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THE ISOLATION AND SYNTHESIS OF COLUMBAMINE

D I S S E R T A T I O N

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

By

Thomas Arnold Reed, B. S.

*****

The Ohio State University
1962

Approved by

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HISTORICAL

The history of columbamine (I) is so closely linked with that of the quaternary alkaloids \(^1\) palmatine (II), berberine (III), and jatrorrhizine (IV), that it is necessary to trace the history of all four and of tetrahydrocolumbamine (V), tetrahydropalmatine (VI), tetrahydroberberine (VII), and tetrahydrojatrorrhizine (VIII) in order to see columbamine (I) in its proper perspective.

In 1826 Chevalier and Pelletan \(^2\) isolated a substance from Xanthoxylon Clave herculis which they called "xanthopicrit." Again in 1837 the same compound was isolated from the root bark of Berberis vulgaris (barberry) by Buchner and Herberger \(^3\) who, not realizing its identity with xanthopicrit, named it berberine. Perrins \(^4\) established the identity of berberine and xanthopicrit in 1862. During the remaining years of the nineteenth century compounds reported to be berberine were isolated from a great

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\(^1\) The quaternary alkaloids are shown with a general cation "X" in the illustrations. An anionic name will not be included in the text unless it is necessary for clarity.


\(^3\) H. Buchner and J. E. Herberger, *Ann.*, 24, 228-238 (1837).

Columbamine and Related Alkaloids.

Columbamine (I)

Palmatine (II)

Berberine (III)

Jatrorrhizine (IV)

For the free base $X^- = \text{OH}^-$ otherwise $X^- = \text{I}^-$ or $\text{Cl}^-$ as designated in the text.

Figure 1
Tetrahydrocolumbamine and Related Alkaloids.

Tetrahydrocolumbamine (V)

Tetrahydropalmatine (VI)

Tetrahydroberberine (VII)

Tetrahydrojatrorrhizine (VIII)

Figure 2
variety of sources by many workers. William Perkin, Jr. established the structure of berberine (III) in 1890.

H. M. Gordin (Wm. S. Merril Co., Cincinnati, Ohio) doubted that the reports of the presence of berberine were correct in all cases because the methods of characterization varied widely and were in general not specific for berberine. One of the most widely used methods was to treat the aqueous or alcoholic plant extract with concentrated hydrochloric acid. If a yellow precipitate formed and if an aqueous solution of the precipitate gave a red color with chlorine water, then berberine was deemed present. In the case of such a complex substance, Gordin felt that such tests offered insufficient proof.

After detailed review of the literature, he devised a method based on the insolubility of berberine iodide, and the precipitation of the berberine-acetone addition product from basic solution. Testing against standard solutions of berberine, he readily detected as little as 0.001 g. Using this method, he investigated a number of plants reported to contain berberine, among them Jatropha palmata.

7 H. M. Gordin, Arch. Pharm., 242, 146-149 (1902).
Boedeker studied the root of Jatrorrhiza palmata, commonly referred to as the "Columba root." His report of berberine's presence in Columba root is based on the analysis of an alkaloid chloride precipitated as a yellow solid by treatment of the plant extract with concentrated hydrochloric acid. The results compared reasonably well with the analysis of berberine chloride obtained by Fleitman from Berberis vulgaris (Table 1). Bocchiola had published the quantitative analysis for berberine in Columba root (Table 2). Despite this previous evidence, Gordin's analysis showed "not a trace" of berberine in the extract of Jatrorrhiza palmata.

Upon examining Gordin's evidence, Gadamer reached the conclusions that either the Columba root does not always contain berberine, or Columba root contains berberine but Gordin's test does not detect it, or there are in Columba root alkaloids which though they are the same as berberine in many of their reactions, are none the less not identical with it. To solve this dilemma, Gadamer

8 C. Boedecker, Ann., 69, 40 (1849).
Table 1
Analysis for Berberine-like Alkaloids

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<th>Fleitman (1846)</th>
<th>Boedeker (1849)</th>
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<td>Jatrorrhiza palmata</td>
<td>62.89%</td>
<td>62.53%</td>
</tr>
<tr>
<td>Berberis vulgaris</td>
<td>62.78%</td>
<td>62.78%</td>
</tr>
<tr>
<td>C</td>
<td>5.44</td>
<td>5.07</td>
</tr>
<tr>
<td>H</td>
<td>5.87</td>
<td>5.07</td>
</tr>
<tr>
<td>Cl</td>
<td>9.13</td>
<td>8.80</td>
</tr>
<tr>
<td></td>
<td>9.06</td>
<td>9.06</td>
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Table 2
Determination of "Berberine" in Columba Root

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<th>Weight % in Bark</th>
<th>Weight % in Wood</th>
</tr>
</thead>
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<tr>
<td>0.98</td>
<td>2.05</td>
</tr>
<tr>
<td>1.38</td>
<td>1.02</td>
</tr>
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</table>
obtained Columba root from E. Merck and set out to prove or disprove Gordin's work. He succeeded in confirming it in every respect and extended it. Recrystallization of the total alkaloid iodides gave one readily soluble in alcohol and one difficultly soluble. This difficultly soluble iodide was deep golden yellow, looked like berberine, but gave no acetone product. It was readily soluble in ammonia with a red color, where berberine is not. The nitrate prepared from it crystallized as prisms, whereas berberine nitrate forms needles. Upon reduction with zinc and sulfuric acid, a colorless base was obtained which melted at 137-138°. The easily soluble iodide was not completely pure. Its nitrate when reduced with zinc and acid gave a mixture of bases one of which could be crystallized out and melted at 204°. Berberine on zinc-acid reduction 12 gives tetrahydroberberine (VII) which melts at 166-167°.

On the basis of his experimental work Gadamer reached four conclusions: the Columba root contains at least two berberine-like alkaloids which are not identical to berberine; these are colored yellow and upon reduction go over to colorless "hydrocompounds" which unlike the starting material are ether soluble; berberine itself is not

contained in Columba root; and finally, the Columba alkaloids are quaternary bases which on reduction are converted to tertiary bases.

Investigation of the Columba root was now taken up by Gunzel. After precipitation of the alkaloid iodides he extracted them with alcohol, leaving behind an orange crystalline solid which he recrystallized from alcohol to get golden-yellow needles melting at 224°. He named this material "columbamine." After several days the alcohol extract yielded darker yellow crystals melting at 210° which he designated as "alkaloid B." "Columbamine" analyzed for \( \text{C}_{21}\text{H}_{22}\text{NO}_{5}\text{I} \) with four methoxyl groups. On reduction with zinc and acid, "columbamine" gave a tetrahydro base melting at 142° which analyzed for \( \text{C}_{21}\text{H}_{25}\text{NO}_{5} \) with four methoxyl groups.

The task Feist set himself was the determination of empirical and if possible structural formulae for the alkaloids discovered by Gadamer. By methods involving recrystallization of the iodides from alcohol he succeeded in isolating three products. The first was Gunzel's

13 E. Gunzel, Arch. Pharm., 244, 257-269 (1906).

14 The name "columbamine" was at first used to designate a substance which was later proved to be a mixture of two alkaloids.

15 K. Feist, Arch. Pharm., 245, 586-637 (1907).
"columbamine" (m.p. 224°). He obtained an analysis for C_{21}H_{22}N_{5}O_{5}I as compared with an analysis for berberine iodide of C_{20}H_{18}N_{4}O_{7}I. While he correctly inferred that the protoberberine ring system (IX) of 17 carbons was involved, he was forced to wrong conclusions about the substituents because his analyses were inaccurate. The "columbamine" he was working with at this time was a mixture, a fact which future investigations were to force him to acknowledge. He agreed with Gunzel that columbamine had four methoxyl groups.

The second alkaloid isolated was Gunzel's alkaloid B which Feist designated jatrorrhizine. Jatrorrhizine iodide (IV) was believed to exist as a monohydrate (m.p. 208-210°). The reddish-yellow needles it formed were darker than those formed by the chloride (m.p. 206°). The essential agreement of these constants with presently accepted values indicates that his sample was jatrorrhizine, despite the inaccuracies of his elemental analysis which gave a value of C_{20}H_{20}O_{5}NI. A value indicative of three methoxyl groups in the molecule suggested that the remaining two oxygen atoms were present as phenolic hydroxyl groups. Numerous jatrorrhizine salts and derivatives were prepared.


Although palmatine (II), the third alkaloid present, was analyzed incorrectly as $C_{22}H_{24}O_5$NI, the presence of four methoxyl groups was correctly detected. Its melting point of 238-240° was in agreement with present values.\(^{16}\)

The methyl ethers of jatrorrhizine and columbamine were oxidatively degraded with dilute alkaline potassium permanganate. Improper identification of the products coupled with misleading analyses lead Feist to conclude that columbamine iodide methylether was represented by either (X) or (XI) which show the same ring system as berberine (I) but have one additional substituent.

In general it may be concluded that in this first paper on the subject, the value of Feist's excellent separations was negated by the unreliability of his analyses.

The second paper \(^{18}\) in Feist's series on Columba root was delayed eleven years by the intervention of the First World War. Indeed, Sanstede, the co-author had volunteered for the artillery and was killed in 1915, his section of the work being published posthumously. In this paper, jatrorrhizine dimethylether and columbamine methylether were assigned the incorrect structure (XI) based on further degradation studies. A series of degradations was attempted on jatrorrhizine which did not give conclusive results.

However, one piece of excellent work was completed which was to be the foundation for the structure of jatrorrhizine. On the bases of oxidation products and reliable analyses, the relationship between berberine (III) and palmatine (II) was established. Palmatine proved to be berberine with the methylenedioxy group replaced by two methoxyl groups.

Späth and Lang \(^9\) confirmed Feist's structure for (II) by synthesis from berberine. Tetrahydroberberine (VII) was treated with methanolic potassium hydroxide in a sealed tube at 180°. Recrystallization of the product from which oxygen was carefully excluded gave an ortho diphenol (XII) which on methylation with dimethyl sulfate in base, gave tetrahydropalmatine (VI). Palmatine itself was obtained by oxidation of the tetrahydro derivative with iodine in alcohol.

The confusion resulting from Feist's \(^{15}\) early incorrect analyses was cleared up in 1922 by Späth and Bohm \(^{20}\) who methylated jatrorrhizine and "columbamine." The product in each case was palmatine. Identity was further confirmed by comparison of the tetrahydro products. Jatrorrhizine was thus recognized as palmatine in which one of the methoxyl groups was replaced by a hydroxyl group.

\(^{19}\) E. Späth and N. Lang, *Ber.*, 54, 3064-3074 (1921).

Figure 3
Confusion still surrounded colubamine, the analysis of which indicated four methoxyl groups.

Reanalysis of columbamine iodide by Feist and Dschug gave an iodine content of 25.6% and a methoxyl value of 25.04%, for an empirical formula $\text{C}_{21}\text{H}_{22}\text{NO}_5\text{I}$. Palmatine iodide gave theoretical values of 26.5% and 25.9% respectively for iodine and methoxyl in an empirical formula of $\text{C}_{21}\text{H}_{22}\text{O}_4\text{NI}$. Since palmatine iodide (II) is the methyl-ether of "columbamine," one might expect (II) to have higher methoxyl value but lesser percentage iodine than "columbamine" iodide. When, as is seen by analysis, both the iodine and methoxyl percentages of palmatine iodide are greater than for "columbamine" iodide, something must be wrong either with the analyses or the basic assumption. Being sure of his analyses, Feist came to the conclusion that what he had been calling "columbamine" was actually a mixture of palmatine and a phenolic base. He formally withdrew the name "columbamine" from use and suggested it be held in reserve until a phenolic base different from jatrorrhizine was discovered.

Späth came to a similar conclusion by examination of the melting points and appearance of "columbamine" and several of its derivatives. He felt Feist's "columbamine" was impure palmatine. His paper reports a private communication with Feist in which they agreed on the withdrawal

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of columbamine as a proposed compound.

The alkaloid jatrorrhizine (IV) was isolated in a purer form.\textsuperscript{21} Tetrahydrojatrorrhizine was prepared and methylated to give tetrahydropalmatine, thus confirming the work of Späh and Bohm\textsuperscript{20} in this direction. Degradative studies showed jatrorrhizine to be palmatine (II) with a methoxyl group replaced by a hydroxyl group as shown in (IV).

The name "columbamine" was not long left in retirement. By recrystallizing the hydrochloride of tetrahydrojatrorrhizine (VIII) Späh and Berger\textsuperscript{22} obtained 0.25 g. of a compound isomeric with (VIII) but melting at 223-224°. This compound which was named tetrahydropalmatine (VI).

The new tetrahydro compound was treated with diazomethane to give tetrahydropalmatine (VI).

The new tetrahydro compound was treated with diazomethane to give an ethyl derivative (XIII). Upon oxidation with potassium permanganate in dilute base 6-methoxy-7-ethoxy-1-keto-1,2,3,4-tetrahydroisoquinoline (XIV) was isolated. This result was further confirmed by oxidation of (XIII) to hemipinic acid (XV) and the methyl ethyl ether of normetahemipinic acid (XVI). These products show that the free hydroxyl of (V) is in the same ring as that of tetrahydrojatrorrhizine but since it is different from

\textsuperscript{22} E. Späh and G. Burger, \textit{Ber.}, \textbf{59}, 1486-1496 (1926).
Figure 4
(VIII) the structure is again proved by elimination. The quaternary alkaloid from which tetrahydrocolumbamine was obtained was named columbamine (I).

The discovery and structural determination of columbamine in Jatrorrhiza palmata lead Späth and Polgar to look for it in the root bark of Berberis vulgaris during the course of a total alkaloid analysis. The phenolic quaternary alkaloids related to berberine were isolated and reduced to a mixture of tetrahydro bases which were separable by recrystallization from methanol to yield both tetrahydrojatrorrhizine and tetrahydrocolumbamine. Späth remarks in this article that he considers it impossible to obtain pure columbamine by recrystallization of unreduced quaternary salts. Once again columbamine was not isolated as the quaternary salt, nor was it prepared by oxidation of tetrahydrocolumbamine. While the existence and structure of (I) were established beyond doubt by Späth and his students in two different plants, the alkaloid itself was never seen.

The numerous reports of the isolation and characterization will now be examined. Table 3 lists them chronologically and indicates the method of characterization.

Tetrahydrocolumbamine has a structure established by degradation and analysis.\textsuperscript{22} Hence there is little doubt that the melting point of this compound is 223-224°.

\textsuperscript{23} E. Späth and N. Polgar, \textit{Monats.}, 52, 117-128 (1929).
Table 3
Reported Isolation of Columbamine

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<td>Iodide (m.p. 224°).</td>
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<td>Spath and Polgar 23</td>
<td>berberine, palmatine, berberrubine, jatrorrhizine</td>
<td>Tetrahydrocolumbamine (m.p. 223-224°) and mixed m.p. on same.</td>
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<td>Berberis thunbergii</td>
<td>Kondo and Tomita 29</td>
<td>berberine, oxyberberine, jatrorrhizine, shobakunin</td>
<td>Iodide (m.p. 223-224°).</td>
</tr>
<tr>
<td>Archangelisia flava</td>
<td>Santos 26</td>
<td>berberine, palmatine, jatrorrhizine</td>
<td>Tetrahydrocolumbamine (m.p. 223-224°).</td>
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</tbody>
</table>
Table 3 (cont'd.)

<table>
<thead>
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<th>Plant</th>
<th>Author(s)</th>
<th>Alkaloids</th>
<th>Remarks</th>
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<td>Berberis heteropodia</td>
<td>Orechoff 27</td>
<td>berberine, palmatine, jatrorrhizine</td>
<td>Tetrahydrocolumbamine (m.p. 220-221°C).</td>
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<td>Berberis floribunda</td>
<td>Chatterjee 30</td>
<td>palmatine, jatrorrhizine</td>
<td>Iodide (m.p. 223-224°C). Analysis for nitrogen.</td>
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<tr>
<td>Berberis lambertii</td>
<td>Chatterjee and Banerjee 31</td>
<td>palmatine, jatrorrhizine</td>
<td>Iodide (m.p. 223-224°C). Mixed melting point.</td>
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<tr>
<td>Coptis chinensis</td>
<td>Schramm and Tang 32</td>
<td>berberine, palmatine, jatrorrhizine</td>
<td>Absolutely none.</td>
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</table>

24 Only alkaloids related to berberine.
The use of tetrahydrocolumbamine to show the presence of columbamine in Berberis vulgaris is also unequivocal. The evidence in this case is the mixed melting point with a genuine sample of tetrahydrocolumbamine obtained by Späth from Jatrorrhiza palmata.

At first glance, the use of the tetrahydro derivative to show columbamine's presence would appear to be a method not subject to error. However, unless a mixed melting point with a genuine sample of tetrahydrocolumbamine has been taken, or some additional evidence has been supplied, there is still room for confusion. An example of this is the work of Resplaudy with Burassaia madagascariensis. He states that his tetrahydrocolumbamine (m.p. 222-224°) is a mixture of tetrahydrojatrorrhizine and tetrahydrocolumbamine. This belief is borne out by his melting point for columbamine chloride (200-202°) which is different from that obtained in this work (239.5-240°), but quite close to the established value of jatrorrhizine chloride (206°).

All of the investigators who used tetrahydrocolumbamine as an indication of columbamine in their sources followed the method of Späth and his students closely. There can be little doubt that the work of Santos, 26


Orechoff,\textsuperscript{27} and Resplaudi\textsuperscript{25} is correct. Unfortunately, not one of these authors thought to oxidize his pure sample of tetrahydrocolumbamine to columbamine iodide and establish the properties of the alkaloid itself.

The remaining workers characterized columbamine by the melting point of columbamine iodide (223-224°). This work rests on much less certain ground. Murayama and Shinozuki\textsuperscript{28} published their work in 1926 before the work of Späth and Burger.\textsuperscript{22} They felt that their "columbamine iodide," like Feist's\textsuperscript{15} was a mixture of palmatine iodide and the iodide of a phenolic base. The reliance of Murayama and Shinozuki on the columbamine iodide melting point as determined by Gunzel\textsuperscript{13} and Feist\textsuperscript{15} is understandable. However, the remaining claimants based their evidence on the melting point of this iodide long after it had been shown a mixture and even after a reasonably good derivative had been brought forward (tetrahydrocolumbamine).

Kondo and Tomita\textsuperscript{29} give no reason for their choice of 223-224° as the correct melting point for columbamine iodide. It must be assumed then that in stating this value they are referring to that of the so-called

\textsuperscript{27} A. Orechoff, \textit{Arch. Pharm.}, 271, 323-327 (1933).


\textsuperscript{29} H. Kondo and M. Tomita, \textit{Arch. Pharm.}, 268, 549-559 (1930).
"columbamine iodide" of Feist \textsuperscript{15} and Gunzel \textsuperscript{13} which was not only later established to be a mixture;\textsuperscript{16,21} moreover the analysis for this "columbamine iodide" had shown the presence of four methoxyl groups while the established structure (I) had been shown to have but three.

D. R. Chatterjee \textsuperscript{30} in 1951 again states the melting point of columbamine iodide as being 223-224°. No reference is given, but this value agrees with that of a fraction of alkaloid iodide obtained by recrystallizing jatrorrhizine iodide from \textit{Berberis floribunda}. His analysis for nitrogen was 3.2\% while that of columbamine iodide is theoretically 3.0\%.

R. Chatterjee and A. Banerjee \textsuperscript{31} used the same value without reference, but substantiate their identification with a mixed melting point. Unfortunately the source of the true columbamine iodide which they used as a standard is not given in the notes.

The best which can be said for a general conclusion regarding the investigators who used columbamine iodide as evidence for columbamine's presence is that they had the same mixture which Feist \textsuperscript{15} and Gunzel \textsuperscript{13} had.


Schramm and Tang\textsuperscript{32} establish the presence of berberine by chemical tests. They state the presence of palmatine, jatrorrhizine and columbamine in Coptis chinensis, but give no reason, experimental or otherwise for entertaining these beliefs.

INTRODUCTION
AND
STATEMENT OF THE PROBLEM

While the structure of columbamine has been established\textsuperscript{22} and some knowledge of tetrahydrocolumbamine exists, columbamine has never been examined and its properties are unknown.

This research was undertaken to develop a standard procedure for isolating columbamine from Columba root. The chemical and spectral properties were to be determined. Columbamine was to be synthesized from berberine.
DISCUSSION

A sample of Columba root was extracted with methanol in a Soxhlet extractor. The alcohol was removed and the dried extract dissolved in water. After purification, treatment of the aqueous solution with sodium iodide produced a brown crystalline precipitate which, in the light of the "Historical" section is considered a mixture of palmatine iodide (II), jatrorrhizine iodide (IV), and possibly columbamine iodide (I). A portion of this iodide mixture was converted to a mixture of chlorides and chromatographed on alumina by the method of gradient elution. The initial elution of this column with methanol-free chloroform showed three bands (Figure 5). Subsequent elution with increasing percentages of methanol in chloroform removed these bands separately.

The first (2nd Fraction) of these bands gave a bright yellow, semicrystalline solid (0.7997 g.) on evaporation. Recrystallization from water produced fine yellow needles having a melting point of 202.5-203.5° (d.). This compound could have been palmatine chloride (m.p. 205°, d.), jatrorrhizine chloride (m.p. 206°, d.), or columbamine chloride (m.p. not recorded in the literature). It was probably not berberine chloride (m.p. 160°). Reduction of sample (0.3329 g.) of the first band with zinc and acid gave 0.1250 g. of a tetrahydro derivative.
Alumina Column After First Elution

Layer of sand.

Dark brown, with dark brown fluorescence. Alumina with adsorbed sample.

Orange, with red-brown fluorescence. — Jatrorrhizine.
Dark yellow, with tan fluorescence. — Columbamine.

Yellow, with yellow fluorescence. — Polmatine.

Figure 5
(m.p. 148°). The possibility that the yellow quaternary alkaloid from the first band was columbamine chloride or jatrorrhizine chloride was eliminated. Tetrahydrojatrorrhizine has been found to melt at 217-218° \(^{15}\), while tetrahydrocolumbamine melts at 223-224° \(^{22}\). The melting point of tetrahydropalmatine (144° \(^{18}\)) agrees with this observed value. Further confirmation is obtained by examining the melting points of the iodide salts of jatrorrhizine and palmatine, which are 208-210° \(^{15}\) and 241° \(^{20}\) respectively. The quaternary iodide of the first band melts at 239.5-240°. Thus the first band removed from the column has been identified as palmatine chloride (II) on the basis of the melting points of its chloride and iodide salts and its tetrahydro derivative.

The infrared and ultraviolet spectra of the iodide of this material further confirm the identification. The infrared spectrum (Figure 6) does not show the bands characteristic of phenolic hydroxyl. The ultraviolet spectrum (Figures 7 and 8) has the characteristic shape and maxima of the protoberberine alkaloids,\(^{33}\) but it fails to show a bathochromic shift in base characteristic of phenol groups. These spectra will be discussed in more detail below. The identification of the first yellow band (Fraction 2) as palmatine chloride was

\(^{33}\) K. Feist, W. Awe and H. Etzrodt, Arch. Pharm., 272, 817-826 (1934).
Figure 6
Figure 7

- Palmatine Iodide - neutral
- " - acid
- Jatrorrhizine Iodide - " - "
- Columbamine Iodide - " - "

Log E

\[ \lambda \]

220 260 300 340 380 420 460 500
Log $E$

Figure 8
established on an even firmer basis by synthetic methods to be discussed in detail later.

The second band (Fraction 3) was columbamine chloride. Removed from the column it produced a bright yellow semi-crystalline mass which was readily differentiable from berberine, palmatine, or jatrorrhizine chlorides by its unusual melting point. Upon recrystallization from water, it gave fine yellow needles (m.p. 239.5-240.5°, d.). This value is much higher than that of the chlorides of either palmatine or jatrorrhizine (205° and 206° respectively). The tetrahydro derivative of this substance melts at 221-222° which is in agreement with Späth and Burger's value of 223-225° for tetrahydro-columbamine. The iodide of the quaternary base (Fraction 3) melts at 228-228.5° which is sufficiently different from palmatine iodide (m.p. 241°) and jatrorrhizine iodide (m.p. 208-210°) to avoid confusion.

In acid and neutral solutions the ultraviolet spectra (Figures 7 and 9) closely resemble the typical protoberberine spectra. In basic solutions these spectra show a bathochromic shift. The spectral data confirm the melting point values in identifying the second band on the column as columbamine.

The third and final band on the column was removed to product an orange semicrystalline material which on recrystallization from water was readily identified as
Figure 9

- Columbamine Iodide – neutral
- " – weak base
- " – strong base

Log E

\(\lambda\)

220 260 300 340 380 420 460 500
jatrorrhizine chloride by its melting point and that of its iodide salt. The melting point of the tetrahydro derivative (VIII) was 216-216.5° which is in agreement with the accepted value of 217-218.5°.¹⁵

The relationship between berberine, palmatine, columbamine and jatrorrhizine is reflected in the ultraviolet spectra of their respective tetrahydro derivatives. The spectra of tetrahydroberberine and tetrahydropalmatine are the same in acid, neutral and basic solution (Figures 10 and 11). The spectra of tetrahydrocolumbamine and tetrahydrojatrorrhizine undergo a bathochromic shift in basic solution (Figures 12 and 13). These relations show that while berberine and palmatine have no free hydroxyl group, columbamine and jatrorrhizine both have such a function.

The similarity of the chromophores of the four tetrahydro compounds is shown by examining their ultraviolet spectra in neutral solution (Figure 14). The close relation of tetrahydrocolumbamine and tetrahydrojatrorrhizine and the differences between these alkaloids and tetrahydropalmatine is shown by the similarity of the spectra of the two hydroxyl group containing derivatives in basic solution (Figure 15).

Jatrorrhizine and columbamine are isomers with very similar properties. This similarity caused Späth to express doubt that columbamine and jatrorrhizine could be
Figure 10
Figure 11

- Acid Tetrahydropalmatine
- Basic ""
- Neutral""
Figure 12
separated by recrystallization of their quaternary salts. When both isomers were available in pure form for comparison, small differences became evident. Jatrorrhizine chloride forms orange needles. Columbamine chloride forms yellow needles. In appearance the latter are indistinguishable from palmatine chloride. Aqueous solutions of jatrorrhizine chloride and columbamine chloride are both yellow. When treated with a few drops of dilute sodium hydroxide, both give a strong red color. If, however, instead of dilute sodium hydroxide, dilute sodium bicarbonate is used, only the jatrorrhizine solution turns red. The columbamine solution remains unchanged. In all cases, the red solutions may be converted back to the original yellow color by addition of acid. Similar phenomena are displayed in the ultraviolet spectra. The spectra of both jatrorrhizine and columbamine are nearly identical in acid and neutral solution as might be expected, since the spectra of jatrorrhizine, palmatine, and berberine are the same in acid and neutral solution\textsuperscript{33,34}.

In weakly basic alcoholic solution, the ultraviolet spectrum of columbamine remains the same as that of the neutral and acid solutions (Figures 9 and 16). However, the spectrum of jatrorrhizine in weakly basic alcohol is entirely different from those in neutral and acid

\textsuperscript{34} D. W. Spiggle, M.S. thesis, Department of Chemistry, The Ohio State University, June 1960.
Figure 16

Log E

--- Palmatine Iodide - weak base
--- Calumbamine Iodide - " "
--- Jatrarrhizine Iodide - " "

220 260 300 340 380 420 460 500

λ
solutions, and quite similar to the spectrum of this compound in alcoholic sodium hydroxide (Figures 16 and 17). Columbamine is alcoholic sodium hydroxide shows a spectrum different from the neutral columbamine one, but also different from that characteristic of jatrorrhizine in base. This difference is visually apparent, columbamine being yellow and tan in alcoholic sodium bicarbonate and sodium hydroxide respectively, while jatrorrhizine displays a strong red color in both.

It is apparent from the above physical evidence that in basic solution jatrorrhizine and columbamine form stable phenoxide ions which differ from each other in their chromophores. Further, the phenoxide ion of jatrorrhizine forms much more readily than that of columbamine. The columbamine phenoxide (XVII) will have structures (XVIII) and (XIX) as contributing resonance species. The resulting hybrid will not have the same chromophore as columbamine itself. Since one of the four rings in the molecule has quinoid resonance contributors, the phenoxide ion would be expected to be more strongly colored than the neutral molecule. This is seen in the tan color of the basic solution. The jatrorrhizine phenoxide ion (XX) has the negative charge in conjugation with the positive charge of the quaternary nitrogen. Thus, in addition to a contributing species (XXI) analogous to species (XVIII) and (XIX) of columbamine in having positive and negative
Figure 17
Phenoxide Ion Resonance Systems

Figure 19
charges, the jatrorrhizine phenoxide ion has an internally neutralized structure (XXII). This species (XXII) would contribute an unusually large share to the overall properties of the jatrorrhizine phenoxide ion because its bond system is still conjugated, but the separated charges have neutralized each other. In (XXII) all three aromatic rings have quinoid character. The phenoxide ion of jatrorrhizine should therefore have a much stronger color than that of columbamine. The unusual stability and quinoid character of jatrorrhizine anion account for the deep red color which forms even in weakly basic solutions.

The observed order of elution from alumina may now be explained. On Woelm Acid Alumina, palmatine chloride was removed by chloroform containing little or no methanol, berberine chloride required still more, columbamine chloride required about six percent methanol in chloroform and jatrorrhizine chloride was removed last with twelve percent methanol in chloroform.

Woelm Acid Alumina is anionotropic. In order to rationalize the order of removal of a series of molecules, we must examine their centers of negative charge or highest electron density. Hence, the oxygen atoms of these four molecules must be considered. The methoxyl groups of the "D" ring in each molecule are the same. Their effect in determining the adsorption will be about the same for all four molecules. The differences between the "D" ring
oxygen will be neglected. It is in the "A" ring substituents that large differences lie. Palmatine has no possibility of forming a phenoxide ion. Any anionotropic effect of the column packing will only involve the free electron pairs of the oxygen atoms of the "A" ring. Palmatine is, therefore, more readily removed than molecules which form phenoxide ions. In berberine, as in palmatine, no phenoxide ion may be formed. Where the electrons of the ethereal oxygens were shielded by the methyl groups of palmatine, the methylene group of berberine offers much less hinderance to interaction with the alumina. Berberine would be expected to be more strongly adsorbed than palmatine, but still less strongly adsorbed than phenoxide-forming molecules. Columbamine can form a phenoxide ion and will be more strongly adsorbed than palmatine or berberine. Jatrorrhizine forms a phenoxide ion more easily than columbamine and is the most strongly adsorbed of the four molecules considered.

The ultraviolet spectra of berberine and palmatine are essentially the same in acid, neutral, and weakly basic solutions (Figure 8). In dilute sodium hydroxide solution both of these compounds lose their yellow color, and their spectra become quite different from their respective spectra in neutral solution. Since palmatine and berberine have the same chromophore, and since the ultraviolet curves for each in strong base are the same,
(Figure 20) it seems likely that the same phenomenon occurs in each case.

This color change in the case of berberine was examined spectroscopically by Tinkler \(^4^4\) and by spectral and electrochemical measurements by Skinner.\(^4^5\) Both came to the conclusion that in basic alcoholic solutions berberine readily formed the pseudo base (XXIII). Tinkler, in 1911, arrived at these conclusions on the basis of comparisons of ultraviolet spectra of the alcoholic berberine solution and the spectra of dihydroanhydroberberine (XXIV). Ultraviolet techniques were rather unreliable at this early date, and the spectrum of (XXIV) has not been determined since. Therefore, dihydroanhydroberberine was prepared from berberine by reduction with sodium borohydride and the spectrum was redetermined in alcohol.

A comparison of the spectra of berberine in neutral alcohol, berberine in basic alcohol, and dihydroanhydroberberine in neutral alcohol (Figure 22) shows a strong resemblance in the location of the maxima of basic berberine solution and dihydroanhydroberberine. Skinner \(^4^5\) observed that a concentration of 0.25 N potassium hydroxide was necessary to convert a \(10^{-5}\) molar solution of berberine in alcohol entirely to the presumed pseudobase form and proved this by observing alterations of berberine's spectrum


Figure 20

- **Palmatine Iodide** - neutral
- """" - strong base
- """"Berberine Iodide"""" - """"
Figure 21
Figure 22: Absorption Spectra of Berberine Iodide and Related Compounds. The graph shows the absorbance (Log E) as a function of wavelength (λ) for Berberine Iodide, a strong base, and a neutral compound, as well as Dihydroanhydroberberine. The absorbance values range from 1 to 4 on the vertical axis, and the wavelength range is from 220 to 500 nm on the horizontal axis.
in various base concentrations. The berberine concentration in our work was also about \(10^{-5}\) molar but the solution was 0.001 N in sodium hydroxide. The maxima and curve shapes agree closely with those of Skinner obtained for similar base concentrations in which he showed the spectrum observed to be due to berberine pseudobase in the presence of small amounts of unchanged berberine. When it is considered that the curves for berberine pseudobase are slightly distorted due to the presence of unchanged berberine, and that the spectrum of dihydroanhydroberberine would undergo small changes due to the presence of a hydroxyl group on the carbon adjacent to the nitrogen, it is felt that the present studies bear out the conclusions of Skinner and Tinkler. Berberine is thus in equilibrium with its pseudobase (XXIII) in basic solution. Palmatine is in equilibrium with a similar pseudobase (XXV) in basic solution.

These results are further confirmed by the studies on cotarnine (XXVI). Skinner has shown that the spectral changes of (XXVI) in basic solution are similar to those undergone by berberine solutions. Dobbie, Lauder and

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Tinkler have studied the spectra of cotarnine and related compounds. Both studies conclude that in basic alcohol, cotarnine (XXVI) forms a pseudobase (XXVII) analogous to (XXIII) and (XXV). Freund, Hantzsch and Kalb have shown that $\psi$-cotarnine cyanide prepared by the action of alkali cyanide on (XXVI) has the structure (XXVIII).

Demethylenberberine (XXIX) is a derivative of berberine which has two hydroxyl groups. One of these is analogous to that of columbamine, the other to that of jatropha. If the hydroxyl group in the same position as that of columbamine ionizes to form a phenoxide ion (XXX), structures (XXXI) and (XXXII) would be expected to contribute to the phenoxide resonance hybrid. If the jatropha-like hydroxyl ionizes, a phenoxide ion (XXXIII) similar to that of jatropha is formed. In addition to a resonance species (XXIV) involving charge distribution within the "A" ring only, formation of a neutralized internal zwitterion (XXXV) is possible. This neutral resonance contributor would make phenoxide ion (XXXIII) considerably more stable than ion (XXX).

Demethylberberine (XXIX) was prepared from

49 M. Freund, Ber., 33, 380-389 (1900).
50 A. Hantzsch and M. Kalb, Ber., 33, 2201-2208 (1900).
Figure 23

Cotarnine $\text{XXVI}$

Cotarnine Pseudobase $\text{XXVII}$

Cotarnine Cyanide $\text{XXVIII}$

Demethyleneberberine $\text{XXXIX}$
berberine by the method of Spath and Quietensky. It was crystallized as the iodide, a deep red salt. In order to ascertain the freedom of this product (XXIX) from the starting material (III) it was converted to the chloride salt with IRA 410 ion exchange resin and chromatographed on Woelm Acid Alumina by the gradient elution method. A negligible amount of berberine was obtained, indicating the suitability of the demethyleneberberine for further studies.

In acid or neutral alcohol solutions (Figure 26) the ultraviolet spectrum of (XXIX) was identical to that of berberine and related alkaloids. The color of these solutions was yellow. An alcoholic solution of (XXIX) made basic with sodium bicarbonate turned red and had a spectrum (Figure 27) similar to that of alcoholic solutions of jatrorrhizine treated with sodium bicarbonate. In strong base (Figure 28) the ultraviolet spectrum of demethyleneberberine was "flatter" than that of jatrorrhizine in strong base, but the same peaks were still visible. This spectral evidence shows that (XXIX) ionizes to form (XXXIII) in preference to (XXX).

In order to establish that the hydroxyl groups of demethyleneberberine ionize in stepwise fashion, a mixture of demethyleneberberine chloride and standard hydrochloric

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Demethylenberberine Phenoxide Ions

Figure 24
Demethyleneberberine Phenoxide Ions.
Figure 27

- Demethylene Berberine Iodide — weak base
- Jatrorrhizine Iodide — " "
- Columbamine Iodide — " "

Log E

220 260 300 340 380 420 460 500

λ
Figure 28

- Demethylene Berberine — acid
- " — neutral
- " — weak base
- " — strong base
acid was titrated with standard base. The derivative of the pH with respect to the volume was plotted against volume (Figure 29). Two peaks were obtained. The first and larger one corresponded to the equivalence point of the standard hydrochloric acid present. The second was due to reaching the equivalence point of the first ionization of demethyleneberberine. The $PK_A$ for the first ionization was calculated from the pH, volume of base and total volume of solution at equivalence. The value for the first ionization of demethyleneberberine was 7.29 which was quite close to the $PK_A$ of jatrorrhizine (7.09) and distinctly different from that determined for columbamine (8.54). These data show that (XXIX) ionizes in a stepwise manner with the first phenoxide ion formed being (XXXIII), which confirms the information obtained from the study of spectra.

Several attempts were made to methylate (XXIX) in aqueous sodium bicarbonate with methyl sulfate. The most selective runs occurred at low temperature (15°). While small amounts of palmatine and jatrorrhizine were obtained, the principle product was columbamine. The reaction rate was low. Twenty hours sufficed to convert only about twenty percent of the starting material to the product mixture. At 40° the reaction was more rapid. Eight hours of agitation at this temperature produced twenty-five percent conversion of the starting material. The results in this
Derivative Curve for Titration of Demethyleneberberine and HCl with Standard NaOH.
case were less selective, but columbamine was still the predominant product.

The separation curve for the products of a 40° run are shown in Figure 30. After separation, these products were recrystallized separately as their respective iodides. These iodides showed no melting point depressions when mixed melting points were run with samples of the pure naturally-occurring alkaloids. These melting points, however, occur with decomposition and are felt to be not as strong evidence as the infrared spectra.

The infrared spectra of the iodides of synthetic palmatine, Columbamine and jatrorrhizine were compared with those of the pure naturally-occurring alkaloids. In the case of each compound, the natural alkaloids and synthetic products had spectra which were super-imposable in all details. Palmatine, jatrorrhizine and columbamine have very similar structures. As might be expected, there are many features in the spectra of all three which are nearly identical. In certain regions each of the three shows definite differences from the other two. These regions are 2.75-3.75 microns, 6.50-6.75 microns, 8.00-8.50 microns, 11.00-11.50 microns, and 12.00-12.50 microns. As can be seen from Figure 6, these differences are sufficiently large to eliminate any possibility of confusion.

The synthesis of columbamine accomplishes several valuable goals. It establishes the structure of columbamine.
Figure 30
The formation of the by-products jatrorrhizine and palmatine in the synthesis confirms the identity of the ring systems and "D" ring substituents of berberine, palmatine, jatrorrhizine, and columbamine. It further confirms the predicted selective ionization of demethylenberberine (\(\text{XXIX}\)) to the phenoxide ion (\(\text{XXXIII}\)). Though the yields at the present time are low, it affords a ready route to columbamine from the common alkaloid berberine in the event that the production of large amounts of columbamine should ever become necessary. Finally, because of the simplicity of the method, it may be used to provide pure samples of palmatine, jatrorrhizine, and especially columbamine for comparison with natural material isolated from plants. In this way the confusion which plagued early investigators in this area may be avoided.
EXPERIMENTAL

Spectroscopy and Melting Points

All ultraviolet spectra were obtained with a Cary Model "14" Spectrophotometer using alcohol as solvent. The solutions for measurement were prepared by diluting 1.00 ml. of an alcoholic stock solution of the alkaloid to 10.00 ml. with grain alcohol. In acid, weakly basic and strongly basic measurements, 1 ml. of 0.1 N alcoholic HCl, NaHCO₃ or NaOH was diluted with the 1.00 ml. of stock alkaloid solution. Thus, the acid, weakly basic and strongly basic solutions were taken in 0.01 N alcoholic HCl, NaHCO₃ and NaOH respectively. Spectra marked "basic" rather than "strong base" or "weak base" are strongly basic solutions. Since the Cary "14" is a double beam instrument, the "blank" tube in each case contained grain alcohol, or alcoholic HCl, NaHCO₃ or NaOH as required.

Infrared spectra were run on a Perkin Elmer Model "21" Spectrophotometer. They were all taken as KBr windows. The alkaloid and KBr used in preparing each window were weighed to assure standard concentrations throughout the measurements. Since mixed melting points of compounds which decompose on melting are sometimes unreliable, the comparison of natural and synthetic alkaloids depended on the accuracy and reproducability of the infrared measurements.
Melting points were determined in capillaries in an oil bath system with corrected Anschütz thermometers. Melting points of tetrahydro derivatives were run in evacuated, sealed capillary tubes. Literature and observed values for melting points of the berberine alkaloids are shown in Table 4.

Table 4

<table>
<thead>
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<th>Chloride</th>
<th>Iodide</th>
<th>Tetrahydro Deriv.</th>
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</thead>
<tbody>
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<td>Berberine</td>
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<td>no listing</td>
<td>167°</td>
</tr>
<tr>
<td>Literature</td>
<td>162-163° (d.)</td>
<td>162°</td>
<td>162.5-1.64°</td>
</tr>
<tr>
<td>Observed</td>
<td>205° 18</td>
<td>241° (d.) 20</td>
<td>144° 18</td>
</tr>
<tr>
<td>Palmatine</td>
<td>202.5-203.5°</td>
<td>239.5-240°</td>
<td>148°</td>
</tr>
<tr>
<td>Literature</td>
<td>206° 15</td>
<td>208-210° 15</td>
<td>217-218° 15</td>
</tr>
<tr>
<td>Observed</td>
<td>205.0-205.5°</td>
<td>213.0-213.5°</td>
<td>216.0-216.5°</td>
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<tr>
<td>Jatrorrhizine</td>
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<td>no listing</td>
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</tr>
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<td>239.5-240.5°</td>
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Paper Chromatography

Preliminary work in paper chromatography was done using downward elution on strips of Whatman #1 paper in the usual manner. In the present work this method had the disadvantage that "tailing" prevented resolution of spots having similar Rf values.

To avoid this difficulty, recourse was made to horizontal techniques. A "jar" was prepared by grinding the lips of two Pyrex baking dishes (18 inc. long, 12 in. wide, 3 in. deep) until they were flat and fitted together evenly. A hole (1/2 in. dia.) was drilled in the side of the bottom dish for the introduction of solvent (Figure 31). A sheet of chromatographic paper (Whatman 3M, 46 cm. x 57 cm., cut from a standard 46 x 57 cm. sheet) was marked with diagonals to determine the center. A pencil mark was made on each radius (2 cm. from the center spot). A solution of the substance under investigation was then placed on that mark by means of a capillary (spot dia. approximately 0.2 cm.). The concentration and number of overlapping spots was varied depending on the type of substance. Drying was accomplished with a portable hair dryer. By using only four diagonals as paths for spot movement, the difference in movement rate


53 These techniques were outlined to Dr. M. P. Cava by various members of the staff of Smith, Kline and French, Philadelphia, Pa.

54 Oster Electric Hair Dryer Model 202CH.
Equipment for Paper Chromatography

Top View

Chromatographic tray.
Three Petrie dishes.
Wick from paper to center of Petrie dish.
Chromatography paper.

Side View (in cross-section)

Upper tray.
Chromatography paper.
Wick.
Access hole.
Lower tray.
Three Petrie dishes.

Detail of Chromatographic Paper

Position for sample spots.
Hole for wick.
Diagonal path for development.
Additional paths for development (dotted lines)

approx. scale
one inch = 20 cms.

Figure 31
along the two major axes of the paper was equalized. In cases where exact Rf values were not important, but in which a large number of separations had to be run, additional radii were drawn from the center. When the sample spots had dried, a small hole was made at the center of the paper, and a string wick knotted near one end was introduced. The knot was wedged firmly in the hole.

Three Petri dishes (3 in. dia.) were placed in the bottom chromatographic tray (Figure 31). The aqueous phase (50 ml.) of the eluting mixture was poured into the bottom tray taking care to get none in the Petri dishes. The Petri dish at each end was filled with the nonaqueous phase of the eluting mixture. Thus the paper was equilibrated against both the aqueous and nonaqueous phases.

The previously prepared chromatographic paper was now laid across the top of this tray with its wick resting in the center (this dish contained no liquid) Petri dish. The covering tray was then put in place. A seal was formed by the bottom and top trays pressing on the filter paper. This also served to support the paper during subsequent operations. A cork was placed in the access hole of the lower tray and the system allowed to stand at constant temperature for three hours. At the end of this equilibrium period, the cork was removed and the nonaqueous phase of the eluting system placed in the center dish. The cork was replaced and elution allowed to continue until a circle
between 9 and 11 cm. in radius had formed. This required from five to fifteen hours, depending on the viscosity of the eluent.

When elution was complete, the wick was pulled from the paper by means of a pair of long forceps introduced through the access hole. The paper was then removed from the tray and hung to dry. Rf values were calculated in the customary way for these chromatograms. Spot detection was by means of Dragendorff's solution and examination under ultraviolet light.

Six solvent systems were used to study the Columba root alkaloids. They are: "A", 100 ml. butanol, 100 ml. chloroform 100 ml. water; "B", 160 ml. butanol, 20 ml. glacial acetic acid, 100 ml. water; "C", 50 ml. secondary amyl alcohol, 50 ml. iso amyl alcohol, 140 ml. 28% formic acid, 36 ml. chloroform; "D", 92 ml. pyridine, 225 ml. ethyl acetate, 200 ml. water; "E", 200 ml. butanol, 100 ml. 7% ammonium hydroxide; and "F", 160 ml. propanol, 40 ml. 1% ammonium hydroxide. Though systems "C", "D", and "E" show reasonably large differences between the Rf values for columbamine and jatrorrhizine, only system "E" produced complete separations of mixtures of the two alkaloids.

Ammonia fumes turn jatrorrhizine spots red, while only turning columbamine spots tan. In this way it could be seen that systems "C" and "D" separated mixtures of
columbamine and jatrorrhizine to a small extent. In these cases, however, the spots of (I) and (IV) overlapped each other sufficiently to show only one spot under ultraviolet examination. System "E" caused isomer mixtures to separate clearly into two spots with a clear area of paper separating them.

The $R_f$ values of palmatine and berberine were never sufficiently different to permit separation. $R_f$ values for all systems are listed in Table 5.

**Alumina Column Chromatography**

A small wad of glass wool was placed at the constriction of a Pyrex column equipped with a stopcock (Figure 32). The column was filled with solvent in which the packing was to take place. It is most convenient to choose one in which the sample to be chromatographed is insoluble. For working with quaternary alkaloids, chloroform was used. Sufficient room must be allowed at the top of the column for introduction of the funnel and valve. A slow, constant flow of alumina (Woelm Grade I, Acid, Basic, or Neutral) was maintained by using a glass ball valve in

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55 The techniques are based on those demonstrated in a documentary film prepared by F. Sorm of the Czecho-Slovakian Academy of Sciences, Prague.

56 Glass wool was used rather than cotton, since alkaloids readily adsorb on the latter.
Table 5
Rf Values of Alkaloid Chlorides

<table>
<thead>
<tr>
<th>Solvent System</th>
<th>Berberine</th>
<th>Palmatine</th>
<th>Jatrorrhizine</th>
<th>Columbamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>.38</td>
<td>.35</td>
<td>.27</td>
<td>.24</td>
</tr>
<tr>
<td>B</td>
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<td>.75</td>
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<td>E</td>
<td>.53</td>
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</tr>
<tr>
<td>F</td>
<td>.53</td>
<td>.53</td>
<td>.38</td>
<td>.37</td>
</tr>
</tbody>
</table>
Equipment for Column Chromatography.

Column during packing.

- Glass ball valve.
- Alumina
- Funnel
- Top of chloroform layer.
- PYREX chromatographic column.
- Layer of alumina already in place.
- Glass wool plug.
- Stopcock

Detail of Valve.

- Glass pulled to a point.
- Blown glass ball.

Top of Column During Elution.

- Reservoir (support not shown)
- Solvent
- Sand
- Alumina with sample.
- Alumina for chromatography.

Figure 32
the funnel. To compensate for the volume of the alumina introduced, the stopcock was opened slightly and solvent allowed to flow out slowly. If alumina is allowed to flow in too rapidly, lumps will be formed and air trapped in the column. Alumina sticking to the sides of the column was washed down with a long-nosed pipette containing the packing solvent. It was unnecessary to tap the column with a stick or one's finger during the packing procedure. If the column itself is vertical, the top of the alumina layer will be parallel to the ground. In the case where realignment of the column failed to produce a level alumina surface, the filling with alumina was completed before the column was tapped to flatten the upper surface. Normally the weight of alumina was 25 to 100 times the weight of the sample to be chromatographed.

When sufficient alumina had been added, the funnel and valve were removed. Though the column had been packed under liquid, small bubbles of air still adhered to the alumina. If the solvent flow were stopped and the column allowed to stand overnight, these bubbles would coalesce to form large spaces which would ruin the column. Thus, an additional volume of packing solvent was passed through the column by means of a flask with a tapered neck inserted in the top of the column (a "bird feeder"). The volume of the solvent passed should be about ten times the volume of the column. The column was now complete and could be allowed to stand overnight if necessary, without damage.
The sample to be chromatographed was dried and ground to powder in a mortar and pestle. This powder was ground with five to ten times its weight of Woelm Alumina (Grade I, of the same type used in the column). Sufficient solvent was added dropwise to make a thick paste and the mixture was simultaneously stirred with a spatula. In the case of quaternary alkaloids, the solvent was methanol. The paste was then dried for one or two hours over phosphorous pentoxide in an evacuated desiccator. The dried powder was broken up with a spatula and run through a sieve (50 mesh). It was placed on top of the column with the funnel and valve, using the same packing solvent as was used with the column itself. A layer of sea sand was placed on top of the added sample to prevent the surface from being disturbed by changing the solvent reservoirs. At no time during preparation or use was the level of the liquid allowed to fall below the level of alumina in the column.

**Extraction of Jatrorrhiza Palmata**

Dried, pulverized root of *Jatrorrhiza palmata* (800 g.) was extracted with methanol in a Soxhlet extractor for twenty hours. The dark-brown methanolic extract was evaporated in a rotary evaporator under reduced pressure (5-10 mm. Hg) at a bath temperature of 50°C. When the extract had been reduced to a few hundred ml. of thick syrup, heating was discontinued and the receiver of the evaporator
cooled with Dry-Ice-acetone slush. The flask containing the resulting resinous gum was placed overnight in a vacuum desiccator with phosphorous pentoxide. The dry residue was a dark brown, brittle resin, honeycombed with air bubbles.

Solution with agitation in boiling water (500 ml.) gave a bright yellow, opaque, highly viscous suspension. The pH was adjusted to approximately three (Hydrion test paper) by the addition of 6N HCl and the solution was placed on a steam bath for two hours. The suspended material precipitated as yellowish-white curds. After filtration while hot through a Buchner funnel ("Filtercel") and washing the filter cake with a hot water (200 ml.) about one liter of dark brown, clear solution was obtained.

This aqueous extract was heated to boiling, allowed to cool very slightly, and to it was slowly added hot sodium iodide solution (70 g. of reagent grade NaI in 100 ml. water). The immediate yellow precipitate rapidly became brown needle-shaped crystals. The solution was allowed to cool to room temperature and the crystals filtered out on a Buchner funnel. After air drying they weighed 6.0921 g.

**Conversion of Columba Iodide Mixture to Chlorides**

An ion exchange column was prepared from the chloride form of Amberlite IRA 410 resin (2 cm. dia., 20 cm. length). After washing with distilled water (500 ml.) it was
equilibrated by washing with acetone-water mixture (1:1, 500 ml.). Following this equilibration the liquid flow from the column was stopped with about 4 cm. of solution remaining above the surface of the resin. The resin granules were stirred with a long thin spatula until all air bubbles had escaped. The liquid was then brought level with the top of the resin.

The crystalline mixed Columba alkaloids (2.2456 g.) were dissolved in warm acetone-water (1:1, 100 ml.). The solution was cooled to room temperature and passed slowly through the exchange column. The column was washed with additional acetone-water mixture until no yellow color was visible in the eluent. After evaporation under vacuum (10 mm. Hg) on a rotary evaporator (bath temp. approximately 50°) a yellow powder weighing 1.7745 g. was obtained.

**Column Chromatography of Columba Root Alkaloids**

A chromatographic column was prepared from Woelm Acid Alumina Grade I (150 g., 2 cm. dia., 45 cm. length) using chloroform as the solvent. Columba Root alkaloid chlorides (1.7745 g.) were adsorbed on Woelm Acid Alumina Grade I (9 g.), dried for two hours in vacuum over phosphorous pentoxide and applied to the top of the column.

1st Elution: Chloroform (800 ml.) containing no methanol was run through the column at a rate of 300 ml./hr. At the completion of the first elution the column showed three bands (Figure 5). The lowest band (bright yellow
with bright yellow fluorescence) was tentatively designated palmatine chloride (II). The intermediate band (dark yellow with tan fluorescence) was tentatively designated columbamine chloride (I). The slow-moving band on the column (orange with dark brown fluorescence) was tentatively designated jatrorrhizine chloride (IV). A fourth band had moved out of the column almost with the chloroform front. This thin, colorless, blue fluorescent band was not examined further.

1st Fraction: The chloroform was removed on a rotary evaporator (bath temp. 50°) to give 0.0137 g. of a brown oil which was discarded.

2nd Elution: One liter of chloroform containing 3% methanol was passed through the column at a rate of 300 ml./hr. The lowest band (II) was removed. The three bands were sufficiently separated to permit visual fraction cutting.

2nd Fraction: The solvent was removed at reduced pressure on a rotary evaporator (bath temp. 50°). The product (II) was a yellow semicrystalline solid weighing 0.7997 g.

3rd Elution: One liter of chloroform containing 7% methanol was passed through the column at a rate of 300 ml./hr. Near the end of this elution it appeared that some jatrorrhizine chloride (IV) might be carried through with the columbamine chloride (I), hence two fractions were cut.
3rd Fraction: The solvent was removed as previously to give 0.2043 g. of a yellow semicrystalline solid (I).

4th Fraction: After solvent removal, a brownish yellow solid weighing 0.0915 g. was obtained. This material was rechromatographed as indicated below.

4th Elution: Two liters of chloroform containing 12% methanol were run through the column at 300 ml./hr. The third band was removed, though the column still contained a uniform faint yellow color.

5th Fraction: Jatrorrhizine chloride (IV) was obtained as an orange semicrystalline mass weighing 0.0140 g.

5th Elution: In an attempt to remove the remaining uniform yellow color from the column, an additional 500 ml. of chloroform containing 12% methanol was run through the column. This produced a final fraction which was also jatrorrhizine chloride (IV).

6th Fraction: The jatrorrhizine chloride weighed 0.0298 g.

The total weight of alkaloids obtained from the column was 1.7723 g. as compared with 1.7745 g. of mixed Columba root alkaloid chlorides applied. This means that 98.8% of the applied alkaloids were recovered from the column.
Rechromatography of a Mixture of (I) and (IV)

The mixture (0.0915 g.) of columbamine chloride and jatrorrhizine chloride designated fraction 4 above was adsorbed on Woelm Acid Alumina Grade I (1 g.), dried in vacuum over phosphorous pentoxide and applied to a column of Woelm Acid Alumina Grade I (10 g., 1 cm. dia., 11 cm. length) packed in chloroform. The column was eluted successively with methanol-free chloroform (100 ml.), chloroform with 4% methanol (200 ml.), and chloroform with 12% methanol (700 ml.).

Three fractions were taken. The first contained 0.0226 g. of columbamine chloride (I). The second contained 0.0268 g. of a mixture of (I) and (IV). The third contained 0.0421 g. of jatrorrhizine chloride (IV).

The Separation of Berberine and Palmatine

Berberine chloride (III), 0.3700 g., (S. B. Pannick) and palmatine chloride (II, 0.1913 g.) were ground together and the total alkaloids (0.5613 g.) were adsorbed on Woelm Acid Alumina Grade I (6 g.). After adsorption they were dried for two hours in vacuum over phosphorous pentoxide and were deposited on the top of an alumina chromatographic column (100 g. Woelm Acid Alumina Grade I, 2.5 cm. dia., 37 cm. length). The column was developed at 180 ml./hr. with methanol-free chloroform (500 ml.). At the end of this time the column contained two distinct yellow zones, both showing yellow fluorescence under ultra-
violet light. The column was placed on an automatic fraction cutter set to cut 40 ml. fractions, and development was continued with methanol-free chloroform until fraction number 25, at which time development with chloroform containing 2% methanol was begun. Development was discontinued after fraction number 40, at which time 0.5381 g. (95.8% recovery) of total alkaloids had been obtained. The fractions were dried by air jet over steam. Fractions numbers 1 through 24 (0.1864 g., 97.5% recovery) were palmatine chloride (II). Recrystallized from water (II) gave a melting point of 203-204° which agrees with the literature value of 205°. Fractions 25 through 28 (0.0065 g.) were considered a mixture. Fractions 29 through 40 (0.3402 g., 94.5% recovery) were considered to be berberine chloride. Recrystallized from water, they decomposed at 162°, which agrees with Perkin's value of about 160°. The weights of the fractions were combined in groups of four and the weights of each group (number of last fraction in each group is shown) plotted against weight in Figure 34.

Palmatine Chloride (II)

Chromatographically prepared semicrystalline palmatine chloride (approximately 0.1 g.) was dissolved in boiling water (approximately 25 ml.) producing a deep yellow, faintly turbid solution. It was filtered while hot through a "Filtercel" pad on a Buchner funnel. The resulting clear
Elution Curve for Berberine and Palmatine.
solution was allowed to cool slowly to room temperature. The large yellow needles (m.p. 202.5-203.5° d.) were collected on a Buchner funnel and air dried. Feist and Sandstede report a melting point of 205° (d.) for palmatine chloride trihydrate. Paper chromatographic data are summarized in Table 5.

Jatrorrhizine Chloride (IV)

Jatrorrhizine chloride was prepared in the same way as palmatine chloride. The product crystallized from hot water as orange needles (m.p. 205.0-205.5° d.). Feist reports a melting point of 206° (d.) for the monohydrate. Paper chromatographic data are summarized in Table 5.

Columbamine Chloride (I)

Columbamine chloride (I) was prepared in the same manner as palmatine chloride. The product crystallized as yellow needles (m.p. 239.5-240.5° d.). Paper chromatographic data are summarized in Table 5.

Analyzed for: C_{20}H_{20}O_{4}NCl·2H_{2}O

Calcd.: C, 58.60%; H, 4.90%; O, 23.42%; N, 3.42%; Cl, 8.65; OCH_{3}, 22.71.

Found: C, 58.77%; H, 4.90%; O, 23.35%; N, 3.25%; Cl, 8.73%; OCH_{3}, 23.03%.
Berberine chloride (III)

Berberine chloride was prepared in the same way as palmatine chloride. The product crystallized as yellow needles which, after air drying, melted with decomposition at 162-163°. No literature value is recorded for the decomposition or melting of berberine chloride, but Perkin reports a melting point of 160° (d.) for berberine itself.

Palmatine Iodide

Chromatographically prepared palmatine chloride (0.2159 g.) was dissolved in water (20 ml.) and filtered ("Filtercel") to remove suspended material. The filtrate was heated to boiling and hot sodium iodide solution (saturated at 20°) was added dropwise until a faint cloudiness persisted. The flask was allowed to cool slowly to room temperature. The palmatine iodide was obtained as yellow needles (0.2003 g.) which, when collected on a Buchner funnel and air dried gave a melting point of 239.5-240.2°. Späth and Bohm list a melting point of 241° (d.) for palmatine iodide dihydrate. The infrared spectrum is shown in Figure 6.

Ultraviolet Spectra: λ max (log E)

<table>
<thead>
<tr>
<th>Type</th>
<th>λ max (log E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td>431(3.79), 350(4.47), 272(4.44), 224(4.59)</td>
</tr>
<tr>
<td>Neutral</td>
<td>431(3.76), 348(4.45), 264(4.43), 217(4.58)</td>
</tr>
<tr>
<td>W. Base</td>
<td>433(3.77), 349(4.46), 267(4.44), 224(4.58)</td>
</tr>
<tr>
<td>S. Base</td>
<td>353(4.38), 273(4.12)</td>
</tr>
</tbody>
</table>
**Jatrorrhizine Iodide**

Chromatographically prepared, semicrystalline jatrorrhizine chloride (0.1714 g.) was treated as in the preparation of palmatine iodide. The iodide (m.p. 213-213.5° d.) was obtained as clumps of fine orange needles. After drying, the total weight was 0.1926 g. Feist reports the melting point of jatrorrhizine iodide monohydrate as 208-210°. The infrared spectrum is shown in Figure 6.

Ultraviolet Spectra: \( \lambda_{\text{max}} \) (log E)

- **Acid**: 435(3.76), 350(4.46), 265(4.41), 223(4.44)
- **Neutral**: 437(3.74), 345(4.44), 260(4.39), 215(4.56)
- **W. Base**: 396(4.28), 354(4.30), 262(4.34), 245(4.38), 220(4.53)
- **S. Base**: 498(4.54), 393(4.57), 247(4.45), 217(4.55)

**Columbamine Iodide**

Chromatographically prepared, semicrystalline columbamine chloride (0.0997 g.) was treated as in the preparation of palmatine iodide to yield 0.1130 g. of yellow-brown needles (m.p. 228-228.5°, d.). The infrared spectrum is shown in Figure 6.

Ultraviolet Spectra: \( \lambda_{\text{max}} \) (log E)

- **Acid**: 433(3.74), 349(4.44), 265(4.46), 223(4.58)
- **Neutral**: 432(3.73), 348(4.43), 261(4.45), 216(4.58)
- **W. Base**: 432(3.75), 349(4.34), 266(4.46), 223(4.57)
- **S. Base**: 383(4.19), 330(4.23), 273(4.57), 243(4.42), 220(4.58)
Analyzed for: C_{20}H_{20}O_{4}NI.H_{2}O

Calcd.: C, 49.70%; H, 4.59%; O, 16.55%; N, 2.90%; I, 26.26%

Found: C, 49.39%; H, 4.75%; O, 15.31%; N, 3.04%; I, 27.51%

**Berberine Iodide**

Chromatographically prepared, semicrystalline berberine chloride (0.2052 g.) was treated as in the preparation of palmatine iodide. The iodide (m.p. 162°, d.) was obtained as a mass of yellow needles. The weight after drying was 0.1989 g. No melting point for berberine iodide was found in the literature. The infrared spectrum is shown in Figure 33.

**Ultraviolet Spectra:** \( \lambda_{\text{max}} \) (log E)

- **Acid:** 430(3.74), 344(4.42), 265(4.40), 224(4.54)
- **Neutral:** 432(3.74), 347(4.43), 272(4.40), 266(4.41), 223(4.55)
- **W. Base:** 432(3.71), 348(4.43), 273(4.40), 266(4.40), 223(4.54)
- **S. Base:** 355(4.35), 274(4.08)

**Tetrahydropalmatine (VI)**

Tetrahydropalmatine (VI) was prepared by the method of Awe and Unger.\(^{57}\) An amalgamated mixture of zinc and

Cadmium was made by treating ten grams of mossy zinc - mossy cadmium mixture (3:1) for one hour with 5% mercuric chloride solution and washing twice with distilled water. Palmatine chloride trihydrate (0.3329 g.) was dissolved in a mixture of aqueous acetic acid (5 ml., 50%) and 2 N sulfuric acid (3 ml.) in a 25 ml. round-bottom flask. Amalgamated zinc-cadmium mixture was added (4 g.) and the mixture refluxed until it was colorless (2 hr.). The hot liquid was separated from the metal residue by pouring through a small funnel containing a bit of glass wool. The flask and residue were washed twice with boiling 10% acetic acid (5 ml. each washing), placed in a stoppered flask and cooled in ice water. Concentrated ammonium hydroxide was added dropwise until the initially-formed precipitate of zinc hydroxide had dissolved leaving behind it a curdy white deposit of tetrahydropalmatine.

The reaction mixture was placed in a separatory funnel and extracted with three portions (25 ml. each) of methylene chloride. After drying over granular anhydrous sodium sulfate, the methylene chloride extract was evaporated to dryness at reduced pressure on a rotatory evaporator.

The yellowish-white solid product was recrystallized once from ethanol-water mixture by dissolving it in boiling ethanol (95%) and adding hot water dropwise until a turbidity remained. After slow cooling to room temperature the mixture was placed in a refrigerator overnight. The fine, colorless plates (VI) which resulted were
crystallized a second time by dissolving them in hot chloroform (10 ml.) and adding methanol dropwise until crystallization began. Tetrahydropalmatine was obtained as large hexagonal plates (0.1256 g., 50.4% yield) which melted at 148°. Feist and Sandstede report a melting point of 144°. The infrared spectrum of (VI) is shown in Figure 35.

Ultraviolet Spectra: \( \lambda_{\text{max}} \) (log E)

Acid 283(3.76), 231(4.23)
Neutral 282(3.73)
Basic 281(3.71)

Tetrahydrocolumbamine (V)

Crystalline columbamine chloride (0.2437 g.) was converted to tetrahydrocolumbamine by the same method used for (VI). After recrystallization from ethanol-water and a second recrystallization from chloroform-methanol mixtures, small white prisms were obtained (0.0901 g., 42.8% yield) which melted at 221-222°. Spath and Burger report a melting point of 223-224°. The infrared spectrum of (V) is shown in Figure 35.

Ultraviolet Spectra: \( \lambda_{\text{max}} \) (log E)

Acid 283(3.73), 225(4.17)
Neutral 283(3.71)
Basic 301(3.71), 284(3.55)
Figure 35
Tetrahydroberberine (VII)

Crystalline berberine chloride (0.1982 g., containing 5.5 molecules of water of crystallization per molecule berberine chloride) was converted to tetrahydroberberine by the same method as employed for the preparation of (VI). After crystallization from ethanol-water mixture and chloroform-methanol, large colorless prisms (0.1000 g., 72.4%) were obtained which melted at 162.5-164°. McDavid, Perkin and Robinson\textsuperscript{12} record a melting point for (VII) of 167°. The infrared spectrum is shown in Figure 33.

Ultraviolet Spectra: $\lambda$ max (log E)

Acid 286(3.58), 230(4.04)
Neutral 284(3.73), 252(2.71)
S. Basic 285(3.73), 216(4.23)

Tetrahydrojatrorrhizine (VIII)

Crystalline jatrorrhizine chloride (VIII) monohydrate (0.2419 g.) was converted to tetrahydrojatrorrhizine by the same method as used for the preparation of (VI). After crystallization from ethanol-water mixture and chloroform-methanol, small white prisms (0.1162 g., 55.5% yield) were obtained, which melted at 216-216.5°. Faist\textsuperscript{15} reports a melting point of 217-218°. The infrared spectra is shown in Figure 35.

Ultraviolet Spectra: $\lambda$ max (log E)

Acid 282(3.74), 222(4.18)
Demethyline Berberine Iodide (XXIX)

Demethyline berberine iodide was prepared by a modification of the method of Späth and Quietensky. Phloroglucinol (22.5 g.), sulfuric acid (150 ml., 98%), distilled water (250 ml.), and berberine bisulfate (20 g.) were placed in a one liter round-bottom flask equipped with a reflux condenser. Boiling the mixture over an open flame failed to cause complete solution. Additional sulfuric acid (35 ml., Conc. acid:water::4:1) was added to effect solution. The flask was heated in a thermostated oil bath (105-110°C) under reflux for nineteen hours.

The contents of the flask were allowed to cool slightly and poured into water (1 liter). The tarry residue in the flask became hard on cooling. It was removed and ground in a mortar with hot water until the aqueous extract failed to give a precipitate with sodium iodide solution. The combined aqueous extract and diluted flask contents were neutralized with sodium hydroxide solution (Hydrion test paper) and then made slightly acid with a few drops of 6 N sulfuric acid. The solution now had a volume of about two liters. It was heated nearly to boiling and filtered ("Filtercel"). Without being allowed to cool, it was treated with hot sodium iodide solution (saturated at 20°C) until a permanent precipitate formed. The
large dark red needles were filtered off in a Buchner funnel when the solution was still slightly warm. After air drying, they weighed 11.35 g. (59% yield) and smelled strongly of sulfur dioxide. The filtrate from these crystals was cooled overnight in the refrigerator to obtain a second crop of crystals. However, these were so irregular that they were discarded.

The demethylene berberine iodide (11.35 g.) was dissolved in boiling water (approximately one liter), treated with a small amount of activated charcoal (Darco) and filtered while still hot ("Filtercel"). Upon cooling to room temperature this solution deposited 8.7866 g. (45.6% yield) of dark red needles which no longer smelled of sulfur dioxide. The infrared spectrum is shown in Figure 33. No melting point is recorded in the literature for this compound. Because of its dark color and ease of decomposition, the compound does not lend itself to accurate melting point determination.

Ultraviolet Spectra: \( \lambda_{\text{max}} (\log E) \)

- Acid: 443(3.73), 351(4.41), 266(4.41), 223(4.54)
- Neutral: 440(3.69), 351(4.36), 266(4.40), 223(4.55)
- W. Base: 500(3.62), 389(4.38), 271(4.33), 245(4.38), 219(4.40)
- S. Base: 390(4.13), 218(4.55)
Figure 33
Analyzed for: $C_{19}H_{18}O_{4}NI\cdot H_2O$

Calcd.: C, 48.55%; H, 4.29%; O, 17.02%; N, 2.98%;
I, 27.00%; OH$_3$, 13.21%.

Found: C, 48.47%; H, 4.26%; O, 17.24%; N, 2.85%;
I, 27.18%; OH$_3$, 11.11%

**Chromatography of Demethylene Berberine (XXIX)**

The above product (XXIX) was to be used for the preparation of palmatine, jatrorrhizine and columbamine. Therefore, it was necessary to know if it were free from berberine which might cause confusion during the chromatography of subsequent synthetic mixtures.

Demethylene berberine iodide (approximately 0.2 g.) was dissolved in acetone-water mixture (1:1) and passed slowly through a column (1 cm. dia., 8 cm. length) of Amberlite IRA 410 resin (chloride form) which had been equilibrated after washing with water by treatment with acetone-water mixture (1:1). The solution of demethylene berberine chloride was evaporated at reduced pressure in a rotary evaporator. The chloride was deposited on alumina (Woelm Acid, Grade I, 2 g.) in the usual fashion, and after drying was placed on top of a column (1 cm. dia., 10 cm. length) of similar alumina packed under chloroform. Elution with chloroform containing 5% methanol produced a faint yellow band which was assumed to be berberine. This rapidly moved out of the column. Because of the extreme
weakness of the fluorescence, the amount of berberine was assumed to be negligible. The amount of methanol in the eluent was not sufficient to cause the main body of the alkaloid to move from its position at the top of the column.

**Effect of Base on Columbamine and Jatrorrhizine**

Jatrorrhizine chloride (IV, 0.0085 g.) was dissolved in water (5 ml.) producing a yellow solution. Columbamine chloride (I, 0.0098 g.) was dissolved in water (5 ml.) also producing a yellow solution. Two milliliters of each solution was treated with sodium hydroxide solution (0.5 ml., 1%). Both the jatrorrhizine and the columbamine solutions were turned red by this treatment. Two milliliters of each solution were treated with sodium bicarbonate solution (0.5 ml., 1%). The jatrorrhizine solution was turned red by this treatment. The columbamine solution remained yellow.

**Dihydroanhydroberberine (XXIV)**

Dihydroanhydroberberine (XXIV) was prepared from berberine chloride by a modification of the methods of Bose, Schmid and Karrer. Berberine (0.2504 g.) was dissolved in hot water (50 ml.) and cooled to room temperature. Solid sodium borohydride was added in small portions from the tip of a micro-spatula with agitation after each addition. At the first addition, a pale yellow
precipitate formed. Sodium borohydride was added until the dark yellow color of berberine had vanished, leaving only a pale yellow solid in suspension. The solution was heated on a hot plate and portions of alcohol added until all the suspended material had gone into solution. The pale yellow solution was now brought to boiling and water added dropwise until a permanent turbidity formed. Cooling to room temperature produced pale yellow needles (0.1683 g., 75% yield, m.p. 166-166.5°). Bose reports a melting point of 166-167°. Schmid and Karrer report 166-167°. No infrared spectrum or paper chromatography was carried out. The ultraviolet spectra in neutral alcohol showed \( \lambda_{\text{max}} \) (log E) of 370(3.62) and 283(3.84).

**Synthesis of Columbamine at 15°**

Demethylenberberine chloride (0.1171 g., 3.25\(\times\)10\(^{-4}\) mols) was dissolved in water (50 ml.) in a 100 ml. round-bottom flask equipped with magnetic stirring bar. Methyl sulfate (1.0 ml.) and saturated aqueous sodium bicarbonate solution (5 ml.) were added. The flask was supported in a water bath (15°) and stirring was begun. The flask was checked at half-hour intervals. If the solution had become acid, an additional 5 ml. of saturated sodium bicarbonate solution was added. If a separate methyl sulfate phase did not exist, an additional 1-ml. portion of methyl sulfate was added.
The reaction was allowed to proceed for twenty hours. At the end of this time, the reaction mixture was evaporated to dryness on a rotary evaporator, dissolved in water (50 ml.), and neutralized (Hydrion paper). The neutral solution was filtered to remove suspended matter ("Filtercel") and the alkaloids precipitated as their iodide salts with aqueous sodium iodide solution.

The mixed iodide salts were collected on a Buchner funnel and then dissolved in acetone-water mixture (1:1). The resulting solution was passed through a short column of IRA 410 ion exchange resin (chloride form) to convert it to the chloride form. After evaporation, the mixed chlorides were evaporated on a rotary evaporator (15 mm. Hg pressure, 50° bath temperature) and deposited on one gram of alumina (Woelm, Acid, Grade I).

This alumina with adsorbed alkaloids was deposited on the top of a column of Woelm Acid Alumina (10 g.). Elution of the column with chloroform containing successively larger amounts of methanol caused the removal of three separate bands. These bands were palmatine chloride, columbamine chloride, and jatrorrhizine chloride. A dark red-brown band consisting of starting material was allowed to remain on the column.

The first elution with chloroform containing two percent methanol gave palmatine chloride (0.0010 g., 2.61\times10^{-6} \text{ mols.}). The second elution with chloroform
containing six percent methanol gave columbamine chloride (0.0181 g., $4.97 \times 10^{-5}$ mols). The third elution with chloroform containing ten percent methanol gave jatrorrhizine chloride (0.0051 g., $1.37 \times 10^{-5}$ mols). The individual alkaloids were identified by their color in visible light on the column, and their order or removal. The total conversion was 20.3% of demethyleneberberine.

**Synthesis of Columbamine at 40°**

The previous synthesis of columbamine was repeated with the water bath held at 40°. Demethyleneberberine (0.1597 g., $4.43 \times 10^{-4}$ mols) was treated with methyl sulfate and sodium bicarbonate for eight hours. After isolation as the iodide and conversion to the chloride, it was adsorbed on Woelm Acid Alumina (1 g.) and deposited on a column containing ten grams of the same adsorbent.

In order to obtain a graph of weight of fraction versus fraction number, showing the alkaloids removed separately but in succession, the percentage of methanol in each successive portion of chloroform was rapidly increased as shown in the solvent graph of Figure 30.

Fraction four was palmatine chloride (0.004 g., $1.043 \times 10^{-5}$ mols). It was dissolved in water (2 ml.) and precipitated as palmatine iodide with sodium iodide solution. A mixed melting point ($239-240°$) of this synthetic palmatine iodide and natural palmatine iodide showed no
depression. The infrared spectra of the two palmatine iodide samples were superimposable in all details.

Fractions six to twenty-one inclusive were combined to give a product considered to be columbamine chloride (0.0313 g., 8.43 \times 10^{-5} \text{ mols}, 19.1\% yield). This material converted to its iodide with sodium iodide solution showed no melting point depression (mixed m.p. 228-229\(^\circ\)) with a sample of natural columbamine iodide. The infrared spectra of the two columbamine iodide samples were superimposable in all details.

Fractions twenty-six to thirty-two inclusive were combined to give a product considered to be jatrorrhizine chloride (0.0118 g., 3.18 \times 10^{-5} \text{ mols}). This material gave an orange crystalline iodide which gave a mixed melting point of 213-214\(^\circ\) with natural jatrorrhizine iodide. (m.p. 213-213.5\(^\circ\)). Infrared spectra of the two jatrorrhizine iodide samples were superimposable.

Figure 30 shows the weight curve for the separation of this mixture of products. The total products represent 29.5\% conversion of the demethylenberberine.

**Titration of Demethylenberberine**

Demethylenberberine chloride (0.1670 g., 4.05 \times 10^{-4} \text{ mols}) was dissolved in water (55 ml.) to which was added 5.00 ml. of standard HCl (0.1997 N). The mixture was stirred with a magnetic stirrer. A Model "G" Beckman pH meter with glass electrodes was used to measure the pH.
Standard base (0.1000 N NaOH) was added from a microburette calibrated in 0.02 ml. divisions. The plot of $dpH/dV$ against volume of base added is shown in Figure 29. The first peak was obtained at 9.35 ml. The second peak was obtained at approximately 14.5 ml. The theoretical value for the location of the second peak is 14.05 ml.

**Calculation of $pK_A$ for Demethyleneberberine**

The data used in this calculation were obtained in the above titration of demethyleneberberine. Since the second peak obtained above corresponds to the equivalence point of demethyleneberberine, the theoretical value for the equivalence point (14.05 ml.) was used to calculate $pK_A$. The pH shown in the calculation below is the value obtained in the titration when 14.05 ml. of base was added. Total volume solution 74.65 ml.

- Grams alkaloid plus alkaloid anion 0.1670
- Mols alkaloid plus alkaloid anion $4.65 \times 10^{-4}$
- Molarity alkaloid plus alkaloid anion $6.22 \times 10^{-3}$
- $pH$ 9.52
- $pOH$ 4.48
- Molarity hydroxyl ion $3.32 \times 10^{-5}$
- Molarity alkaloid $3.32 \times 10^{-5}$
- Molarity alkaloid anion $6.19 \times 10^{-3}$
- $K_{\text{hydrolysis}}$ $1.70 \times 10^{-7}$
- $K_A$ $5.89 \times 10^{-8}$
- $pK_A$ 7.23
Calculation of $pK_A$ for Columbamine Chloride Dihydrate

A solution containing a known amount of columbamine dihydrate was prepared. The calculated equivalent of base (0.1000 N NaOH) was added to it. The pH of the resulting solution was measured on a Beckman Model "G" pH meter. From these data $pK_A$ of columbamine chloride was determined.

Total volume solution 54.35 ml.
Grams alkaloid plus alkaloid anion 0.1784
Mols alkaloid plus alkaloid anion $4.35 \times 10^{-4}$
Molarity alkaloid plus alkaloid anion $8.05 \times 10^{-3}$
$pH$ 10.22
$pOH$ 3.78
Molarity hydroxyl ion $1.65 \times 10^{-4}$
Molarity alkaloid $1.65 \times 10^{-4}$
Molarity alkaloid anion $7.89 \times 10^{-3}$
$K_{hydrolysis}$ $3.47 \times 10^{-6}$
$K_A$ 2.88 $\times 10^{-9}$
$pK_A$ 8.54

Calculation of $pK_A$ for Jatrorrhizine Chloride Dihydrate

The $pK_A$ for a solution of jatrorrhizine chloride was determined in the same manner as that of columbamine. While the number of molecules of water of hydration are normally taken to be one for jatrorrhizine, the molecular
weight was calculated assuming two molecules of water of hydration to bring the structure of crystalline jatro-rhizine chloride more in line with that of columbamine chloride.

<table>
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<th>Property</th>
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<td>Grams alkaloid plus alkaloid anion</td>
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</table>
AUTOBIOGRAPHY

I, Thomas Arnold Reed was born in Hampton, Virginia, on August 31, 1928. I received my primary and secondary education in the public schools of Hudson, Ohio, Cleveland, Ohio, Park Ridge, Illinois, and Evanston, Illinois. I obtained my undergraduate training at University of Illinois and was awarded a Bachelor of Science degree from that institution in 1950. I began graduate work at the University of Nebraska at Lincoln, Nebraska, in 1950. This work was interrupted in 1953 for service in the United States Army. I served in Texas and Korea as a 1st Lt. until 1956. In March 1956 I entered the Graduate School of The Ohio State University. While completing the requirements for the degree Doctor of Philosophy, I held the following positions: Assistant in the Department of Chemistry, 1956-1958; Research Assistant in the Department of Chemistry, 1958-1960; Research Fellow, 1960-1962.