} \text{MeOH} = 391 \text{m\textmu} \ (\log \epsilon 3.83), \)
\( 311 \ (4.21), \ 263.5 \ (4.71), \ 236 \ (4.49), \ \lambda_{\text{min.}} \text{MeOH} = 356 \text{m\textmu} \ 281 \text{m\textmu}, \ 244 \text{m\textmu}, \)
\( 214 \text{m\textmu} \) with shoulders at 343 m\textmu, 256 m\textmu and 225 m\textmu; \( \lambda_{\text{max.}} \) Methanolic HCl
\( 286 \text{m\textmu} \ (\log \epsilon 3.82), \ 311 \ (4.22), \ 264.5 \ (4.74), \ 237 \ (4.48); \ \lambda_{\text{min.}} \) 0.01N Methanolic HCl
\( 357 \text{m\textmu}, \ 288 \text{m\textmu}, \ 244 \text{m\textmu} \) and 214 m\textmu with shoulders at
\( 343 \text{m\textmu}, \ 257 \text{m\textmu} \) and 225 m\textmu; \( \lambda_{\text{max.}} \) 0.01N Methanolic KOH
\( 291 \text{m\textmu} \ (\log \epsilon 3.82), \ 312 \ (4.19), \ 264 \ (4.71), \ 237 \ (4.49); \ \lambda_{\text{min.}} \) 0.01N Methanolic KOH
\( 357 \text{m\textmu}, \ 282 \text{m\textmu}, \ 244 \text{m\textmu}, \ 223 \text{m\textmu} \) with shoulders at 340 m\textmu, 256 m\textmu. The infrared spectrum showed the presence of a very strong carbonyl band at approximately 1740 cm\(^{-1}\). The mass spectrum showed a molecular ion peak at m/e 380, a base peak at m/e 58 and other intense peaks at m/e 365, m/e 320 and m/e 307.

4. Separation and isolation of the tertiary phenolic alkaloids.

a. Preliminary purification and fractionation of the crude tertiary phenolic alkaloid fraction.

The crude tertiary phenolic alkaloid residue (13 gm.) was dissolved in 5 hydrochloric acid (100 ml.) and shaken twice with ether (100 ml. each time). The combined ethereal solutions were dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a small amount of crude residue which gave negative alkaloid tests with Valser's and Dragendorff's reagents.

The acidic solution was basified with concentrated ammonium hydroxide to pH 8-9 and extracted six times with ether (100 ml. each time). The combined ether solutions were dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a glassy brown tertiary phenolic residue (2.64 gm.).
i. The isolation of alkaloid M (Thalifendine iodide)

The ammoniacal solution was then extracted three times with chloroform (100 ml. each time). The pooled chloroform solutions were dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a crude dark red residue (2.90 gm.). Thin layer chromatography of this chloroform fraction using benzene-acetone-concentrated ammonium hydroxide (16:16:1) as developing solvent revealed only a single, heavy alkaloidal spot at the origin. Since quaternary alkaloids do not move away from the origin in this solvent system, the possibility of a quaternary phenolic alkaloid was suspected. The dark residue (2.90 gm.) was dissolved in methanol (10 ml.) and to this solution was added, dropwise, a saturated aqueous potassium iodide solution (10 drops). Upon standing at room temperature, dark red needles (22.1 mg.) crystallized from the solution. This alkaloid was recrystallized from methanol to yield a quaternary, red alkaloidal iodide (17.6 mg.). This alkaloid was designated alkaloid M. m.p.

207°C decomp., $\lambda_{\text{max.}}^{\text{MeOH}}$ 436 m\(\mu\) (log $\varepsilon$ 3.68), 351 (4.33), 278 (4.39), 228 (4.50); $\lambda_{\text{min.}}^{\text{MeOH}}$ 311 m\(\mu\), 249 m\(\mu\), with shoulders at 380 m\(\mu\), 337 m\(\mu\) and 265 m\(\mu\); 0.01N Methanolic HCl $\lambda_{\text{max.}}$ 436 m\(\mu\) (log $\varepsilon$ 3.84), 346 (4.41) 264 (4.47), 228 (4.56); 0.01N Methanolic HCl $\lambda_{\text{min.}}$ 379 m\(\mu\), 303 m\(\mu\), 248 m\(\mu\) with shoulders at 339 m\(\mu\) and 270 m\(\mu\); 0.01N Methanolic KOH $\lambda_{\text{max.}}$ 474 m\(\mu\) (log $\varepsilon$ 3.62), 373 (4.41), 287 (4.53); 0.01N Methanolic KOH $\lambda_{\text{min.}}$ 325 m\(\mu\), 258 m\(\mu\), with shoulders at 246 m\(\mu\) and 230 m\(\mu\). The NMR spectrum (in trifluoroacetic acid with tetramethylsilane as internal standard) of the chloride salt showed one methoxy group at 4.22 $\delta$ (3H), one methylenedioxy group at 6.10 $\delta$ (2H) and five aromatic protons at 6.91 $\delta$ (1H), 7.47 $\delta$ (1H), 7.90 $\delta$ (2H) and 8.44 $\delta$ (1H). An additional aromatic proton was suspected farther downfield (around 9.5 $\delta$) as ultraviolet data
suggested a protoberberine alkaloid, but the spectrum was only run from 0-95. The mass spectrum showed a molecular ion peak at m/e 321 (measured 321.0997 and calculated 321.1001 for C19H15NO4), a base peak at m/e 307 and other intense peaks at m/e 278, m/e 142 and m/e 127.

A thin layer chromatogram of the ether extractable tertiary phenolic alkaloids (2.64 gm.) using a 2% phosphomolybdic acid spray (in acetone) as the detecting reagent revealed the presence of four alkaloids with the following Rf values: 0.34, 0.23, 0.13 and 0.07. The developed and air dried plate was sprayed with the phosphomolybdic reagent, dried and exposed to ammonia vapors. The four phenolic compounds gave dark blue to green spots immediately upon exposure to ammonia vapors. Chart III shows the purification and fractionation of the crude tertiary phenolic alkaloid fraction.
CHART III
Flow Sheet for the Purification and Fractionation of the

Crude Tertiary Phenolic Alkaloid Fraction

Crude tertiary phenolic alkaloid residue

5 HCl
Ether

Ether
Discard

Acid

Ammonia to pH 8-9
Ether

Ammonia Solution

Chloroform

Chloroform
Ammonia

Na₂SO₄
Filter
Evaporate

Discard

Dark red residue
(2.90 gm.)

Tertiary phenolic alkaloid residue
(2.64 gm.)
b. Column chromatography of the tertiary phenolic alkaloid fraction

A column (2.7 cm. x 80 cm.) was packed in chloroform with a silicic acid-celite 545 (4:1) mixture (100 gm.) to a height of 45 cm. as previously described. The tertiary phenolic alkaloid fraction (2.64 gm.) was dissolved in chloroform (25 ml.) and applied to the column. Elution was begun using chloroform as the initial eluting solvent and collecting 20 ml. fractions via a fraction collector. Thin layer chromatography was employed using phosphomolybdic acid spray and ammonia as detecting reagents. The results of the column chromatography are shown in Table 10.
### TABLE 10

Results of the column chromatography of the tertiary phenolic alkaloid fraction

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Eluent composition (methanol)</th>
<th>Alkaloid Rf values</th>
<th>Weight (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 47</td>
<td>0-1%</td>
<td>None</td>
<td>179</td>
</tr>
<tr>
<td>48 - 53</td>
<td>2</td>
<td>0.33</td>
<td>26</td>
</tr>
<tr>
<td>54</td>
<td>2</td>
<td>0.39</td>
<td>3</td>
</tr>
<tr>
<td>55 - 61</td>
<td>2</td>
<td>---</td>
<td>43</td>
</tr>
<tr>
<td>62 - 63</td>
<td>2</td>
<td>0.50, 0.39, 0.27, 0.11, 0.05</td>
<td>28</td>
</tr>
<tr>
<td>64 - 65</td>
<td>2</td>
<td>0.50, 0.27, 0.18, 0.11</td>
<td>18</td>
</tr>
<tr>
<td>66 - 69</td>
<td>2</td>
<td>---</td>
<td>21</td>
</tr>
<tr>
<td>70 - 71</td>
<td>2</td>
<td>0.61</td>
<td>41</td>
</tr>
<tr>
<td>72 - 79</td>
<td>2</td>
<td>0.61, 0.55</td>
<td>69</td>
</tr>
<tr>
<td>80 - 84</td>
<td>4</td>
<td>---</td>
<td>40</td>
</tr>
<tr>
<td>85 - 87</td>
<td>4</td>
<td>0.07</td>
<td>29</td>
</tr>
<tr>
<td>88 - 89</td>
<td>4</td>
<td>0.33, 0.07</td>
<td>22</td>
</tr>
<tr>
<td>90 - 105</td>
<td>4</td>
<td>0.33</td>
<td>198</td>
</tr>
<tr>
<td>106 - 111</td>
<td>4</td>
<td>0.38, 0.30, 0.33</td>
<td>---</td>
</tr>
<tr>
<td>112 - 133</td>
<td>8</td>
<td>0.38, 0.30</td>
<td>---</td>
</tr>
<tr>
<td>134 - 149</td>
<td>16</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>150 - 151</td>
<td>16</td>
<td>0.43</td>
<td>---</td>
</tr>
<tr>
<td>152 - 153</td>
<td>16</td>
<td>0.39</td>
<td>---</td>
</tr>
<tr>
<td>154 - 157</td>
<td>16</td>
<td>0.39, 0.20, 0.13</td>
<td>---</td>
</tr>
<tr>
<td>158 - 159</td>
<td>16</td>
<td>0.20, 0.13</td>
<td>---</td>
</tr>
</tbody>
</table>

Total 717
After observing the thin layer chromatographic results, the fractions were pooled as follows: 40-47, 48-52, 53-59, 60-65, 66-70, 71-87, 88-105, 105-138 and 139-153.

i. The isolation of alkaloid N (Thalifoline)

Long needles deposited from a concentrated chloroform solution of fractions 60-65 upon standing open at room temperature for one hour. The needles were rinsed with cold chloroform and recrystallized from methanol to yield white rods (5.2 mg.). This alkaloid was designated alkaloid N. m. p. 210-211°C; $\lambda_{\text{max.}} \text{MeOH} 302 \text{m}\mu$ ($\log \varepsilon 3.77$), 261 (3.87), 223.5 (4.41); $\lambda_{\text{min.}} \text{MeOH} 280 \text{m}\mu, 234 \text{m}\mu, 212.5 \text{m}\mu$, with a shoulder at 267 m\mu; $\lambda_{\text{max.}} 0.01\text{N Methanolic HCl} 303 \text{m}\mu$ ($\log \varepsilon 3.77$) 261 (3.86), 223.5 (4.41); $\lambda_{\text{min.}} 0.01\text{N Methanolic HCl} 281 \text{m}\mu, 249 \text{m}\mu, 213 \text{m}\mu$, with a shoulder at 268 m\mu; $\lambda_{\text{max.}} 0.01\text{N Methanolic KOH} 329 \text{m}\mu$ ($\log \varepsilon 3.66$), 238 (4.41); $\lambda_{\text{min.}} 0.01\text{N Methanolic KOH} 292 \text{m}\mu, 221 \text{m}\mu$, with a shoulder at 270 m\mu. The infrared spectrum in KBr indicated the possibility of a $C=O$ stretch of six membered lactam (N substituted) at 1640 cm. $^{-1}$. The NMR spectrum (140 scans C-1024 Time Averaging Computer) indicated the possibility of one N-methyl group at $3.27\delta (3H)$, one methoxy group at $4.03\delta (3H)$, and two aromatic protons at $6.63\delta (1H)$ and $7.68\delta (1H)$. The mass spectrum showed a molecular ion peak at m/e 207 (measured 207.0885 and calculated 207.0895 for $C_{11}H_{13}NO_3$), a base peak at m/e 164 and other intense peaks at m/e 191, m/e 177 and m/e 136.

5. Separation and isolation of the quaternary alkaloids

a. Fractionation of the crude quaternary alkaloidal reineckates into 50% acetone-water soluble and insoluble fractions

The crude quaternary alkaloid reineckates (200 gm.) were shaken and stirred with a 50% acetone-water solution (1 L.). The
insoluble residue was suction filtered and a fresh acetone-water (1 L.) added to it and the process repeated. This process was repeated twice again leaving a bright yellow insoluble residue. The 50% acetone-water soluble portions were pooled, filtered and passed through an anion exchange resin column (IRA-410 (Cl)) (725 gm. or ca. 1075 ml.\textsuperscript{−1}) at a rate of approximately one drop per five seconds. The eluate, which consisted of crude quaternary alkaloid chlorides, was evaporated in vacuo at 40°C to leave a dark brown residue.

i. The isolation of alkaloid P (Magnoflorine iodide)

A small aliquot of the above mentioned dark brown residue (crude quaternary alkaloid chlorides) was dissolved in methanol and to this solution was added a saturated aqueous potassium iodide solution (10 ml.). A copious, white crystalline mass formed upon standing and was suction filtered. Recrystallization from hot methanol yielded white needles of quaternary alkaloid iodide (883 mg.). This alkaloid was designated alkaloid P. m.p. 249.5-251°C decomp.; 

\[
[a]_{D}^{2.7} + 198.2^\circ (c 0.202, \text{MeOH}); \frac{\lambda}{\lambda_{\text{max}}^{\text{MeOH}}} 323 \, \text{mp} (\log \varepsilon 3.90), 279 (3.88), 224.5 (4.65); \frac{\lambda}{\lambda_{\text{min}}^{\text{MeOH}}} 288 \, \text{mp}, 263 \, \text{mp} \text{ with a shoulder at } 270 \, \text{mp};
\]

\[
0.01\text{N Methanolic HCl} \frac{\lambda}{\lambda_{\text{max}}^{\text{HCl}}} 303 \, \text{mp} (\log \varepsilon 3.86), 268 (4.14), 222 (4.72); \frac{\lambda}{\lambda_{\text{min}}^{\text{HCl}}} 289 \, \text{mp}, 248 \, \text{mp} \text{ with a shoulder at } 276 \, \text{mp};
\]

\[
0.01\text{N Methanolic KOH} \frac{\lambda}{\lambda_{\text{max}}^{\text{KOH}}} 323 \, \text{mp} (\log \varepsilon 3.93), 277 (3.90), 269 (3.90); \frac{\lambda}{\lambda_{\text{min}}^{\text{KOH}}} 289 \, \text{mp}, 263 \, \text{mp} \text{ with a shoulder at } 225 \, \text{mp}.
\]

ii. The isolation of alkaloid Q (Berberine iodide)

The 50% acetone-water insoluble alkaloid reineckate was dissolved in acetone and passed through an anion exchange resin column (IRA-410 (Cl)) (200 gm.) at a rate of approximately one drop per
five seconds. The column eluate was treated with a saturated solution of potassium iodide in acetone and long yellow needles of quaternary alkaloidal iodide immediately formed. The crystalline material was filtered by suction and recrystallized from hot methanol as fine yellow needles. This alkaloid was designated alkaloid Q, m.p. 260-262°C decomp., after darkening at 240°C; $\lambda_{\text{MeOH}}^\text{max.}$ 429 m$\mu$ ($\log \varepsilon$ 3.78), 350 (4.44), 266 (4.46), 226 (4.56); $\lambda_{\text{MeOH}}^\text{min.}$ 382 m$\mu$, 302 m$\mu$, 250 m$\mu$, with a shoulder at 337 m$\mu$; $\lambda_{\text{0.01N Methanolic HCl}}^\text{max.}$ 429 m$\mu$ ($\log \varepsilon$ 3.78), 350 (4.40), 266 (4.46), 227.5 (4.57); $\lambda_{\text{0.01N Methanolic KOH}}^\text{max.}$ 351 m$\mu$ ($\log \varepsilon$ 4.41), 266 (4.38); $\lambda_{\text{0.01N Methanolic KOH}}^\text{min.}$ 302 m$\mu$, 249 m$\mu$, with shoulders at 390 m$\mu$, 341 m$\mu$, 275 m$\mu$ and 227 m$\mu$.

iii. The synthesis of tetrahydro alkaloid Q (Tetrahydroberberine)

Alkaloid Q (941 mg.) was dissolved in 50% acetic acid (100 ml.) by heating on a steam bath. Zinc dust (3 gm.) was added to the solution. An air condenser was employed. The solution was heated on a steam bath for three hours with intermittent shaking. At that time, additional zinc dust (1 gm.) was added and the solution heated for two more hours. The very pale yellow solution was filtered, cooled in an ice bath, basified with concentrated ammonium hydroxide to pH 8-9 and shaken with ether three times (200 ml. each time). The ether extracts were pooled, dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a crystalline residue. This residue was recrystallized from hot methanol to yield yellowish white prisms (392 mg.). An additional recrystallization from hot methanol yielded white prisms (61.6 mg.) of tetrahydro alkaloid Q (Tetrahydroberberine). m.p. 171-172°C; $\lambda_{\text{MeOH}}^\text{max.}$ 284 m$\mu$ ($\log \varepsilon$ 3.42); $\lambda_{\text{MeOH}}^\text{min.}$ 258 m$\mu$, with a shoulder at 294 m$\mu$. 
CHART IV
Flow Sheet for the Separation and Isolation of the
Quaternary Alkaloids

Crude Quaternary Alkaloid
Reineckates

50% acetone-water

<table>
<thead>
<tr>
<th>Soluble portion</th>
<th>Insoluble portion</th>
</tr>
</thead>
<tbody>
<tr>
<td>filter</td>
<td>dissolve in acetone</td>
</tr>
<tr>
<td>IRA-410 (Cl)</td>
<td>IRA-410 (Cl)</td>
</tr>
<tr>
<td>evaporate</td>
<td>KI in acetone</td>
</tr>
<tr>
<td>Crude quaternary</td>
<td>Precipitate</td>
</tr>
<tr>
<td>alkaloid chlorides</td>
<td>mother liquors</td>
</tr>
<tr>
<td>Small aliquot in methanol</td>
<td>Alkaloid Q</td>
</tr>
<tr>
<td>saturated aqueous KI soln.</td>
<td>(berberine iodide)</td>
</tr>
<tr>
<td>Alkaloid P</td>
<td>(magnoflorine iodide)</td>
</tr>
</tbody>
</table>
6. Treatment of the acid-neutral fraction. A portion (10 gm.) of the acid-neutral fraction (383 gm.) was put aside and the other 373 gm. was dissolved in chloroform (1 L.). The chloroform solution was shaken four times with 1% hydrochloric acid (250 ml. each time), the chloroform removed, dried over anhydrous sodium sulfate, filtered, and the solvent removed in vacuo at 40°C to leave a dark green oil (245 gm.). The acid solutions were combined, basified with concentrated ammonium hydroxide to pH 9 and shaken four times with chloroform (1 L. each time). The combined chloroform solutions were dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a residue (7.0 gm.).

a. Column chromatography of an aliquot (10 gm.) of the acid-neutral fraction.

The aliquot (10 gm.) of the acid-neutral fraction that was put aside was dissolved in chloroform and chromatographed on a small column of grade V neutral alumina (Woelm).

i. The isolation of neutral compound A (β-sitosterol)

Elution of the above column with chloroform afforded a greed oil, which, upon the addition of methanol deposited needles (220 mg.). Recrystallization of this compound from methanol yielded nonalkaloidal white needles (190 mg.). m.p. 131.5 - 133°C; [α]D 28 -38.8° (c 0.52, CHCl₃); \( \lambda_{\text{max.}} \text{MeOH} \) 293 m\( \mu \) (logε 2.27), 282 (2.48), 271.5 (2.46); \( \lambda_{\text{min.}} \text{MeOH} \) 289 m\( \mu \), 276 m\( \mu \), 237.5 m\( \mu \), with shoulders at 263 m\( \mu \) and 252.5 m\( \mu \). The infrared spectrum in chloroform indicated the possibility of absorption at 3600 cm\(^{-1}\). The mass spectrum showed a molecular ion peak at m/e 414 (measured 414.3865 and calculated 414.3861 for C₂₉H₅₀O), and a base peak at m/e 43. The NMR spectrum showed an envelope of peaks from
0.5-2.0 but no other absorptions further downfield. Chart V shows the treatment of the acid-neutral fractions.

ii. The preparation of the acetate of neutral compound A (β-sitosterol acetate).

Neutral compound A (166 mg.) was dissolved in pyridine (5 ml.). Acetic anhydride (5 ml.) was added, with shaking, and the solution was heated on a steam bath for 15 minutes. After cooling, the solution was poured into 25 ml. of ice water. The resulting precipitate was filtered, washed with cold 2% hydrochloric acid, washed with cold water and then recrystallized several times from hot ethanol to yield the acetate of neutral compound A (β-sitosterol) (112.6 mg.) m.p. 125.0-125.5°C; [α]_D^{27} +242° (c 0.1, CHCl₃).
CHART V

Flow Sheet for the Treatment of the Acid-Neutral Fraction

Neutral fraction (383 gm.)

373 gm. 10 gm.

Chloroform 1%HCl

Chloroform HCl

Sodium sulfate filter evaporate

Ammonia to pH 9 Chloroform neutral compound A (β-sitosterol)

Basic soln. Chloroform

Discard Sodium sulfate filter evaporate

Residue (7 gm.)
E. General extraction and fractionation of *Thalictrum minus* L. var. *adiantifolium* Hort. top alkaloids

1. Extraction of the total alkaloids. Dried *Thalictrum minus* L. var. *adiantifolium* Hort. tops (15 kg.) were extracted in a continuous extraction apparatus as previously described with 95% ethanol. This cyclic percolation procedure yielded, upon concentration and evaporation, a dark green sirupy extract (523 gm.).

2. Fractionation of the total alkaloid extract into an ether soluble tertiary alkaloid fraction, ether insoluble-chloroform soluble tertiary alkaloid fraction, quaternary alkaloid fraction and acid-neutral fraction. The dark green sirupy extract (523 gm.) was poured with vigorous stirring into a warm 2% citric acid solution (1 L.). The solution and extract were stirred vigorously for 30 minutes and filtered. The insoluble residue was discarded. The filtrates were combined (2 L.) and shaken three times with equal volumes of ether. The ether extracts were combined, dried over anhydrous sodium sulfate, filtered and the solvent removed *in vacuo* at 40°C to yield a yellow oil (12.99 gm.). This oil was designated the acid-neutral fraction. The acid solution (2 L.) was basified with concentrated ammonium hydroxide to pH 9-10 and shaken six times with equal volumes of ether. The ether extracts were combined, dried over anhydrous sodium sulfate, filtered and the solvent removed *in vacuo* at 40°C to leave an orange oil (4.10 gm.). This oil was designated the ether soluble tertiary alkaloid fraction.

The remaining alkaline solution was shaken three times with equal volumes of chloroform (2 L. each). The chloroform extracts were combined, dried over anhydrous sodium sulfate, filtered and the solvent removed *in vacuo* at 40°C to leave a brown oil (4.75 gm.). This oil was designated the chloroform soluble-ether insoluble tertiary alkaloid fraction. Thin layer chromatography using benzene-acetone-concentrated
ammonium hydroxide (16:16:1) as developing solvent revealed the presence of at least four alkaloids in this fraction (Rf 0.97, 0.63, 0.04 and 0.0).

The remaining alkaline solution was cooled and cautiously acidified to pH 2 with concentrated hydrochloric acid. To this acidic solution was added a saturated aqueous solution of ammonium reinecate until precipitation was complete. The precipitate was filtered by suction, washed with ether and air dried to yield a dark pink, crude quaternary alkaloid reinecate (17.4 gm.) fraction. This fraction was designated the quaternary alkaloid fraction. The filtrate was discarded.

a. Fractionation of the ether-soluble tertiary alkaloid fraction into phenolic and nonphenolic fractions.

The ether-soluble tertiary alkaloid fraction (4.10 gm.) was dissolved in chloroform (500 ml.) and shaken three times with equal volumes of 5% sodium hydroxide solution. The chloroform was then shaken with an equal volume of water, the water discarded, the chloroform dried over anhydrous sodium sulfate, filter and the solvent removed in vacuo at 40°C to leave a residue (3.2 gm.). This fraction was designated the ether-soluble tertiary nonphenolic alkaloid fraction. Thin layer chromatography revealed the presence of eleven alkaloids with the following Rf values: 0.97, 0.93, 0.88, 0.76, 0.63, 0.48, 0.43, 0.33, 0.18, 0.13, and 0.0.

The sodium hydroxide solution was cooled in ice, acidified with hydrochloric acid to pH 5, basified with concentrated ammonium hydroxide to pH 8-9 and shaken three times with equal volumes of chloroform. The chloroform extracts were combined, dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a
141

residue (679 mg.). This residue was designated the ether-soluble tertiary phenolic alkaloid fraction. Thin layer chromatography using benzene-acetone-concentrated ammonium hydroxide (16:16:0.1) as a developing solvent revealed the presence of six alkaloids with the following R_f values: 0.48, 0.40, 0.33, 0.22, 0.08 and 0.0.

The remaining alkaline solution was acidified to pH 3 with concentrated hydrochloric acid and treated with a solution of 2% ammonium reineckate. No precipitation occurred, therefore, the solution was discarded.

3. Separation and isolation of the ether soluble tertiary non-phenolic alkaloids.

a. pH gradient separation of the ether soluble tertiary nonphenolic alkaloid fraction.

The ether soluble tertiary nonphenolic alkaloid fraction (3.2 gm.) was dissolved in benzene (500 ml.) and shaken with 0.1M citric acid solution (500 ml.). The benzene solution was dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a residue (674 mg.). The acid solution (pH 2.7) was treated with concentrated ammonium hydroxide to pH 2.9 and shaken three times with equal volumes of benzene (500 ml.) each time. The benzene solutions were combined, dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a residue (129 mg.). This pH gradient extraction process was subsequently repeated at 0.5 pH increments through pH 6.9 and then at pH 7.9. The alkaline solution remaining after the pH 7.9 extraction was discarded, since no precipitation resulted upon acidification and addition of ammonium reineckate solution to a small amount of the alkaline solution. The results are summarized
in Table 11 and a drawing of the thin layer chromatogram of the pH gradient separation shown in Figure 11. Note the presence of twelve alkaloids in Figure 11.


**TABLE II**

*Results of the thin layer chromatography of the ether-soluble tertiary nonphenolic pH gradient alkaloid fractions*

<table>
<thead>
<tr>
<th>pH gradient fraction</th>
<th>Weight (mg.)</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; values of the alkaloids</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.7</td>
<td>674</td>
<td>None</td>
</tr>
<tr>
<td>2.9</td>
<td>139</td>
<td>None</td>
</tr>
<tr>
<td>3.4</td>
<td>129</td>
<td>0.77, 0.68, 0.59, 0.49, 0.38</td>
</tr>
<tr>
<td>3.9</td>
<td>184</td>
<td>0.84, 0.77, 0.68, 0.59, 0.49, 0.38</td>
</tr>
<tr>
<td>4.4</td>
<td>431</td>
<td>0.68, 0.59, 0.49, 0.38, 0.29</td>
</tr>
<tr>
<td>4.9</td>
<td>214</td>
<td>0.68, 0.59, 0.49, 0.38, 0.29</td>
</tr>
<tr>
<td>5.4</td>
<td>237</td>
<td>0.59, 0.49, 0.38, 0.29, 0.25, 0.18, 0.09, 0.0</td>
</tr>
<tr>
<td>5.9</td>
<td>125</td>
<td>0.59, 0.49, 0.38, 0.29, 0.25, 0.18, 0.09, 0.0</td>
</tr>
<tr>
<td>6.4</td>
<td>84</td>
<td>0.49, 0.43, 0.38, 0.29, 0.25, 0.18, 0.09, 0.0</td>
</tr>
<tr>
<td>6.9</td>
<td>69</td>
<td>0.49, 0.18, 0.09, 0.0</td>
</tr>
<tr>
<td>7.9</td>
<td>31</td>
<td>None</td>
</tr>
</tbody>
</table>

Solvent: Benzene-acetone-concentrated ammonium hydroxide (16:16:0.5) as developing solvent.
FIGURE 11

Thin Layer Chromatogram of the pH Gradient Alkaloid Fractions (tops)

2.7  2.9  3.4  3.9  4.4  4.9  5.4  5.9  6.4  6.9
Front

The numbers represent the intensity of the stain:

+3 heavy
+2 medium
+1 light

Solvant: Benzene-acetone-concentrated ammonium hydroxide
(16:16:0.5)
b. Column chromatography of the combined pH gradient fractions

After observing the thin layer chromatogram of the pH gradient alkaloids, it was decided that the pH gradient had not worked very efficiently in fractionating the alkaloids. Therefore, the pH gradient fractions were pooled and subjected to column chromatography.

A column (2.7 cm. x 72 cm.) was packed in chloroform with silicic acid-celite 545 (4:1) mixture (100 gm.) to a height of 57 cm. as previously described. The pooled mass (1.47 gm.) was dissolved in chloroform (30 ml.) and applied to the column. Elution was begun using chloroform as an initial eluent and collecting 20 ml. fractions via a fraction collector. Thin layer chromatography was employed using benzene-acetone-concentrated ammonium hydroxide (16:16:0.1) as a developing solvent. The results of the column chromatography are shown in Table 12.
TABLE 12

Results of the column chromatography of the combined pH gradient fractions

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Eluent composition (methanol)</th>
<th>Alkaloid Rf values</th>
<th>Weight (mg.)</th>
<th>Weight of crystalline alkaloid (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 - 87</td>
<td>2%</td>
<td>None</td>
<td>148</td>
<td></td>
</tr>
<tr>
<td>88 - 89</td>
<td>2</td>
<td>0.38</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>90 - 91</td>
<td>2</td>
<td>0.52, 0.38</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>92 - 93</td>
<td>2</td>
<td>0.52</td>
<td>6</td>
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<td>94 - 97</td>
<td>4</td>
<td>0.70, 0.52</td>
<td>15</td>
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<td>98 - 99</td>
<td>4</td>
<td>0.70</td>
<td>6</td>
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<td>100 - 101</td>
<td>4</td>
<td>0.70, 0.52</td>
<td>5</td>
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<td>102 - 106</td>
<td>4</td>
<td>0.70, 0.52, 0.41</td>
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<td>107</td>
<td>4</td>
<td>0.70, 0.57, 0.41</td>
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<td>108 - 109</td>
<td>4</td>
<td>0.57, 0.41</td>
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<td>110 - 111</td>
<td>4</td>
<td>0.85, 0.69, 0.57, 0.38</td>
<td>6</td>
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<tr>
<td>112 - 113</td>
<td>4</td>
<td>0.57, 0.38, 0.19</td>
<td>6</td>
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<tr>
<td>114 - 121</td>
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<td>0.38, 0.19, 0.12</td>
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<td>122 - 123</td>
<td>4</td>
<td>0.69, 0.56, 0.19</td>
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<td>124 - 125</td>
<td>4</td>
<td>0.69, 0.56, 0.47, 0.19</td>
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<td>126 - 147</td>
<td>8</td>
<td>0.69, 0.56, 0.47</td>
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<td>148 - 157</td>
<td>16</td>
<td>0.69, 0.56, 0.47, 0.37</td>
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<td>158 - 159</td>
<td>16</td>
<td>0.69, 0.56, 0.47, 0.37</td>
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<td>160 - 163</td>
<td>16</td>
<td>0.56, 0.47, 0.37, 0.24, 0.15</td>
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<tr>
<td>164 - 165</td>
<td>16</td>
<td>0.56, 0.47, 0.37, 0.24</td>
<td>21</td>
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<tr>
<td>166 - 176</td>
<td>32</td>
<td>0.56, 0.47, 0.24</td>
<td>102</td>
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<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>889</strong></td>
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After observing the thin layer chromatographic results, the fractions were pooled as follows: 1-30, 31-64, 65-70, 71-86, 87-94, 95-100, 104-106, 108-109, 110-111, 112-120, 121-146 and 147-179.

i. The isolation of alkaloid R (Adiantifoline)

Yellow alkaloidal rosettes (3mg.) deposited from a concentrated petroleum ether (30-60°C) solution of fractions 110-111. This alkaloid was designated alkaloid R. m.p. 98-100°C; \( \lambda_{\text{max.}} \text{MeOH} \) 311 \( \mu m \) (log \( \varepsilon \) 4.09), 301 (4.14), 282 (4.28); \( \lambda_{\text{min.}} \text{MeOH} \) 254 \( \mu m \) with shoulders at 262 \( \mu m \) and 221 \( \mu m \). The spectra did not change in 0.01N methanolic HCl or 0.01N methanolic KOH. The infrared spectrum in a potassium bromide pellet was superimposable with that of alkaloid D (Adiantifoline).


a. Column chromatography of the ether-insoluble chloroform-soluble tertiary alkaloid fraction.

A column (3.8 cm. x 73 cm.) was packed in chloroform with a silicic acid-celite 545 (4:1) mixture (250 gm.) as previously described. The ether-insoluble chloroform-soluble tertiary alkaloid fraction (4.39 gm.) was dissolved in chloroform (90 ml.) and added to the column. Elution was begun with chloroform as the initial eluting solvent and 20 ml. fractions were collected via a fraction collector. Thin layer chromatography was employed using benzene-acetone-concentrated ammonium hydroxide (16:16:0.1) as a developing solvent for fractions 1-239 and methanol-concentrated ammonium hydroxide-water (8:1:1) for fractions 240-on. The results of the column chromatography are shown in Table 13.
**TABLE 13**

Results of the column chromatography of the ether insoluble-
chloroform soluble tertiary alkaloid fraction

<table>
<thead>
<tr>
<th>Fractions (mg.)</th>
<th>Eluent composition (methanol)</th>
<th>Alkaloid R_f values</th>
<th>Weight (mg.)</th>
<th>Weight of crystalline alkaloid (mg.)</th>
</tr>
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<tr>
<td>0 - 84</td>
<td>4 %</td>
<td>None</td>
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<tr>
<td>85 - 86</td>
<td>4</td>
<td>0.69, 0.54</td>
<td>177</td>
<td>8</td>
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<td>87 - 99</td>
<td>4</td>
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<td>100 - 115</td>
<td>4</td>
<td>0.88</td>
<td>243</td>
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<td>116 - 121</td>
<td>4</td>
<td>0.10</td>
<td>115</td>
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<tr>
<td>122 - 151</td>
<td>8</td>
<td>0.79</td>
<td>328</td>
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<tr>
<td>152 - 163</td>
<td>8</td>
<td>0.79, 0.70</td>
<td>238</td>
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<tr>
<td>164 - 168</td>
<td>8</td>
<td>0.79</td>
<td>---</td>
<td></td>
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<tr>
<td>169 - 259</td>
<td>16</td>
<td>None</td>
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<td>260 - 310</td>
<td>32</td>
<td>0.23</td>
<td>688</td>
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<tr>
<td>311 - 393</td>
<td>64</td>
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<td>394 - 427</td>
<td>75</td>
<td>0.75</td>
<td>---</td>
<td>8</td>
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<tr>
<td>427 - on</td>
<td>100% methanol</td>
<td>None</td>
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</tr>
<tr>
<td>427 - on</td>
<td>2% methanolic HCl 0.23</td>
<td>70</td>
<td>70</td>
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</tbody>
</table>

**Total** 1.935 gm.
After observing the thin layer chromatographic results, the fractions were pooled as follows: 1-80, 81-84, 85-86, 87-99, 100-107, 114-115, 150-162, 164-259, 260-310, 311-390, and 394-427.

i. The isolation of alkaloid S.

Light orange alkaloidal needles (8 mg.) deposited from an ethereal solution of fractions 85-86. This alkaloid was designated alkaloid S. m.p. 236.5-237.5°C decomp.; \( \lambda_{\text{MeOH max.}} \) 317 m\( \mu \), (log\( e \) 3.75), 278 (3.71), 232 (4.21); \( \lambda_{\text{MeOH min.}} \) 293 m\( \mu \), 254 m\( \mu \), 219 m\( \mu \); \( \lambda_{0.01\text{N Methanolic HCl max.}} \) 317 m\( \mu \), (log\( e \) 3.77), 277 (3.72), 232 (4.20); \( \lambda_{0.01\text{N Methanolic HCl min.}} \) 292 m\( \mu \), 253 m\( \mu \), 219 m\( \mu \); \( \lambda_{0.01\text{N Methanolic KOH max.}} \) 317 m\( \mu \), (log\( e \) 3.79), 279 (3.77), 230 (4.23); \( \lambda_{0.01\text{N Methanolic KOH min.}} \) 293 m\( \mu \), 254 m\( \mu \), 227 m\( \mu \). The nuclear magnetic resonance spectrum (100 mc) indicated the possibility of two methoxy groups at \( 3.78^\delta \) (3H) and \( 3.85^\delta \) (3H), one methylenedioxy group at \( 6.00^\delta \) (2H) and approximately seven aromatic protons (via integration). There is a singlet at \( 5.13^\delta \) (1H) and an apparent AB system (quartet) centered at \( 6.88^\delta \) and \( 7.05^\delta \), \( J_{\text{app}} = 8 \) cps.

The mass spectrum showed a molecular ion peak at \( m/e \) 381 (measured 381.1226 and calculated 381.1212 for \( C_{21}H_{16}NO_6 \)), a base peak at \( m/e \) 352 and other intense peaks at \( m/e \) 338, 322, 176 and 148. The infrared spectrum showed intense peaks at 1690 cm.\(^{-1}\) and 1640 cm.\(^{-1}\), both in the carbonyl region of the spectrum.

ii. The isolation of alkaloid T (Berberine iodide).

Yellow alkaloidal needles (300 mg.) were obtained from a methanolic solution of fractions 260-310 after treating this solution with a saturated aqueous potassium iodide solution. This alkaloid was
designated alkaloid T. m.p. 258-260°C decomp. The infrared spectrum was superimposable with that of berberine iodide. In addition, there was no mixed melting point depression nor any difference in thin layer Rf in two different solvent systems.

iii. The isolation of alkaloid U (Thalifendine iodide)

Yellow alkaloidal needles (8 mg.) were obtained from a methanolic solution of fractions 394-427 after treating this solution with a saturated aqueous potassium iodide solution. This alkaloid was designated alkaloid U. m.p. 237-238°C decomp. The infrared spectrum was superimposable with that of thalifendine iodide. In addition, there was no mixed melting point depression nor any difference in thin layer Rf in two different solvent systems.

iv. The isolation of alkaloid V (Berberine chloride)

Yellow alkaloidal needles (70 mg.) were obtained from a methanolic solution of the 2% methanolic hydrochloric acid eluate. This alkaloid was designated alkaloid V. m.p. 194-195°C decomp. The infrared spectrum was superimposable with that of berberine chloride. In addition, there was no mixed melting point depression nor any difference in thin layer Rf in two different solvent systems. Treatment of a methanolic solution of alkaloid V with a saturated aqueous potassium iodide solution produced yellow needles of quaternary alkaloidal iodide whose infrared spectrum was superimposable with that of berberine iodide.
F. Degradation of Alkaloid D (Adiantifoline) and Thalicarpine

1. Sodium-liquid ammonia cleavage of alkaloid D (Adiantifoline). A ground glass three-necked 300 ml. round bottom flask equipped with a nitrogen gas inlet, a dry ice-acetone cold finger condenser and a dropping funnel was placed in a dry ice-acetone bath mounted on a magnetic stirring device. Dried ammonia gas was introduced into the flask via the nitrogen gas inlet to a volume of approximately 50 ml. of liquid ammonia. A stirring magnet was added to the flask followed by a small amount of freshly cut metallic sodium to color the solution blue. Alkaloid D (Adiantifoline) (75 mg.) was dissolved in dry toluene (4.0 ml.). The reaction was carried out under a nitrogen atmosphere by adding alternately small pieces of the metallic sodium and small portions (ca. 0.5 ml.) of the toluene solution to the reaction vessel so that the blue color of the reaction mixture was maintained. The bath temperature was maintained by occasional addition of dry ice to the bath. The reaction mixture turned from a dark blue to yellow twenty minutes after the addition of the last of the toluene solution. An additional chunk of sodium was added and the blue color returned and was maintained an additional 20-30 minutes before the mixture was allowed to stand in the hood overnight to evaporate the ammonia and solvent. The total amount of sodium used in the reaction was 58 mg.

After standing overnight, the residue was dissolved in 2% hydrochloric acid (20 ml. ) and this acidic solution shaken with ether 20 ml. ). The ether layer was separated and shaken with water (20 ml.). The ether layer was discarded and the water washing added to the acidic solution. The acidic solution was then basified with concentrated ammonium hydroxide to pH 8-9 and shaken four times with ether (75 ml. each time). The ammoniacal layer was discarded. The
ether solutions were pooled, dried over anhydrous sodium sulfate, filtered and evaporated in vacuo at 40°C to a volume of approximately 50 ml.

The concentrated ether solution was extracted twice with 2% potassium hydroxide solution (25 ml. each time) to remove the phenolic products. The remaining ether solution was separated, shaken twice with water and the water washings discarded. The ether layer was dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a semi-crystalline solid (22 mg.), which was designated the nonphenolic fraction.

The potassium hydroxide solution was cooled, acidified with hydrochloric acid to pH 5-6, basified with concentrated ammonium hydroxide to pH 8-9 and extracted four times with ether (75 ml. each time). The ether solutions were pooled, dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a yellow oil (23 mg.) which was designated the phenolic fraction.

Thin layer chromatography of the nonphenolic fraction using benzene-acetone-concentrated ammonium hydroxide (16:16:0.1) as a developing solvent revealed the presence of three compounds with the following Rf values: 0.63, 0.47, and 0.17. Thin layer chromatography of the phenolic fraction under the same conditions revealed the presence of one compound at Rf 0.63. Using phosphomolybdic acid as a spray reagent (followed by exposure of the dry plate to ammonia vapors), demonstrated that the compound at Rf 0.63 was phenolic in both cases. It was thus assumed that the potassium hydroxide extraction of the phenolic cleavage product was incomplete and that some of this phenolic product remained in the nonphenolic fraction.
a. The phenolic cleavage product.

The phenolic fraction (23 mg.) was treated with hydrochloric acid. The acidic solution was stirred and warmed on a water bath at 40°C. The soluble portion was decanted from the insoluble residue and this decantate treated with saturated aqueous potassium iodide solution (3 drops). Immediate turbidity developed and an orange oil deposited. After standing overnight, a yellowish white precipitate formed and the mother liquor was decanted away. The precipitate was dissolved in acetone and allowed to sit overnight in the freezer, whereupon, white crystals deposited. These crystals were very soluble in water, gave negative alkaloid tests with Valsers and Dragendorff's reagents and did not char upon heating. They were most likely potassium iodide and were discarded. The acetone mother liquor was decanted from the potassium iodide crystals and nitrogen gas blown across the acetone solution to reduce its volume. After standing overnight at room temperature, clumps of whitish crystals of the alkaloid hydriodide salt (5.4 mg.) deposited. m.p. 194-196°C, after darkening at 185-187°C; \( \lambda_{\text{max}} \) MeOH 289 m\( \mu \) (log\( \epsilon \) 4.03), \( \lambda_{\text{min}} \) MeOH 257 m\( \mu \). A second crop of crystals (5.4 mg.) was obtained upon further concentration of the mother liquors.

b. The nonphenolic cleavage products.

As the nonphenolic semicrystalline residue (22 mg.) was treated with ether (3 ml.) to effect solution, colorless needles (as rosette clumps) immediately formed (ca. 5 mg.). After standing overnight, the mother liquor was decanted, the crystals rinced with cold ether and the rinsing added to the mother liquor. The crystalline compound was designated nonphenolic cleavage product A. m.p. 166-
169.5°C; $\lambda_{\text{max, MeOH}}$ 287.5 μm ($\log \varepsilon$ 3.42), 280 (3.44); $\lambda_{\text{min, MeOH}}$ 250 μm, with a slight shoulder at 324 μm. The infrared spectrum showed a carbonyl absorption at 1715 cm$^{-1}$ which is characteristic of a six
membered cyclic ketone. The mass spectrum showed a molecular ion peak at m/e 285 (measured 285.1730 and calculated 285.1729), a base peak at m/3 285 and other intense peaks at m/e 270, 242, 227 and 164.

The total mother liquor was evaporated to dryness in vacuo at 40°C. The residue was dissolved in chloroform, streaked onto a preparative thin layer silica gel G plate (200 x 200 mm.) and the plate developed in benzene-acetone-concentrated ammonium hydroxide (16:16:0.1). Three alkaloidal zones ($R_f$ 0.63, 0.47, 0.17) were observed by spraying only a thin section of each side of the plate.

The zones were scraped from the plate and put aside. The zone with $R_f$ 0.17 was placed in a small flask and slurried with a chloroform-methanol (1:1) mixture (50 ml.) for 30 minutes. The solution was filtered and the filtrate evaporated in vacuo at 40°C to leave clear oil. Addition of ether to this oil afforded colorless needles whose thin layer $R_f$, melting point and infrared spectrum were identical with those of the nonphenolic cleavage product A.

The middle zone was placed in a small flask and slurried with a chloroform-methanol (1:1) mixture (50 ml.) for 30 minutes. The solution was filtered and the filtrate evaporated in vacuo at 40°C to leave a dark yellow residue. This residue was dissolved in methanol (15 ml.), methyl iodide (1 ml.) added and the solution refluxed for six hours. The solvent was removed in vacuo at 40°C to leave a yellow residue (28 mg.) which was designated nonphenolic cleavage product B. $[\alpha]_{D}^{25} +5.3^\circ$ (0.275, MeOH); $\lambda_{\text{max, MeOH}}$ 358 μm, 289 μm, 259.5 μm, 220 μm.
155 m µ, 265 m µ, 255 m µ. The top band, which contained the phenolic fragment, was put aside.

2. Permanganate oxidation of alkaloid D (Adiantifoline). A sample of alkaloid D (Adiantifoline) (75 mg.) was dissolved in acetone (30 ml.) in a 125 ml. erlenmeyer flask. A small amount (3 drops) of a saturated solution of potassium permanganate in acetone was added to the alkaloid and the solution heated to boiling on a steam bath. The solution was removed from the steam bath, allowed to cool to room temperature and a stirring magnet was added. The flask was mounted on a magnetic stirring device and stirring begun. The saturated potassium permanganate solution was added dropwise over a period of five days. The addition of permanganate was stopped when the color of the solution was a bright orange-yellow and when the solution showed only two spots (Rf 0.96, 0.88) on thin layer chromatography using benzene-acetone-concentrated ammonium hydroxide (32:16:1) as a developing solvent. The Rf of the starting product, alkaloid D (Adiantifoline), was 0.24 in this system. The solution was filtered, the precipitated manganese dioxide washed well with acetone and the solvent removed in vacuo at 40°C to leave a crude yellow residue (111 mg.). The residue was dissolved in acetone (20 ml.) and methanol (20 ml.) added. The resulting yellow solution was evaporated in vacuo at 40°C until crystals began to form, and allowed to stand overnight at room temperature, whereupon clumps of red orange needles in rosette form deposited (5.2 mg.). This compound was designated oxidation product A. m.p. 230-231.5°C after darkening from 204-210°C; λMeOH

max. 436 m µ (log e 3.61), 314 (4.16), 274 (4.57); λMeOH

min. 411 m µ, 307 m µ, 256 m µ, with a shoulder at 225 m µ. The infrared spectrum showed a carbonyl absorption at
1680 cm$^{-1}$, which is characteristic of an aromatic aldehyde in a highly conjugated system. The mass spectrum showed a parent ion at m/e 533 and other intense peaks at m/e 517 and m/e 502.

3. Sodium-liquid ammonia cleavage of thalicarpine. The same procedure was followed as outlined under the sodium-liquid ammonia cleavage of alkaloid D (Adiantifoline). The following amounts of reagents were used: liquid ammonia (75 ml.), thalicarpine (250 mg.) in toluene (10 ml.) and freshly cut metallic sodium (193 mg.).

After the reaction mixture stood overnight in the hood, the residue was dissolved in 2% hydrochloric acid (50 ml.) and shaken with ether (50 ml.). The ether layer was discarded. The acidic solution was basified with concentrated ammonium hydroxide to pH 8-9 and shaken three times with ether (75 ml., each time). The ether solutions were pooled, dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to a volume of approximately 50 ml. The ammoniacal layer was discarded. The concentrated ether solution was extracted twice with 2% potassium hydroxide solution (25 ml. each time). The ether layer was separated, shaken twice with water (50 ml.) and the water layer discarded. The ether was dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a crude residue (124 mg.), which was designated the nonphenolic fraction.

The potassium hydroxide solution was cooled, acidified with hydrochloric acid to pH 6, basified with concentrated ammonium hydroxide to pH 8 and extracted three times with ether (50-75 ml., each time). The ether solutions were pooled, dried over anhydrous
sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a crude residue (139 mg.), which was designated the phenolic fraction.

a. The phenolic cleavage product.

The phenolic fraction (139 mg.) was dissolved in ethanol (5 ml.) and hydroiodic acid (47-50%) added dropwise until the pH, upon the addition of one more drop, turned acidic. The solution was warmed slightly on a steam bath and the solvent removed in vacuo at 40°C. The residue was dissolved in acetone (ca. 3 ml.) and the resulting solution allowed to stand overnight in the freezer. Yellowish crystals (82 mg.) of the alkaloid hydriodic salt deposited from the concentrated acetone solution, m.p. 192-195.5°C after darkening at 187°C; [α]**D +77° (c 0.673, MeOH). The infrared spectrum of this compound was superimposable with that of the hydriodic salt of the phenolic cleavage product of alkaloid D.

b. The nonphenolic cleavage products.

Thin layer chromatography of the nonphenolic fraction revealed the presence of nine compounds with the following $R_f$ values and relative strengths: 0.76 (+2), 0.72 (+1/2), 0.52 (+3), 0.40 (+4), 0.24 (+3), 0.17 (+3), 0.12 (+2) and 0.08 (+1). The nonphenolic fraction (124 mg.) was dissolved in chloroform (2-3 ml.) and streaked onto three preparative silica gel G thin layer plates. The plates were developed using benzene-acetone-concentrated ammonium hydroxide (16:16:1) as a developing solvent and a small streak at each side of the plate sprayed with Dragendorff reagent to reveal the presence of eight compounds with the following $R_f$ values and relative strengths: 0.86 (+2), 0.77 (+1), 0.64 (+1), 0.51 (+2), 0.39 (+4), 0.18 (+3),
0.13 (+3) and 0.05 (+3). The ninth compound, which should have been seen at approximately Rf 0.24 was apparently mixed in with one of the lower Rf compounds.

The compound at Rf 0.39 was scraped from the plates and placed in a small flask where it was shaken intermittently with a chloroform-methanol (1:1) mixture (50 ml.) for 30 minutes. The solution was filtered and the solvent removed in vacuo at 40°C to leave a crude oil (27 mg.). The oil was dissolved in methanol (20 ml.), methyl iodide added (1 ml.), and the mixture refluxed for three hours. The solvent was removed in vacuo at 40°C to leave an oil (55 mg.). Addition of acetone (3 ml.) to the oil resulted in the immediate formation of white rosette crystals (7 mg.) of methyl iodide salt. This compound was designated thalicarpine nonphenolic cleavage product A. m. p. 225°C decom., after darkening at 157-163°C; \( \lambda_{\text{max.}} \) MeOH 312 m\( \mu \) (log 3.85), 310 (3.85), 298 (3.80), 273 (4.14), 266 (4.14), 217 (4.71); \( \lambda_{\text{min.}} \) MeOH 290 m\( \mu \), 268 m\( \mu \), 249 m\( \mu \). The infrared spectrum showed a carbonyl absorption at 1715 cm.\(^{-1}\) which is characteristic of a six membered cyclic ketone.

The compound with Rf 0.51 was similarly scraped from the plate and placed in a small flask where it was shaken intermittently with a chloroform-methanol (1:1) mixture (50 ml.) for thirty minutes. The solution was filtered and the solvent removed in vacuo at 40°C to leave a brown oil (4.7 mg.). The oil was dissolved in methanol (10 ml.), methyl iodide (1 ml.) added and the mixture refluxed for two hours. The solvent was removed in vacuo at 40°C to leave a yellow oil (11.4 mg.) which was designated thalicarpine nonphenolic cleavage product B. [\( \alpha \)]\(D\)\(32\) +37.3° (c 0.114, MeOH); \( \lambda_{\text{max.}} \) MeOH 359 m\( \mu \), 289 m\( \mu \), 218 m\( \mu \); \( \lambda_{\text{min.}} \) MeOH 329 m\( \mu \), 263 m\( \mu \), 217 m\( \mu \).
4. Permanganate oxidation of thalicarpine. A sample of thalicarpine (75 mg.) was dissolved in acetone (30 ml.) in a 125 ml. erlenmeyer flask. A small amount of a saturated solution of potassium permanganate in acetone was added to the alkaloid and the solution heated to boiling on a steam bath. The solution was removed from the steam bath, allowed to cool to room temperature and a stirring magnet was added. The flask was mounted on a magnetic stirring device and stirring begun. The saturated potassium permanganate solution was added dropwise over a period of five days. The addition of permanganate was stopped when the color of the solution was a bright orange-yellow and when the solution showed only two spots ($R_f$ 0.97, 0.94) on thin layer chromatography using benzene-acetone-concentrated ammonium hydroxide (32:16:1) as a developing solvent. The $R_f$ of the starting product, thalicarpine, was 0.26 in this system. The solution was filtered, the precipitated manganese dioxide washed well with acetone and the solvent removed in vacuo at 40°C to leave a crude yellow residue (129 mg.). The residue was dissolved in acetone (20 ml.) and methanol (20 ml.) added. The resulting yellow solution was evaporated in vacuo at 40°C until crystals began to form. It was removed from the flash evaporator and allowed to stand overnight at room temperature, whereupon clumps of yellow-orange rosettes deposited (4.8 mg.). This compound was designated thalicarpine oxidation product A. m.p. 201-202°C, after darkening at 196.5-198.5°C; $\lambda_{\text{MeOH max}}$ 436 m$\mu$ ($\log\varepsilon$3.50), 314 (3.96), 273 (4.44); $\lambda_{\text{MeOH min}}$ 304 m$\mu$, 257 m$\mu$, with shoulders at 250 m$\mu$ and 225 m$\mu$. The infrared spectrum in KBr pellet showed a carbonyl absorption at 1680 cm.$^{-1}$, which is characteristic of an aromatic aldehyde in a highly conjugated system.
G. Syntheses of Alkaloid K (Noroxyhydrastinine) and Alkaloid N (Thalifoline)

1. The synthesis of alkaloid K (6,7 methylenedioxy-1-oxo-1,2,3,4-tetrahydroisoquinoline) (CCLIII) (Noroxyhydrastinine).

This compound was synthesized by the alkaline permanganate oxidation of berberine chloride (CCLII) following the method of Perkin (116). Berberine chloride (11.72 gm.) (twice recrystallized) was dissolved in water (800 ml.) at 70°C. Anhydrous sodium carbonate (1 gm.) was added, followed by the dropwise addition of a hot (70°C) aqueous solution of potassium permanganate (14.1 gm. in 800 ml.) with stirring over a period of 1-1/2 hours. The resulting solution was cooled to room temperature and a steam of sulfur dioxide gas was passed throughout the vigorously stirred solution for approximately one hour. During this time, the color of the solution changed from a dark brown (with a brown precipitate) to a yellow-orange (with a heavy yellow precipitate). The precipitate was removed via suction filtration and put aside. The filtrate (approximately 1.6 L.) was evaporated to approximately 500 ml. in vacuo at 65°C and the resulting yellow and white precipitate was suction filtered. The filtrate was shaken three times with ether (500 ml. each time) and then transferred to a continuous liquid-liquid extractor and extracted overnight with ether. The ether was removed and the aqueous portion shaken 16 times with ether (500 ml. each time). The ether extracts were pooled and the volume of the solvent reduced to 1 L. on a steam bath in the hood. The remaining solvent was removed in vacuo at 40°C to leave a residue. Boiling water (10 ml.) was added to the residue, followed by enough sodium carbonate to make the solution distinctly basic (pH 10).
The hot solution was immediately filtered through a prewarmed funnel into a prewarmed flask. The cloudy filtrate was heated briefly on a steam bath until a clear solution resulted and then put aside on the bench for 18 hours. Yellowish tinged white crystals deposited as feathery clumps. The crystalline material was filtered and recrystallized several times from hot methanol to yield large white needles (71 mg.). m.p. 179.5-183°C obs.; 181-182°C lit. (113); 
\[
\begin{align*}
\text{MeOH max.} & \quad 303 \text{ mμ} (\log \varepsilon 3.71), \\
\text{MeOH min.} & \quad 279 \text{ mμ}, \\
\end{align*}
\]
254 mμ, 240 mμ, with shoulders at 340 mμ and 270 mμ.

No melting point depression was observed upon admixing alkaloid K and the synthetic product. The infrared spectrum in a potassium bromide pellet showed the N-H stretch of lactam at 3175 cm.⁻¹ and 3040 cm.⁻¹ and the carbonyl stretch of a six membered lactam at 1670 cm.⁻¹. The infrared spectrum was superimposable with that of alkaloid K. The NMR spectrum showed a methylenedioxy group at 6.02 δ (2H), two aromatic protons at 6.69 δ (1H) and 7.58 δ (1H), and two triplets centered at 2.88 δ and 3.53 δ J_app = 7 cps.

![Chemical Structures](CCLII and CCLIII)
2. The synthesis of alkaloid N (Thalifoline) (2-methyl-6-methoxy-7-hydroxy-1-oxo-1,2,3,4-tetrahydroisoquinoline) (CCLXI).

Alkaloid N (Thalifoline) was synthesized in seven steps, as summarized below. Vanillin (CCLIV) was treated with benzyl chloride and base to obtain O-benzylvanillin (CCLV) which was converted to 3-methoxy-4-benzyloxy-\(\beta\)-nitrostyrene (CCLVI) by treatment with nitromethane and base. The nitrostyrene adduct was reduced to 3-methoxy-4-benzyloxy-\(\beta\)-phenethylamine (CCLVII) by treatment with lithium aluminum hydride in tetrahydrofuran. The amine was converted to its N-formyl analog, N-formyl-3-methoxy-4-benzyloxy-\(\beta\)-phenethylamine (CCLVIII), by treatment with 97-100% formic acid. The N-formyl compound was subsequently cyclized with phosphorous oxychloride to 3-methoxy-7-hydroxy-3,4-dihydroisoquinoline (CCLIX). Treatment of the dihydroisoquinoline compound with methyl iodide produced the methiodide salt (CCLX), which was converted to the desired 2-methyl-6-methoxy-7-hydroxy-1-oxo-1,2,3,4-tetrahydroisoquinoline (CCLXI) via an alkaline potassium ferricyanide oxidation.
a. Synthesis of O-benzylvanillin (CCLV)

A solution of sodium hydroxide (10 gm.) in water (25 ml.) was added to a solution of vanillin (CCLIV) (28.5 gm.) in 95 ethanol (125 ml.). Benzyl chloride (25.6 ml.) was added to the semisolid mass and the mixture refluxed overnight. The solution was decanted from a white solid mass (potassium chloride) at approximately 75°C and the solvent removed in vacuo at 40°C to leave an orange oil, which was poured into a 5% sodium hydroxide solution (50 ml.). Scratching of the oil against the bottom and sides of the flask resulted in crystallization. The crystals (38.2 gm., 88% yield) were filtered, washed with water and recrystallized from hot 95% ethanol. Two recrystallizations from hot ethanol afforded an analytical sample. m.p. 55-58°C. This compound was synthesized according to Buck (117), m.p. 60-63°C.

b. Synthesis of 3-methoxy-4-benzylxy-β-nitrostyrene (CCLVI)

A solution of O-benzylvanillin (CCLV) (30 gm.) in ethanol (1L.) was cooled to 5-10°C in an ice bath and nitromethane (15 ml.) was added. A solution of sodium hydroxide (12.5 gm.) in ethanol (250 ml.) was added dropwise with stirring, while the temperature of the reaction was maintained at 5-10°C. A very fine but voluminous precipitate resulted, which was probably the sodium salt of the nitrostyrene adduct. Ice water was added until the precipitate just dissolved and the solution was poured with stirring into a solution of concentrated hydrochloric acid (150 ml.) in water (225 ml.). The resultant fine yellow precipitate was filtered, washed with water and dissolved in acetone (600 ml.). The acetone solution was concentrated on a steam bath to a volume of 300 ml. Upon standing overnight at room
temperature, long yellow needles (20.8 gm.) crystallized from the solution. A subsequent crop of crystals (10.1 gm.) was obtained from the mother liquors (88% yield, based on both crops). Two recrystallizations from hot acetone afforded an analytical sample, m.p. 120-121°C. This compound was synthesized according to Buck (118), m.p. 120-122°C.

c. Synthesis of 3-methoxy-4-benzylxy-β-phenethylamine (CCLVII)

A solution of 3-methoxy-4-benzylxy-β-nitrostyrene (CCLVI) (70 gm.) in anhydrous tetrahydrofuran (500 ml.) was added dropwise, through a dropping funnel to a well stirred solution of lithium aluminum hydride (19.6 gm.) in anhydrous tetrahydrofuran (500 ml.) in a three liter three necked flask in which a dropping funnel was in the left neck, a stirring bar through the middle neck and a reflux condenser in the right neck. The solution rapidly came to reflux as the nitrostyrene compound was added and after the addition was complete, the mixture was refluxed, with vigorous stirring, on a heating mantle for five hours. The mixture was cooled to room temperature, and a saturated aqueous solution of sodium sulfate was added dropwise, with stirring, until the mixture turned white and no dark particles of decomposing lithium aluminum hydride were observed.

The precipitate of inorganic salts was removed by suction filtration, washed twice with tetrahydrofuran (400 ml. each time) and discarded. The solvent was removed from the filtrate in vacuo at 40°C to leave an orange oil (43 gm., 68% yield). This compound was synthesized according to Buck (119). Upon standing for three to four weeks in the refrigerator, the oil slowly crystallized in long needles.
All attempts to recrystallize this amine immediately after reaction workup were unsuccessful and thus the hydrochloride salt was prepared for analytical purposes. An aliquot (1.92 gm.) of the crude liquid amine was dissolved in 95% ethanol (10 ml.) and concentrated hydrochloric acid was added dropwise (10-15 drops) until, upon the addition of one more drop, the pH turned from basic to acidic. Needles immediately began to separate from solution and after thirty minutes the reaction mixture was a solid mass of needles. The material was filtered by suction, dissolved in ethanol, heated to boiling, decolorized with activated charcoal, filtered and the solvent reduced to approximately 10 ml., whereupon needles slowly deposited from the solution. The needles were suction filtered, rinsed with ether and air dried to leave a mass of white needles (204 mg.) m.p. 174.5-176°C, observed; m.p. 173-175°C, literature (120).

The parent amine was first successfully crystallized by regenerating it from the pure hydrochloride salt. The salt (204 mg.) was dissolved in 5% hydrochloric acid solution (25 ml.) and the acidic solution shaken twice with ether (25 ml. each time). The ether washings were discarded. The acidic solution was basified with concentrated ammonium hydroxide to pH 8-9 and shaken twice with ether (50 ml. each time). The ether layers were combined, dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a clear oil (163 mg.). Addition of ether (10 ml.) to the oil and subsequent refrigeration in the freezer resulted in the formation of long needles as rosettes (85 mg.). m.p. 65.5-66.2°C, observed; 67-69°C, literature (121).

d. Synthesis of N-formyl-3-methoxy-4-benzyl oxy-β-phenethylamine (CCLVIII)

3-methoxy-4-benzyl oxy-β-phenethylamine (CCLVII) (10 gm.)
and 97-100% formic acid (10 ml.) were heated in a Woods Metal bath at 175°C for ten hours under reflux. The reaction mixture was cooled to room temperature and cautiously poured into cold water (20 ml.) while stirring vigorously. An oily, aqueous solution with a brown lower layer resulted. This mixture was transferred to a separatory funnel, the parent flask rinsed with water (50 ml.) and the rinsing added to the separatory funnel. This mixture was shaken five times with benzene (100 ml. each time) and the aqueous layer discarded. The benzene extractives were pooled, dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a dark brown oil (16.59 gm.). The oil was used directly for the next step. The synthesis was patterned after Gullard (122) who synthesized N-formyl-3-methoxy-β-phenethylamine (or formyl β-3-methoxyphenethylamine) from 3-methoxy-β-phenethylamine and formic acid under the same conditions.

e. The synthesis of 6-methoxy-7-hydroxy-3,4-dihydroisoquinoline

(CCLIX)

Phosphorous oxychloride (25 ml.) was added dropwise to crude N-formyl-3-methoxy-4-benzylxoy-β-phenethylamine (CCLVIII) (16.59 gm.) while cooling in an ice bath. After removing the reaction mixture from the ice bath, a vigorous evolution of heat with concurrent bubbling was observed. The mixture was subsequently heated under reflux on a steam bath for 45 minutes. After cooling to room temperature, petroleum ether (30-60°C, 50 ml.) was added to the mixture with vigorous swirling. The petroleum ether was decanted off and hydrochloric acid (50 ml.) was added to the mixture while cooling in an ice bath. About 30-45 seconds after the addition of the acid, a
very vigorous reaction, characterized by heat evolution and violent bubbling, took place. This apparently was the reaction of the un-reacted phosphorous oxychloride with water. A dark brown solution with a great deal of brown sludge resulted after the reaction stopped. The solution was decanted from the sludge, basified with concentrated ammonium hydroxide to pH 7 and shaken twice with benzene (100 ml. each time). The pH was then raised to 8 by the addition of more concentrated ammonium hydroxide and shaken twice more with benzene (100 ml. each time). The same process was repeated at pH 9. The benzene solutions were pooled, dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a crude crystalline mass (2.0 gm., 19% yield—based on pure starting material, which, of course, was not used). The crystalline mass was recrystallized from hot methanol to yield yellowish-white needles (573 mg.).

Further crops of crystals (226 mg. and 200 mg.) were obtained by concentration of the mother liquors. A second recrystallization, this time from hot acetone, yielded long and nearly colorless needles. m.p. 183.5–185.0°C.

Anal. Calcd. for C_{10}H_{11}NO_{2}: C, 67.80; H, 6.21; N, 7.91
Found: C, 68.81; H, 6.37; N, 7.76

The infrared spectrum in chloroform showed the presence of an -OH function at 3540 cm.\(^{-1}\) and an imine function (C = N) at 1635 cm.\(^{-1}\). The NMR spectrum showed only two aromatic protons at 6.69\(_{(1H)}\) and 6.90\(_{(1H)}\), thus confirming the cleavage of the benzylxyoxy group at the 7 position of the isoquinoline ring and its subsequent replacement with an -OH group. This synthesis was patterned after Gullard (122) who synthesized 6-methoxy-3,4-dihydroisoquinoline from N-formyl-3-methoxy-\(\beta\)-phenethylamine and phosphorous oxychloride under the same conditions.
f. The synthesis of 6-methoxy-7-hydroxy-3,4-dihydroisoquinoline methiodide (CCLX)

6-methoxy-7-hydroxy-3,4-dihydroisoquinoline (CCLIX) (300 mg.) was dissolved in warm acetone (40 ml.) and methyl iodide (0.8 ml.) added to the solution. After 30 seconds, a copious yellow precipitate formed. The mixture was refrigerated in the freezer for 15 minutes and the precipitate suction filtered and washed with ether to yield the yellow crystalline methiodide (423 mg.) (78% yield).

The methiodide salt was recrystallized from hot methanol several times to afford an analytical sample. m.p. 211-212.5°C, after darkening at 209-210°C.

Anal. Calcd. for C_{11}H_{14}NO_{2}I:  C, 41.38; H, 4.39; N, 4.39
Found:  C, 41.48; H, 4.45; N, 4.29

g. The synthesis of 2-methyl-6-methoxy-7-hydroxy-1-oxo-1,2,3,4-tetrahydroisoquinoline (CCLXI) (Thalifoline)

6-methoxy-7-hydroxy-3,4-dihydroisoquinoline methiodide (CCLX) (289 mg.) was dissolved in water (5 ml.). Potassium ferricyanide (1.54 gm.) was dissolved in 10% potassium hydroxide solution (10 ml.) and the resulting solution placed in an ice bath on a magnetic stirring device. A small stirring magnet was added to the basic solution followed by the dropwise addition, with vigorous stirring, of the methiodide solution over a period of thirty minutes. The solution, whose temperature was always kept between 5-10°C, was stirred an additional thirty minutes and then allowed to warm to room temperature. Glacial acetic acid was added dropwise to the basic
solution until the pH reached 5-6. The acidic solution was shaken five times with ether (100 ml. each time), the ether solutions combined, dried over anhydrous sodium sulfate, filtered and the solvent removed in vacuo at 40°C to leave a yellow oil, which deposited white feathery rosette crystals (82 mg., 44% yield) on standing. One re-crystallization from chloroform-Skellysolve B gave clear feathery plates (9.4 mg.). m.p. 208-211°C; $\lambda_{\text{MeOH}}^{\text{max}}$ 301 μ\(\lambda \) (log ε 3.71), 261 (3.81), 223 (4.37); $\lambda_{\text{MeOH}}^{\text{min}}$ 279 μ\(\lambda \), 242 μ\(\lambda \), 213 μ\(\lambda \), with a shoulder at 269 μ\(\lambda \); $\lambda_{\text{0.01N Methanolic HCl}}^{\text{max}}$ 301 μ\(\lambda \) (log ε 3.71), 261 (3.80), 223 (4.36); $\lambda_{\text{0.01N Methanolic HCl}}^{\text{min}}$ 279 μ\(\lambda \), 247 μ\(\lambda \), 214 μ\(\lambda \), with a shoulder at 269 μ\(\lambda \); $\lambda_{\text{0.01N Methanolic KOH}}^{\text{max}}$ 330 (log ε 3.59), 269 (3.68), 237 (4.35); $\lambda_{\text{0.01 Methanolic KOH}}^{\text{min}}$ 291 μ\(\lambda \), 264 μ\(\lambda \), 222 μ\(\lambda \), with a shoulder at 311 μ\(\lambda \). The infrared spectrum of (CCLXVIII) in a potassium bromide pellet was superimposable with that of alkaloid N. This synthesis was patterned after Sugasawa (188) who synthesized 2-methyl-6,7-dimethoxy-1-oxo-1,2,3,4-tetrahydroisoquinoline from 6,7-dimethoxy-3,4-dihydroisoquinoline and alkaline potassium ferricyanide.

h. The synthesis of berberrubine iodide (CCLXIII)

Berberine iodide (400 mg.) (CCLXII) and urea (800 mg.) were thoroughly mixed and put into a pyrex test tube (2.2 cm. x 15 cm.). The test tube was heated for 45 minutes at 180-190°C in a Woods Metal bath. Upon cooling, the dark residue was dissolved in water (25 ml.) and the resulting dark red solution shaken repeatedly with chloroform (75 ml. each time) until the chloroform layer became nearly colorless. The chloroform solutions were pooled, dried over anhydrous sodium sulfate, filtered and the chloroform removed in vacuo.
at 40°C to leave a dark red residue. This residue was dissolved in warm 1% hydrochloric acid (25 ml.) and filtered. A saturated aqueous potassium iodide solution was added dropwise to the warm filtrate until precipitation was complete. The resulting bright yellow precipitate was filtered by suction and washed with a small volume of water. The precipitate of berberrubine iodide and a small amount of berberine iodide was suspended in methanol (50 ml.), heated, and a small amount of insoluble residue removed by filtration. The final volume of the methanol solution was adjusted to 25 ml. by evaporation on a steam bath. Red orange prisms (139 mg.) (36% yield) of berberrubine iodide deposited from solution upon standing overnight. Two recrystallizations from hot methanol afforded an analytical sample. m.p. 212°C decom. This alkaloid was synthesized by a variation of the method of Frerichs and Stoepel (123).

(CCLXII)  (CCLXIII)
The nuclear magnetic resonance spectrum (in trifluoroacetic acid with tetramethyldisilane as internal standard) of the chloride salt showed the presence of one methoxy group at 4.18 δ (3H), one methylenedioxy group at 6.12 δ (2H), and six aromatic protons at 6.95 δ (2H), 7.52 δ (1H), an apparent AB system centered at 7.81 δ (1H) and 8.02 δ (1H), J_{app} 9 cps, 8.44 δ (1H) and 9.66 δ (1H).
III. DISCUSSION

The alkaloids from the roots of *Thalictrum minus* L. var. adiantifolium Hort. were isolated from both petroleum ether and ethanol extracts, while the alkaloids from the tops were isolated from the ethanol extract. The total extracts were fractionated into tertiary (phenolic and nonphenolic) quaternary and neutral fractions. Further fractionation of the tertiary nonphenolic fractions was accomplished via the pH gradient technique and the tertiary alkaloids were isolated after column chromatography. The quaternary alkaloids were obtained as iodide salts, after converting their reineckate salts to chloride salts by passing through a resin in the chloride form and subsequent treatment with potassium iodide solution.

Eleven different alkaloids were isolated from the plant. Ten alkaloids were isolated from the roots (Alkaloid A, O-Methylthalic-berine, diantifoline, thalifendine iodide, berberine iodide, alkaloid J, noroxyhydrastinine, alkaloid L, thalifoline and magnoflorine iodide) and four from the tops (berberine iodide, thalifendine iodide, diantifoline and alkaloid S), with three of the four from the tops being the same as from the roots (berberine iodide, thalifendine iodide and diantifoline). Alkaloid S was only present in the tops.

Six of the alkaloids were positively characterized (O-Methylthalicberine, berberine iodide, thalifendine iodide, magnoflorine iodide, noroxyhydrastinine and thalifoline) and the other five were partially characterized (diantifoline, alkaloids A, J, L and S).
Of the ten alkaloids isolated from the roots, six were positively characterized (O-methylthalicberine, berberine iodide, thalifendine iodide, magnoflorine iodide, noroxyhydrastinine and thalifoline) and four partially characterized (adiantifoline, alkaloids A, J and L), while two of the four alkaloids isolated from the tops were positively characterized (berberine iodide and thalifendine iodide) and two were partially characterized (adiantifoline and alkaloid S).

Each of the isolated compounds will be discussed in turn. Structures will be assigned where possible and postulated upon where enough evidence is available.

A. Alkaloid A

This compound was found to have a superimposable infrared spectrum, an identical melting point (m.p. 150-151°C) and an identical ultraviolet spectrum with that of alkaloid H (151.5-152.5°C). Thus, both compounds were assumed to be identical and will be discussed together. The infrared spectrum indicated the possibility of an imine function (C = N) at ca. 1640 cm⁻¹. A bathochromic shift in acidic methanol in the ultraviolet spectrum supported this observation. The ultraviolet spectrum of alkaloid A $\lambda_{max}^{MeOH}$ 349 $\mu$m (log $\varepsilon$ 3.80), 261 (4.49); $\lambda_{max}^{0.01N\text{ Methanolic HCl}}$ 284 (log $\varepsilon$ 4.44); $\lambda_{max}^{0.01N\text{ Methanolic KOH}}$ 349 $\mu$m (log $\varepsilon$ 3.80), 262 (4.49) is not typical of a benzylisoquinoline or bisbenzylisoquinoline, since both of the latter show a single maximum at ca. 283 $\mu$m (log $\varepsilon$ 3.7) (100). Neither is the spectrum of alkaloid A typical of a benzylisoquinoline-aporphine dimer (e.g., thalicarpine), since this type of compound exhibits maxima at ca. 282 $\mu$m (log $\varepsilon$ 4.2) and 302 $\mu$m (log $\varepsilon$ 4.1). If however, the C ring of the aporphine moiety of a benzylisoquinoline-aporphine dimer (like thalicarpine, CLXXIX) shows
some unsaturation (as in dehydrothalicarpine, CLXXVI), the maxima become 268 μ (log ε 4.81) and 331 μ (log ε 4.34). The ultraviolet spectrum of thalmethine (which contains an imine function (C = N)), an alkaloid isolated from Bulgarian T. minus, was not reported (83) and thus a comparison with this type of compound cannot be made. The nuclear magnetic resonance spectrum (55 scans, C-1024) indicated the possibility of one N-methyl group at 2.25 δ (3H), four methoxy groups at 3.41 δ (3H), 3.50 δ (3H), 3.65 δ (3H), and 3.77 δ (3H), one methylenedioxy group at 6.01 δ (2H) and approximately five aromatic protons (by integration). The spectrum was not determined further downfield than 8.0 δ, thus any low field protons were missed. The mass spectrum showed a molecular ion at m/e 648, corresponding to an empirical formula of C_{38}H_{36}N_{2}O_{8}, a base peak at m/e 58 and other intense peaks at m/e 633, 618, 442, 422, 403, 324, 220, 204 and 190. Double bond equivalents were calculated to be 22.

Lack of material prevented further work on this alkaloid. Alkaloids C and H were shown to be the same as alkaloid A.

B. Alkaloid B (O-methylthalicberine)

The ultraviolet spectrum (λ_{max}^{MeOH} 278 μ (log ε 3.65) ) of this compound was suggestive of that of a bisbenzyltetrahydroisoquinoline alkaloid. The absence of a bathochromic shift in acidic or basic media was a strong indication that phenolic (Ar-OH) and imine (C = N) functions were absent. The lack of characteristic infrared absorption for these two functions was also observed, thus, it appeared that alkaloid B was a nonphenolic bisbenzylisoquinoline alkaloid. Mass spectral data showed a molecular ion peak at m/e 622 corresponding to an empirical formula of C_{38}H_{42}N_{2}O_{6}. A review of the known Thalictrum bisbenzylisoquinoline alkaloids showed that O-methylthalicberine (CCLXIV) had the
proper empirical formula. The infrared spectrum of alkaloid B was compared with that of authentic O-methylthalicberine and found to be superimposable. In addition, there was no mixed melting point depression nor difference in thin layer chromatographic $R_f$ values.

Further support for this structure was found in the mass spectral data, as the mass spectrum of alkaloid B and that of authentic O-methylthalicberine (124) were identical. The intense peaks observed at m/e 396 and m/e 198 could be attributed to two cleavages (cleavages of the carbon-carbon bonds which are both β to nitrogen and β to two aromatic systems). If two charges are retained on the tetrahydroisoquinoline half of the molecule, a fragment is found at m/e 198, but if only one charge is retained, a fragment is found at m/e 396 (CCLX V). Alkaloid B was therefore shown to be O-methylthalicberine (CCLXIV).
C. Alkaloid D (Adiantifoline)

This alkaloid was named adiantifoline, after the variety of the plant. The ultraviolet spectrum of this alkaloid strongly resembled that of a benzylisoquinoline-aporphine dimer (e.g., thalicarpine). The absence of a bathochromic shift in acidic or basic media was strongly indicative of the absence of a phenolic (Ar-OH) or imine (C=N) function. Characteristic infrared absorptions were also lacking for these two functions. Thus, adiantifoline was believed to be a nonphenolic alkaloid of the dimeric benzylisoquinoline-aporphine type.

The nuclear magnetic resonance spectrum indicated the possibility of eight methoxy groups at 3.59 (3H), 3.78 (9H), 3.82 (3H), 3.89 (3H), 3.94 (3H), and 3.96 (3H), two N-methyl groups at 2.44 (3H) and 2.47 (3H) and six aromatic protons at 6.24 (1H), 6.55 (2H), 6.60 (2H) and 8.08 (1H).

The benzyltetrahydroisoquinoline-aporphine dimer thalicarpine (CCLXVI) very closely resembled adiantifoline in its spectral (ultraviolet, infrared, nuclear magnetic resonance) properties. The only difference between adiantifoline and thalicarpine was that adiantifoline contained one more methoxy group (eight) than thalicarpine (seven).
Assuming that adiantifoline was a benzyltetrahydroisoquinoline-aporphine dimer containing eight methoxy groups, two N-methyl groups, six aromatic protons and one ether bridge, the molecular formula 
\[ C_{42}H_{50}N_2O_9 \] would be appropriate. Elemental analysis showed a close correlation with the expected formula.

Anal. Calcd. for \( C_{42}H_{50}N_2O_9 \):  
- C, 69.41; H, 6.88; N, 3.85  
Found:  
- C, 70.03; H, 7.01; N, 3.96

The double bond equivalents were calculated to be 19.

As a working hypothesis, it was assumed that adiantifoline had the same methoxy substitution pattern and the same ether bridge connection as thalicarpine. The problem of structure determination of adiantifoline would be one of proper placement of the extra methoxy group in one of the four aromatic rings.

From the mass spectral data, an intense peak at m/e 206 indicated the presence of fragment (CCLXVII). In addition, the mass with the highest value (m/e 522) is not the parent ion (molecular weight 726) which is similar to the behavior of thalicarpine (molecular weight 696), which shows the mass with the highest value as m/e 492. It is of interest to note that the difference in the mass units of the above two masses with the highest values is 30 (OCH₂), which is in support of the expected difference (one methoxy group) of adiantifoline and thalicarpine.

\[ \text{CCLXVII} \]
The nuclear magnetic resonance data revealed two important facts. First, a low field signal at 8.08 was observed, which is characteristic of the 11-hydrogen of an aporphine system (125). Thus, the aporphine moiety bears no substituent at C-11. Second, no AB-type quartet, expected for the C-8 and C-9 hydrogen atoms of the aporphine moiety was observed in the aromatic proton region of the nuclear magnetic resonance spectrum.

In order to obtain information about the methoxylation substitution pattern and the placement of the diaryl ether bridge, a sodium-liquid ammonia cleavage of alkaloid D was performed. In addition, a similar cleavage of thalicarpine was performed in order to obtain its cleavage products for comparison with those of adiantifoline.

Cleavage of thalicarpine (CCLXVIII) with sodium in liquid ammonia was reported (126) to yield three products, two of which were nonphenolic (CCLXX and CCLXXI) and the third was phenolic (CCLXIX).
Cleavage of adiantifoline with sodium in liquid ammonia yielded three products, two of which were nonphenolic and the third was phenolic. The infrared spectrum of the hydriodide salt of the phenolic cleavage product of adiantifoline was superimposable with that of the hydriodide salt of the phenolic cleavage product (1-benzyl-3',4'-dimethoxy-6'-hydroxy)-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline) (CCLXIX) of thalicarpine. In addition, the ultraviolet spectrum of the former ($\lambda_{\text{max}}^\text{MeOH} 289 \text{ m} \mu (\log \varepsilon 4.03)$) closely resembled that reported (122) for the latter ($\lambda_{\text{max}}^\text{MeOH} 289 \text{ m} \mu (\log \varepsilon 3.76)$). It can be stated, therefore, that the phenolic cleavage product of thalicarpine and of adiantifoline are structurally identical. Since only small amounts of the latter were obtained, no specific rotation was performed and thus no stereochemical assignment can be made.

The nonphenolic ketonic cleavage product obtained from the cleavage of adiantifoline appeared identical in every respect to that described in the literature (122). The carbonyl absorption at 1715 cm. $^{-1}$ in the infrared spectrum, the molecular ion peak at m/e 285 and the maxima in the ultraviolet spectrum at 287.5 m$\mu$ ($\log \varepsilon 3.42$) and 281 m$\mu$ ($\log \varepsilon 3.44$) were all in good agreement with the reported values (122) of 5.85 m$\mu$ (1709 cm. $^{-1}$), M$^+$ at m/e 285, and 289 m$\mu$ ($\log \varepsilon 3.38$) and 281 m$\mu$ ($\log \varepsilon 3.42$).

The methiodide salt of the nonphenolic ketonic cleavage product (CCLXX) of thalicarpine showed an infrared spectrum that was very similar to that of the nonphenolic ketonic cleavage product of adiantifoline. Thus, two of the three sodium-liquid ammonia cleavage products of alkaloid D were found to be identical with those of thalicarpine.

The co-identity of the phenolic products indicated that the extra methoxy group of adiantifoline was not in the benzylisoquinoline half of
the molecule. The co-identity of the nonphenolic ketonic products indicated that the methoxy group was not in the bottom or D ring of the aporphine system. The absence of an AB-type quartet expected for the C-8 and C-9 hydrogen atoms of the aporphine moiety combined with the presence of a low field aromatic proton signal at 8.08δ indicated that the ether bridge was not at position 11 of the aporphine system. Since it was demonstrated that the nonphenolic ketonic cleavage product of adiantifoline was identical with the nonphenolic ketonic cleavage product (CCLXXI) of thalicarpine and since the latter contains a methoxy group at the 10-position, the ether linkage of adiantifoline must either be at position 8 or position 9 of the aporphine system. Since the ether linkage in thalicarpine is at the 9 position and since no known naturally occurring aporphine bears an oxygenated substituent at the 8 position, the 9 position was regarded as the point of attachment of the ether bridge in the aporphine part of adiantifoline. The phenolic cleavage products of thalicarpine and adiantifoline being identical, demonstrated that the other end of the ether linkage is at the 6 position of the benzyl ring of the benzyltetrahydroisoquinoline system.

Permanganate oxidation (in acetone) of thalicarpine (CCLXVIII) has been reported (127) to yield two fragments, one of which is an isoquinolone (CCLXXII) and the other a highly conjugated aromatic aldehyde (CCLXXIII).
Permanganate oxidation of adiantifoline (in acetone) yielded a deep yellow colored compound exhibiting a strong infrared absorption at 1680 cm\(^{-1}\), which is characteristic of an aromatic aldehyde in a highly conjugated system.

The mass spectrum of this aromatic aldehyde showed a molecular ion at m/e 533 and other intense peaks at m/e 517 and m/e 502. Considering the above information, the aromatic aldehyde obtained from the oxidation of adiantifoline may be assigned the tentative structure (CCLXXIV).
Further weight was lent to the belief that the extra methoxy group of adiantifoline was in the aporphine moiety when the correct molecular weight of 533 from the mass spectrum was obtained for structure (CCLXXIV).

After examining all of the above presented evidence, there remains only one position in which the extra methoxy group of adiantifoline may reside and that is the 3 position of the aporphine moiety. Thus, structure (CCLXXV) was postulated as the structure of adiantifoline. Alkaloids E and R were also identified as adiantifoline.

(CCLXXIV)

(CCLXXV)
A plausible synthesis of compound (CCLXXV) would be an Ullmann condensation of 6-bromolaudanosine (CCLXXVI) with 3-methoxy-N-methyl-laurotetane (CCLXXVII). Before a synthesis of diantifoline is attempted to prove its identity without a doubt, the stereochemical assignments for the two asymmetric centers will have to be made. This will require obtaining more of this alkaloid and its cleavage products.

\[ \text{(CCLXXVI)} \quad \text{(CCLXXVII)} \]

D. Alkaloid F (Thalifendine iodide)

The infrared spectrum of this compound indicated the possibility of a hydroxyl group. The ultraviolet spectrum of the compound was very similar to that of the protoberberine alkaloid class and showed a bathochromic shift in basic media. The nuclear magnetic resonance spectrum (in trifluoroacetic acid with tetramethylsilane as internal standard - Varian HA-100 spectrometer) of the chloride salt indicated the possibility of one methoxy group at 4.24 $\delta$ (3H), one methylenedioxy group at 6.12 $\delta$ (2H), and five aromatic protons at 6.92 $\delta$ (1H), 7.48 $\delta$ (1H),
7.92δ(2H) and 8.46δ(1H). An additional proton was suspected farther downfield (around 9.5δ) but the spectrum was only run from 0-9δ. The mass spectrum showed a M-1 peak at m/e 321 (measured 321.0997 and calculated 321.1001) (C₁₉H₁₆NO₄I - HI = C₁₉H₁₅NO₄). From the above data, alkaloid F was suspected of being a protoberberine alkaloid containing a methylenedioxy group, a methoxy group and a hydroxy group.

Two protoberberine alkaloids, thalifendine (CCLXXVIII) and berberrubine (CCLXXIX) had properties resembling those of alkaloid F.

Berberrubine iodide was synthesized from berberine iodide and urea (123). Both the infrared spectrum and the nuclear magnetic resonance spectrum of berberrubine chloride were different from alkaloid F. The reported nuclear magnetic resonance spectrum of thalifendine (128) was in close agreement with that of alkaloid F and the infrared spectrum of an authentic sample of thalifendine iodide was superimposable with that of alkaloid F. In addition, thalifendine iodide and alkaloid F
iodide exhibited identical thin layer behavior, while berberrubine iodide differed from them both. Thus alkaloid F was shown to be thalifendine iodide. Alkaloids M and T were shown to be the same as thalifendine iodide.

E. Alkaloid J

Very little was deduced about the structure of this alkaloid due to the small quantity available and the lack of nuclear magnetic resonance and mass spectral data. The compound indicated the possibility of aromatic character as exemplified by the absorptions at 1600 cm$^{-1}$, 1580 cm$^{-1}$ and 1500 cm$^{-1}$ in the infrared spectrum. Little else can be said about the structure of this compound.

F. Alkaloid K (Noroxyhydrastinine)

The infrared spectrum of this compound indicated the possibility of a six membered cyclic lactam by the characteristic absorptions at 3175 cm$^{-1}$, 3040 cm$^{-1}$ and 1670 cm$^{-1}$. A peak at 925 cm$^{-1}$ was indicative of an aromatic methylenedioxy group. No phenolic absorption was observed in the infrared spectrum nor any bathochromic shift in alkaline medium in the ultraviolet spectrum.

The mass spectrum showed a molecular ion peak at m/e 191 (measured 191.0578 and calculated 191.0582 for $C_{10}H_9NO_3$) which corresponds to an empirical formula of $C_{10}H_9NO_3$.

Coordination of this information led to the postulation of a two ring system, one of which was aromatic and contained a methylenedioxy group and the other of which was a $\delta$-lactam. Since one oxygen atom was in the carbonyl group and the other two in the methylenedioxy group, all three oxygen atoms were accounted for. The nitrogen atom, of course,
was present in the lactam function. All of this data, coupled with the postulated structural intermediates from the mass spectral fragments as shown below at m/e 162 (CCLXXX), m/e 134 (CCLXXXI) and m/e 104 (CCLXXXII) led to the possibility of 6,7-methylenedioxy-1-oxo-1,2,3,4-tetrahydroisoquinoline (noroxyhydrastinine) (115) as the structure of alkaloid K.

\[
\begin{align*}
(C_{19}H_{20}NO_3) & \quad \text{m/e} \, 191 \\
(C_{19}H_{20}NO_3) & \quad \text{m/e} \, 162 \\
(C_{9}H_{6}O_3) & \quad \text{m/e} \, 134 \\
(C_{7}H_{4}O) & \quad \text{m/e} \, 104
\end{align*}
\]

An authentic sample of this tetrahydroisoquinoline compound (CCLXXXIII) was obtained via an alkaline potassium permanganate oxidation of berberine chloride (CCLXXXIV) following the procedure of Perkin (116).
The resulting tetrahydroisoquinolone (CCLXXXIII) had an infrared spectrum superimposable with that of alkaloid K. In addition, the ultraviolet spectra were the same and no melting point depression was observed upon admixture of the two. Thus, alkaloid K was shown to be 6, 7-methyleneedioxy-1-oxo-1,2,3,4-tetrahydroisoquinoline (nor-oxysteratrine) (CCLXXXIII). This is the first reported isolation of this compound from natural sources. The only previous reference to this compound was in the structure determination of berberine via its alkaline permanganate oxidation products (115).

G. Alkaloid L

As with other samples, the small amount of alkaloid material isolated prevented any extensive study of the compound. The infrared spectrum showed a strong absorption at ca. 1740 cm.\(^{-1}\) which could be
attributed to several systems including a five membered cyclic ketone, an ester, and α-keto ester, a substituted four membered cyclic lactam and a five membered α,β-unsaturated lactone. An absorption at 925 cm⁻¹ demonstrated the presence of a possible methyleneedioxy group. The mass spectrum showed a molecular ion at m/e 380. The ultraviolet spectrum showed no resemblance to that anticipated for a benzylisoquinoline, bisbenzylisoquinoline, aporphine, benzylisoquinoline-aporphine dimer or isoquinolone.

H. Alkaloid N (Thalifoline)

The infrared spectrum of this alkaloid showed a strong hydroxy absorption due to its phenolic character and a strong carbonyl absorption at 1640 cm⁻¹, characteristic of a six membered or lactam (tertiary). The ultraviolet spectrum showed a pronounced bathochromic shift in alkaline media due to the phenolic group. This spectrum also closely resembled that of alkaloid K (noroxyhydrastinine, 6,7-methyleneedioxy-1-oxo-1,2,3,4-tetrahydroisoquinoline) except for the bathochromic shift in base. The mass spectrum exhibited a molecular ion peak at m/e 207 (measured 207.0885 and calculated 207.0895 for C₁₁H₁₃NO₃), corresponding to an empirical formula of C₁₁H₁₃NO₃.

One oxygen atom was unaccounted for, since of the three, one is necessary for the phenolic hydroxy group and the other for the carbonyl group. By assuming that the alkaloid might be a tetrahydroisoquinolone, as was alkaloid K, and by assuming that the third oxygen atom was present in a methoxy group, one of two possible structures (CCLXXXV) and (CCLXXXVI) seemed appropriate.
Mass spectral fragments at m/e 164 (CCLXXXVII), m/e 136 (CCLXXXVIII), and m/e 121 (CCLXXXIX) lent weight to this postulation.

Synthesis of compound (CCLXXXV), as described in the experimental section, showed that alkaloid N and compound (CCLXXXV) exhibited superimposable infrared spectra, nearly identical ultraviolet spectra and identical thin layer behavior. Thus, alkaloid N was shown to be 2-methyl-6-methoxy-7-hydroxy-1-oxo-1,2,3,4-tetrahydroisoquinoline (CCLXXXV) and was named thalifoline. This is the first reported isolation of this compound from natural sources. The only previous
reference to this compound (and then, only as the ethoxy ether) was in the structure determination of columbamine via the permanganate (189) oxidation of the ethoxy ether of tetrahydrocolumbamine (isocorypalmine).

I. Alkaloid P (Magnoflorine iodide)

This quaternary alkaloidal iodide was strongly suspected of being magnoflorine iodide, from its physical properties and the almost universal presence of this alkaloid in Thalictrum species.

The infrared spectrum of this compound was found to be superimposable with that of authentic magnoflorine iodide. In addition, there was no mixed melting point depression nor any difference in thin layer behavior in several solvent systems. The ultraviolet spectrum was also in close agreement with the authentic compound. Thus, alkaloid P was shown to be magnoflorine iodide (CCXC).

![CCXC](image)

J. Alkaloid Q (Berberine iodide)

This yellow quaternary alkaloidal iodide was strongly suspected of being berberine iodide due to its abundance, color, fluorescence under longwave ultraviolet light, and thin layer behavior; almost universal presence in Thalictrum species. The infrared spectrum of this compound was found to be superimposable with that of authentic berberine iodide.
There was no melting point depression upon admixture of the two compounds nor any difference in thin layer behavior in several solvent systems. The ultraviolet spectrum was also in agreement with the known compound. Thus, alkaloid Q was shown to be berberine iodide (CCXCI).

In addition, reduction of alkaloid Q with either zinc in acetic acid or sodium borohydride in methanol led to the tetrahydro compound, which was identical in all respects to authentic tetrahydroberberine (CCXCII).

K. Alkaloid S

The infrared spectrum of this compound showed two strong peaks at 1690 cm.$^{-1}$ and 1640 cm.$^{-1}$. These absorptions, both in the carbonyl region, could be due to a number of varied groupings. The absorption at 1690 cm.$^{-1}$ could be attributed to an $\alpha,\beta$-unsaturated acyclic ketone,
and α,β-unsaturated cyclic six membered ketone, an aryl ketone or an α,β-unsaturated aliphatic aldehyde. The 1640 cm$^{-1}$ band could be due to a six membered or lactam (tertiary). The ultraviolet spectrum
\[ \lambda_{\text{max.}}^{\text{MeOH}} 317 \text{m} \mu (\log \varepsilon 3.75), 278 (3.71) \text{ and } 232 (4.21) \] showed a resemblance to that of magnoflorine iodide
\[ \lambda_{\text{max.}}^{\text{MeOH}} 323 \text{m} \mu (\log \varepsilon 3.90), 279 (3.88) \text{ and } 224.5 (4.65). \]

The nuclear magnetic resonance spectrum (Varian HA-100 spectrometer) indicated the possibility of two methoxy groups at 3.78$\delta$ (3H) and 3.86$\delta$ (3H), one methylenedioxy group at 6.00$\delta$ (2H) and seven aromatic protons (via integration). There is a singlet at 5.13$\delta$ (1H) (probably olefinic) and an apparent AB system as a quartet centered at 6.88$\delta$ and 7.05$\delta$, $J_{\text{app}} = 8$ cps, probably due to ortho coupling of aromatic protons ($J = 6-9$ cps).

The mass spectrum showed a molecular ion peak at m/e 381 (measured 381.1226 and calculated 381.1212 for C$_{21}$H$_{19}$NO$_6$) and a base peak at m/e 352 corresponding to the empirical formulas of C$_{21}$H$_{19}$NO$_6$ and C$_{20}$H$_{18}$NO$_5$. Other intense peaks were observed at m/e 338, 322, 205, 190, 176, and 148.

In summary, this compound appears to contain two carbonyl functions, two methoxy groups, a methylenedioxy group and approximately seven aromatic protons. No N-methyl or N-alkyl group was observed in the nuclear magnetic resonance spectrum, as was expected from the 1640 cm$^{-1}$ peak in the infrared spectrum. Lack of material precluded further work on this substance.
L. Alkaloid V (Berberine chloride)

This yellow alkaloidal chloride was suspected of being berberine chloride because of its characteristic yellow fluorescence under long wave ultraviolet light and its characteristic thin layer behavior. The infrared spectrum of this compound was found to be superimposable with that of authentic berberine chloride. No melting point depression upon admixture of the two nor any difference in thin layer behavior was observed.

In addition, preparation of the iodide salt of alkaloid V yielded a compound that was identical in all respects to authentic berberine iodide. Thus, alkaloid V was shown to be berberine chloride.

M. Neutral compound A (β-sitosterol)

The infrared spectrum of this compound indicated the possibility of an -OH group. The mass spectrum showed a molecular ion at m/e 414 (measured 414,3865 and calculated 414,3861 for C_{29}H_{50}O) which corresponds to an empirical formula of C_{29}H_{50}O. This empirical formula and the neutral character of the compound was suggestive of a sitosterol. β-sitosterol was immediately suspected due to its wide distribution in the plant kingdom. The specific rotation of neutral compound A ([\alpha]_{D}^{28.4} -38.8^\circ (c 0.52, CHCl_{3}) ) was quite close to the reported value (129) ([\alpha]_{D}^{25} -37^\circ (c 2, CHCl_{3}) ).

The acetate of neutral compound A was prepared and its specific rotation ([\alpha]_{D}^{27.2} -42^\circ (c 0.1, CHCl_{3}) ) was in close agreement with the reported value (128) ([\alpha]_{D}^{25} -41^\circ (c 2, CHCl_{3}) ). In addition, there was no melting point depression upon admixture of the two (CCXCIII).
Thus, neutral compound A was found to be $\beta$-sitosterol (CCXCIII).

As a final review of the discussion, several general comments are in order. The isolation of $O$-methylthalicberine from the roots of the plant constitutes the first time that this alkaloid has been reported in any other Thalictrum species than $T. \text{thunbergii}$ (130), in which it was originally found.

The isolation of alkaloid D (adiantifoline) from both the roots and the tops of the plant is of interest, for this alkaloid is a new compound of the novel benzylisoquinoline-aporphine dimeric class. Only two alkaloids, thalicarpine (131) and thalmelatine (132) previously comprised this unique class.

The isolation of thalifendine from both the roots and the tops of the plants constitutes only the second Thalictrum species ($T. \text{fendleri}$-first) from which this compound has been isolated. It is of value to note that thalifendine was isolated from tertiary fractions (mainly phenolic) and not quaternary fractions, even though it is a quaternary protoberberine. Thalifendine partitions quite readily into chloroform from a basic aqueous solution, thus necessitating careful testing of aqueous mother liquors before discarding them.
The isolation of two isoquinolone alkaloids (6, 7-methylene-dioxy-1-oxo-1, 2, 3, 4-tetrahydroisoquinoline) (noroxyhydrastinine) and (2-methyl-6-methoxy-7-hydroxy-1-oxo-1, 2, 3, 4-tetrahydroisoquinoline) (thalifoline) constitutes the first reported isolation of these compounds from a natural source.

The isolation of berberine and magnoflorine from the plant was not surprising due to their nearly universal distribution in Thalictrum species.

It should be noted that the tertiary Thalictrum alkaloids are not easily separated or crystallized, especially when present in small amounts (less than 100 mg.). Bisbenzyltetrahydroisoquinoline alkaloids, as the free bases, are especially resistant to crystallization and for this reason the isolation and characterization of these compounds may be greatly facilitated by converting them to various salts of mineral acids.

Because of the great structural similarity of many of the alkaloids, partition chromatography and countercurrent distribution are additional methods which should be investigated thoroughly in future Thalictrum work.
IV. SUMMARY OF FINDINGS

1. A complete literature survey of the alkaloids of the genus Thalictrum was presented.

2. Extraction of the roots with petroleum ether and ethanol and the tops with ethanol yielded alkaloidal extracts which were fractionated into tertiary (nonphenolic and phenolic), quaternary and neutral fractions.

3. The tertiary nonphenolic alkaloid fraction was further fractionated by the pH gradient technique.

4. Column chromatography on alumina and silicic acid-celite (4:1) of the tertiary nonphenolic alkaloid fractions resulted in the isolation of seven tertiary nonphenolic alkaloids, five of which were present in the roots (alkaloid A, O-methylthalicberine, alkaloid J, alkaloid L and noroxyhydrastinine), one in the tops (alkaloid S) and one in both the roots and the tops (adiantifoline).

5. Column chromatography on silicic acid-celite (4:1) of the tertiary phenolic alkaloid fraction from the roots resulted in the isolation of thalifoline.

6. Ion exchange and adsorption chromatography of the quaternary alkaloid fraction resulted in the isolation and identification of three quaternary alkaloids, two of which were present in both the roots and the tops (berberine iodide and thalifendine iodide) and the third in the roots (magnoflorine iodide).
7. Of the seven tertiary nonphenolic alkaloids isolated, two of them were positively identified (O-methylthalicberine and noroxyhydrastinine) while five were partially characterized (alkaloids A, J, L, S and adiantifoline).

8. Noroxyhydrastinine and thalifoline, new tertiary nonphenolic and tertiary phenolic alkaloids from the roots, were characterized and synthesized.

9. Adiantifoline, a new benzyltetrahydroisoquinoline-aporphine dimer, was partially characterized by its sodium-liquid ammonia cleavage products and its permanganate oxidation product.

10. Column chromatography on alumina resulted in the isolation and identification of β-sitosterol from the neutral fraction of the roots.
FIGURE 12

The Infrared Spectrum of Adiantifoline (CHCl₃)
FIGURE 13

The Infrared Spectrum of Adiantifoline (KBr)
FIGURE 14
The Infrared Spectrum of Alkaloid A (CHCl₃)
FIGURE 15
The Infrared Spectrum of Alkaloid J (KBr)
FIGURE 16

The Infrared Spectrum of Noroxyhydrastinine (KBr)
FIGURE 17

The Infrared Spectrum of Synthetic Noroxyhydrastinine (KBr)
FIGURE 18
The Infrared Spectrum of Alkaloid L (KBr)
FIGURE 19

The Infrared Spectrum of Thalifoline (KBr)
FIGURE 20

The Infrared Spectrum of Synthetic Thalifoline (KBr)
FIGURE 21

The Infrared Spectrum of Alkaloid S (KBr)
FIGURE 22

The Nuclear Magnetic Resonance Spectrum of Adiantifoline
FIGURE 23

The Nuclear Magnetic Resonance Spectrum of Alkaloid A
FIGURE 24
The Nuclear Magnetic Resonance Spectrum of Synthetic Noroxyhydrastinine
FIGURE 25

The Nuclear Magnetic Resonance Spectrum of Alkaloid S
FIGURE 26

The Nuclear Magnetic Resonance Spectrum of Alkaloid S


44. Patil, P. N. 1960. Preliminary Pharmacology of *Thalictrum Revolutum* DC. M.S. Thesis. The Ohio State University, Columbus, Ohio. 112pp.


118. Ibid., p. 89.

119. Ibid., pp. 89-90.


121. Ibid.


