THE ALKALOIDS OF OCHROSIA SANDWICENSIS
A. GRAY

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THE ALKALOIDS OF OCHROSIA SANDWICENSIS A. GRAY

By Werner Hans Georg Jordan

A thesis submitted to the Graduate School of the University of Hawaii in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

The endemic Hawaiian tree Ochrosia sandwicensis A. Gray is a member of the family Apocynaceae. Its trivial Hawaiian name is holei and its bark found medicinal use in ancient Hawaii. Previous work established the presence of the alkaloids ellipticine, methoxyellipticine, isoreserpiline and N(b)-methylisoreserpilinium chloride (holeinine) in the leaves and/or bark of the tree.

Renewed investigation of the bark alone resulted in the isolation of two additional quaternary bases.

One of these was isolated in yields of 0.03 % of dry bark, m.p. 322-324° (dec.), [α]_D + 10 (water-methanol), [α]_D + 44 (water-pyridine), and was only sparingly soluble in most common solvents. It was shown to be identical with hunterburnine α-methochloride of molecular formula C_{20}H_{27}N_{2}O_{2}Cl and structure I, which had recently been isolated from Hunteria eburnea and possesses hypotensive activity, thus accounting for this type of activity observed in crude extracts of O. sandwicensis.

The second base was isolated in yields of 0.08 % of
dry bark, m.p. 288-289° (dec.), \([\alpha]_D^0 + 85\) (methanol), molecular formula C\(_{20}\)H\(_{29}\)N\(_2\)O\(_2\)Cl, and is tentatively referred to as W.J.-3. Physical data and chemical evidence are in agreement with a proposed structure II. Further work with this compound was precluded because of its unusually high sensitivity under customary reaction conditions.

Information gained during work on W.J.-3 made it possible to deduce the stereochemistry of the related alkaloid huntrabrine methochloride.

A previously reported yellow base from \(O.\) sandwicensis could be identified as ellipticine hydrochloride.

Another unknown yellow base found in \(O.\) oppositifolia was shown to be methoxyellipticine. A likely position of the methoxyl group in the molecule was derived from spectral analogies.

A novel and rapid scheme for the separation of alkaloids from plant material was developed. It promises to be of general utility and involves extraction with dilute aqueous acetic acid, precipitation of the bases with Mayer's reagent and conversion of the Mayer's complex to chlorides by anion exchange.

Correlations of \(O.\) sandwicensis with other species of the genus, other genera of the family Apocynaceae and \(Strychnos\) melinoniana of the family Loganiaceae are discussed.
I. INTRODUCTION

A. Botanical

The plant family Apocynaceae, consisting of approximately 300 genera, is widely distributed but most abundant in tropical regions.¹ Also referred to as the periwinkle family, its ca. 1500 species occur in the form of perennial herbs, vines, shrubs or trees and are known for their milky latex. They have served man in a variety of ways: as ornament, edible fruit, medicine, arrow poison, fiber, rubber, timber etc.²

Endemic to the Hawaiian Islands are the genus Pteralyxia and some species of the genera Alyxia, Rauvolfia and Ochrosia.²

The genus Ochrosia includes about 36 species,³ distributed from the Mascarene Islands through Malaysia and tropical Australia into Polynesia.⁴ O. sandwicensis is botanically related to O. elliptica and O. oppositifolia.⁴

Ochrosia sandwicensis A. Gray is the only endemic Hawaiian species of the genus.⁴ A most unfortunate state of confusion exists at present concerning the proper nomenclature of the tree. When Goodwin et al.⁵ reported their work, they used the name Ochrosia sandwicensis A. DC. and it can be stated with certainty that their plant material was collected from the same species upon which this present work is based.⁶ According to Rock⁴ Ochrosia
sandwicensis A. DC. is a synonym of Rauvolfia sandwicensis A. DC. The proper authority for the endemic Ochrosia species should be Asa Gray according to the early literature. Bisset, aware of this confusion, notes that Pichon includes Ochrosia sandwicensis A. DC. in his synonymy of Aspidosperma tuberculatum without further comment. Since Pichon's oldest synonym is Macaglia tuberculata Vahl (1810), Bisset suggests the new combination Ochrosia tuberculata (Vahl) Pich.

Such an argument is not useful as long as it remains uncertain whether Pichon had the Rauvolfia or Ochrosia species in mind. Rock's clear distinction between the two species offers an effective guide for the collection of plant material. He observes that the ripe fruits of the Hawaiian Rauvolfia are small and black. The Hawaiian Ochrosia, on the other hand, may be easily recognized by its large twin fruits of a light yellow to orange color.

There is no problem, therefore, regarding the taxonomy of the plant in question. There is confusion only in regard to its correct nomenclature. In this paper the name Ochrosia sandwicensis A. Gray is used since this name is in general use among Hawaiian botanists at the present time.

Ochrosia sandwicensis A. Gray is a small tree or shrub growing to a height of 10-25 feet. The dark green
leaves are oblong and arranged in whorls of three or four. The yellowish flowers are very fragrant, and the wood is hard, fine grained and dark yellowish brown. Although the tree has now become rather scarce, it can be found in dry areas of higher elevation on the leeward sides of the islands. The Hawaiian name for Ochrosia sandwicensis is holei, and the natives extracted a yellow dye from the bark and roots to stain their tapa.

Another source reports that the tree grows to an enormous size. The meat of the nut is described as very delicious. It was used to relieve general debility while steamed extracts of the bark and leaves were beneficial in sweat bath treatments.

B. Chemical

The family Apocynaceae includes numerous alkaloid-producing members. Some of the genera which are outstanding in this respect are Hunteria, Pleiocarpa, Aspidosperma, Vinca, Rauwolfia and Ochrosia.

Very little chemical work on the genus Ochrosia had been reported before 1959. In 1890 Greshoff noted the presence of alkaloids in O. acuminata, ackeringae, coccinea and elliptica. He was able to distinguish three different bases by their color and solubility.

In 1958 Buzas et al. isolated and characterized a yellow base from Ochrosia oppositifolia.
The year 1959 marked the beginning of a more intensive and fruitful investigation of the genus. Goodwin et al. reported the presence of isoreserpiline in *Ochrosia elliptica* and isolated and characterized the three new alkaloids ellipticine, methoxyellipticine and elliptinine from the same plant.\(^5\) Isoreserpiline is a rather common constituent of many *Rauvolfia* species. It is of the yohimbine type and was first isolated from *Rauvolfia canescens*.\(^{14}\)

The properties of ellipticine showed that the structure of the compound must markedly differ from those of the familiar alkaloids of the yohimbine-ajmalicine-corynantheine and strychnos types. Woodward et al. proposed a structure and confirmed it by synthesis.\(^{15}\) Reports on further syntheses along different routes and by other groups of workers followed soon.\(^{16-18}\) The compound represents the first example of a novel pyridine-carbazole ring system and possesses structure I.

![Structure I](image1)

It should be mentioned as a noteworthy coincidence that, during the same period of time, the alkaloid olivacine was isolated from the *Aspidosperma* species *oliv-
aceum, longipetiolatum and australe and also from Tabernae-montana psychotria. A structure was again proposed and confirmed by two different syntheses.\textsuperscript{19-22} Olivacine was shown to be a structural isomer of ellipticine having structure II.

The presence of both ellipticine and olivacine in Aspidosperma subincanum, the Peruvian "Quillo-bordon", was reported by Büchi \textit{et al.} in 1961.\textsuperscript{23}

\textit{Ochrosia coccinea}, also referred to by its synonym \textit{Excavatia coccinea},\textsuperscript{24} was investigated by Macko and Raffauf. They were the first workers to establish the presence of the important alkaloid reserpine in an \textit{Ochrosia} species. Eight additional bases were indicated by paper chromatography.\textsuperscript{25}

Abisch and Reichstein, conducting a survey for alkaloids on numerous Apocynaceae species, obtained a strongly positive alkaloid test with \textit{Ochrosia silvatica}.\textsuperscript{26}

During the years 1960-1964, Moore \textit{et al.} reported on their extensive work on \textit{Ochrosia poweri}.\textsuperscript{27-29} From the leaves of the tree they obtained reserpine, isoreserpiline and four new indole alkaloids including elliptamine. The stem bark furnished again isoreserpiline and elliptamine and three new indole alkaloids not found in the leaves. Isoreserpiline and elliptamine proved to be rather common within the genus \textit{Ochrosia}. The same group of workers detected the presence of these two compounds in \textit{O}. 
elliptica, moorei, glomerata and coccinea. Although ellipticine and methoxyellipticine were also found in these four species, Moore was not able to detect them in Ochrosia poweri.

Ochrosia sandwicensis was first examined by Swanholm, St. John and Scheuer in 1959. During their survey for alkaloids in Hawaiian plants the species gave strong color tests. 30

Goodwin et al. investigated a small quantity of leaves and isolated ellipticine, methoxyellipticine and a new colorless base. 5

In 1961 Scheuer and Metzger reported the isolation of a new colorless alkaloid from the bark. They named it holeinine and showed it to be N(b)-methylisoreserpilinium chloride of structure III. 31 It is the first recorded occurrence of a quaternary N-methochloride of the ajmalicine type in the family Apocynaceae and represents a noteworthy link between melinonine A (IV) from Strychnos melinoniana of the Loganiaceae 32 and isoreserpiline (V) of the Apocynaceae. 14, 31

\[ \text{III} \]

\[ \text{IV} \]
Scheuer and Metzger also mention the isolation of a new yellow base not further examined at the time.

A recent re-investigation of the leaves revealed the presence of isoreserpiline. Three further and possibly new bases were isolated in small quantities and some physical data obtained.\textsuperscript{33}

Continuation of work on \textit{Ochrosia sandwicensis} appeared attractive for a variety of reasons. Thin-layer chromatography tests supported the belief that further alkaloids could still be isolated, characterized and their structures determined. Such results should not only contribute to the existing knowledge of recorded compounds but also aid in further establishment of biogenetic relationships between plant genera and families.

Buzas \textit{et al.} indicated that their yellow base from \textit{Ochrosia oppositifolia} may be identical with methoxy-ellipticine.\textsuperscript{13} Clarification of this possibility, of the exact position of the methoxyl group in methoxy-ellipticine and the nature of the yellow base mentioned by Scheuer and Metzger\textsuperscript{31} offered three more problems of interest.
Finally, it was known that the crude alkaloid extract of *Ochrosia sandwicensis* exhibits hypotensive properties while no such activity could be detected in any one of the bases thus far described. The prospect of identifying the principle responsible for the physiological effect added greatly to the challenge of this work.

C. Acknowledgments

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Grateful acknowledgment is also made to the following people:

Dr. R. M. Heinicke for kind assistance with the fluorescence spectra; Mr. H. C. Inciong for his help during collection of plant material and for a most memorable automobile ride; Drs. R. E. Moore and F. Werny for many fruitful suggestions; Mr. A. H. Struck for recording the mass spectrum; Dr. W. I. Taylor for helpful correspondence and for furnishing some infrared spectra and authentic samples; and Mr. Donald MacMillan for purchasing countless pitchers of beer during the progress of this work.
II. EXPERIMENTAL

All melting points are uncorrected and were determined by techniques as stated in the text.

Elemental analyses were performed by three different laboratories:

Dr. Alfred Bernhardt, Mikroanalytisches Laboratorium im Max-Planck-Institut für Kohlenforschung, Mülheim, Germany.

Dr. W. Zimmermann, Australian Microanalytical Service, University of Melbourne.

Berkeley Analytical Laboratory, Berkeley, California.

These institutions will be indicated in the text by Bernh., Zimm. and Berkeley.

Ultraviolet and visible absorption spectra were obtained with a Beckman DK-2 ratio recording spectrophotometer.

All infrared absorption spectra were measured as potassium bromide disks, using a Beckman IR-5 automatic recording spectrophotometer.

Nuclear magnetic resonance spectra were determined with a model A-60 Analytical N.M.R. Spectrometer of Varian Associates. The sweep width was 500 c.p.s. in all cases, and the maxima are recorded in delta values, referring to tetramethylsilane as $\delta = 0$. Most of the samples examined during this work were of such a low solubility
that the instrument had to be operated at maximal sensitivity. The resulting ratio of signals to noise was so unfavorable that it could usually not be decided whether the observed peaks were singlets or multiplets. Since such a distinction was never of importance for any of the arguments, the peaks are simply listed according to their average position and no attempt of a more detailed description is made.

Fluorometry readings were taken with an Aminco-Bowman spectrophotofluorometer.

Optical rotation measurements were carried out with an O. C. Rudolph and Sons polarimeter.

The mass spectrum was supplied by the Perkin-Elmer Corporation, Norwalk, Connecticut.

Mayer's and Dragendorff's reagents were employed in the conventional manner\textsuperscript{35} to establish whether a fraction contained alkaloids.

Two brands of anion exchange resin, both identical in type and performance, were used: Amberlite IRA-401, 20-50 mesh and Bio-Rad AG 1-X4, 50-100 mesh, both analytical grade and in the chloride form.

A. Procurement and Preparation of Plant Material

Since this work is not concerned with an investigation of leaf material, only stem bark (always containing a small amount of root bark) was collected.
According to original belief, the island of Maui offered the best supply of plant material. The first major collection was therefore conducted on the lower slopes of Haleakala in the Auahi lava fields, about eight miles from Ulupalakua at an elevation of approximately 3000 feet. Goodwin's^5 small collection was also derived from the same stand of trees.

Subsequent observations in the northern part of the Koolau Range on the island of Oahu showed that more favorable conditions existed along the Pupukea Road and the Laie Trail at elevations of 1000-1500 feet. Not only could a greater number of trees be detected, but they were all within easy reach by automobile, which was not the case on Maui. The second major collection of bark was therefore carried out at Pupukea Road.

In no instance could any botanical or chemical difference be noted between Maui and Oahu material.

The fresh bark was dried in a forced draft oven at 50^\circ \text{C} for 70 hr. It was then ground in a Wiley mill to pass a 1-mm. screen and stored in airtight containers.

B. Extractions and Separations

1. Large Extraction I

Based on numerous small scale preliminary tests the following extraction procedure was found to be promising and is schematically summarized in Fig. 1.
Ground Bark

Methanol Extraction

Marc (discarded)

Methanol Extract

Concentrated

Water and Ammonia to pH 10

Aqueous Ammoniacal Filtrate

Chloroform Extraction

Solid (contaminated with filter aid)

Soxhlet Methanol

Residue (discarded)

Methanol Extract

Evaporated

"Ammonia Precipitate" (rich in tertiary bases)

Chloroform Fraction (forms a precipitate)

Solid Holeinine

Filtrate

Evaporated

"Chloroform Fraction" (rich in tertiary bases)

"Aqueous Fraction"

Concentrated

Carbon Chromatography

Alumina Chromatography

Hunterburnine α-Methochloride

Fig. 1. Scheme for Large Extraction I.
Two kilogram of dried and ground bark (Maul) was extracted in a stainless steel continuous extractor, first with 8 l. of methanol for 1 d., then with 6 l. of fresh methanol for 2 d. and finally with 6 l. of fresh methanol for 3 d. The weak Mayer's test of the last batch of solvent and examination of the plant marc indicated that the extraction was sufficiently complete, and the marc was discarded. All solutions, containing some fine plant material which had fallen through the extractor basket, were combined and concentrated in vacuo to 750 ml. of a viscous, dark brown syrup. After dilution with 1 l. of water the mixture had a pH of 5. Ammonium hydroxide (conc.) was added under stirring until pH 10. A heavy, dark brown precipitate formed immediately and was allowed to settle for better coagulation. Substantial amounts of diatomaceous earth and sand were needed for filtration.

The residue was washed with water, dried and exhaustively extracted in a Soxhlet apparatus with methanol. The solid in the thimble gave negative alkaloid tests and was discarded, while the clear, dark brown methanol extract was evaporated to dryness. A glassy solid (106 g., 5% yield) was obtained. It was rich in tertiary bases and shall hereafter be referred to as the "ammonia precipitate."

The ammoniacal filtrate (2 l.) of pH 10 was extracted in a continuous extractor with chloroform for 3 d.
During this process, a small amount of light brown precipitate formed in the chloroform phase. After filtration and drying, the solid (0.63 g., 0.03 % yield) gave a very strong Mayer's test. It could be purified by partially extracting it into ethyl acetate by refluxing in a Soxhlet apparatus and subsequently crystallizing it from methanol-ethyl acetate. The resulting fine crystals were compared with authentic holeinine\textsuperscript{31} in parallel tests by thin-layer chromatography (alumina, methanol, $R_f$ 0.60), ultraviolet and infrared spectroscopy. Both materials could be shown to be identical.

The clear chloroform filtrate was evaporated to dryness, resulting in 18.2 g. (0.9 % yield) of a dark, reddish-brown solid. It was again rich in tertiary bases and shall be referred to as the "chloroform fraction."

The ammoniacal aqueous phase after chloroform extraction was concentrated in vacuo as far as possible. A stiff, dark red-brown syrup (270 g., ca. 12 % yield) was obtained and shall hereafter be known as the "aqueous fraction."

\begin{itemize}
  \item[a.] The Tertiary Bases
  It appears best to begin this section with a summarizing statement that none of the many efforts to isolate further tertiary bases from the bark resulted in success. The amount of data accumulated during these
attempts is so voluminous and yet of so little relative
interest that a greatly simplified presentation shall be
given, merely indicating the more significant avenues of
investigation and omitting all unnecessary details.

Both the "ammonia precipitate" and "chloroform
fraction" (vide supra) were carefully examined by thin-
layer chromatography on alumina, the solvent systems rang-
ing in polarity from benzene to methanol. The alkaloid
composition was essentially identical in both fractions.
Ellipticine and methoxyellipticine$^5$ occurred in great
abundance, while of the distinctly minor unknown constitu-
ents three were migrating faster and three or more
migrated more slowly than these two compounds. In an
attempt to isolate some or all of the presumably new
tertiary bases, the following exploratory experiments
are examples of different approaches.

(1) A counter-current distribution run was car-
rried out in a Craig-Post counter-current distribution
instrument, model 2-B with 100 cells of 20 ml. volume each.
The aqueous buffer phase of pH 5.0 for the bottom layers
was prepared by dissolving 21 g. of citric acid in 1 l. of
0.2 N sodium hydroxide$^{36}$ and saturating the solution with
ethyl acetate. For the top layers, ethyl acetate was satu-
urated with the above buffer. The sample was prepared by
dissolving a maximum of the "chloroform fraction" (2.52 g.)
in 50 ml. of ethyl acetate, giving a dark brown solution
with the typical strong green fluorescence of ellipticine. After charging the first 5 tubes of the apparatus with this sample and an operating time of 30 hr. (20 shakings and ca. 5 min. settling time per cycle), 47 top layers of 20 ml. each and 100 bottom layers of 10 ml. each were obtained. Based on thin-layer chromatography top fractions 2-8 looked most promising, were combined (1.25 g.) and chromatographed on a column of aluminum oxide G with chloroform as the eluant. The resulting 109 column fractions were examined by thin-layer chromatography and ultraviolet spectroscopy. Some crystallizations were attempted but without success.

(2) In a more comprehensive scheme, the "ammonia precipitate" (106 g.) and the remainder of the "chloroform fraction" (15.4 g.) were combined and exhaustively leached with a total of 1 l. of 1 N hydrochloric acid at room temperature. After filtration, the residue was discarded and the acidic filtrate extracted with chloroform in a continuous extractor for 6 d. On evaporation of the chloroform phase, 13.0 g. of a light brown solid was obtained. A 6.0 g. sample of it was again subjected to a counter-current distribution, this time using a series of 6 separatory funnels. Each funnel contained 125 ml. of the previously mentioned pH 5.0 citrate buffer and 125 ml. of ethyl acetate. After manual operation of the system, 6 top and 6 bottom layers of 125 ml. each were obtained.
Top layers 1-3 (1.82 g. after combining) were converted into 3.03 g. of crude picrates with picric acid in ethanol-water. The picrates were chromatographed in a column of Woelm basic alumina, activity grade I. Solvent polarity was gradually increased from 50% benzene in chloroform to 8% methanol in chloroform until 174 fractions were collected. Fractions 76-92 (70 mg. after combining) were then re-chromatographed over Woelm neutral alumina, activity grade I. The solvent was 50% benzene in chloroform, and 9 fractions were collected. No useful results were derived from this attempt.

(3) The pH of the aqueous phase after chloroform extraction, 1 N in hydrochloric acid, was raised to 4.0 by addition of solid sodium carbonate. A heavy, black-brown precipitate was removed by filtration, and more sodium carbonate was added to the filtrate to attain a pH of 8.0. The new yellowish-brown precipitate was again filtered off and the clear pH 8.0-filtrate exhaustively extracted with a total of 3 l. of ethyl acetate in a separatory funnel.

The ethyl acetate phase was evaporated to dryness. Leaching with 30% methanol in chloroform separated the alkaloidal portion (1.94 g.) from inorganic salts of the residue. It was passed through a column of Woelm neutral alumina, activity grade I. Elution started with pure chloroform, and methanol was gradually introduced up to
5 %. of the 139 collected fractions, the combined fractions 60-112 (226 mg.) were re-chromatographed in a new column of the same type and 5 % methanol in chloroform as the eluant, giving 52 additional fractions.

The numerous residues, solutions, counter-current distribution and chromatography fractions were again thoroughly examined by thin-layer chromatography and ultraviolet and visible spectroscopy. Crystallizations were attempted where promising.

In addition to the above work, original ground bark or a crude methanolic extract of it were successively extracted with hexane, benzene, ether, chloroform and methanol. Isopropyl alcohol and n-butanol were employed in some cases. None of all this work with the tertiary bases resulted in any success.

b. The Quaternary Bases

The "aqueous fraction" of the main extraction, 270 g. (ca. 12 % yield) of a stiff dark syrup, was divided into two equal portions. One of these was spent for various preliminary investigations which resulted in the following chromatographic scheme.

A slurry, prepared by thoroughly mixing 225 g. of Darco G 60 activated carbon, 135 g. of diatomaceous earth and 1.25 l. of water, was packed into a column on top of a bed of pure diatomaceous earth. The remaining portion of
the syrup (135 g.), after dilution with water to a volume of 300 ml., was applied as the sample. Gravity alone did not suffice to operate the column, but good flow rates could be obtained with only a mild aspirator vacuum. Elution started with 10 l. of water (fractions 1-12). In order to prevent fermentation, the fractions were evaporated to a heavy syrup and then diluted with small amounts of methanol for storage. Elution continued with 3 l. of methanol (fractions 13-15) and was completed with 2 l. of isopropyl alcohol (fractions 16-18). Mayer's tests were now negative, and fractions 13-18 were evaporated to small volumes. Thin-layer chromatography suggested combination of fractions 6-18. A precipitate formed in this mixture on standing. It was filtered off and leached with cold 3% hydrochloric acid to dissolve sugars. After filtering and washing with water and methanol, it was recrystallized from methanol and gave 131 mg. of a compound, later shown to be hunterburnine \(\alpha\)-methochloride.\(^{37,38}\)

The filtrate of combined fractions 6-18 was evaporated to dryness and gave 25.3 g. of a brown glass. Redissolving in methanol and evaporation to dryness in the presence of 63 g. of Woelm neutral alumina resulted in a powdery sample for further chromatography.

A column was packed with a slurry of 550 g. of Woelm neutral alumina, activity grade I, in chloroform. The solid sample was placed on top and elution was started
with 1.2 l. of 10% methanol in chloroform (fractions 1-45). Elution continued with 0.7 l. of 20% methanol in chloroform (fractions 46-73) and then with 2.8 l. of 30% methanol in chloroform (fractions 74-184). According to thin-layer chromatography, fractions were combined and a simpler numbering system started as shown in Table I.

**TABLE I**

**CHROMATOGRAPHY LEADING TO HUNTERBURNINE**

<table>
<thead>
<tr>
<th>Old numbers</th>
<th>New numbers</th>
<th>Old numbers</th>
<th>New numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10</td>
<td>discarded</td>
<td>74-76</td>
<td>8</td>
</tr>
<tr>
<td>11-20</td>
<td>1</td>
<td>77-100</td>
<td>9</td>
</tr>
<tr>
<td>21-29</td>
<td>2</td>
<td>101-113</td>
<td>10</td>
</tr>
<tr>
<td>30-44</td>
<td>3</td>
<td>114-121</td>
<td>11</td>
</tr>
<tr>
<td>45-46</td>
<td>4</td>
<td>122-130</td>
<td>12</td>
</tr>
<tr>
<td>47-56</td>
<td>5</td>
<td>131-145</td>
<td>13</td>
</tr>
<tr>
<td>57-65</td>
<td>6</td>
<td>146-155</td>
<td>14</td>
</tr>
<tr>
<td>66-73</td>
<td>7</td>
<td>156-184</td>
<td>15-17</td>
</tr>
</tbody>
</table>

Elution was concluded, with 30% methanol in chloroform, by collecting fractions 18-22 (125 ml. each) and fractions 23 and 24 (2 l. each).

Some of the column fractions 10-24 formed precipitates of crude hunterburnine α-methochloride on standing, while others could be induced to do so by reducing the volume of the methanol-chloroform or by evaporating the solvents completely and crystallizing the residues from small amounts of methanol. Purification was easily accom-
lished by recrystallization from methanol. Methanol-ether and methanol-benzene gave higher yields of crystals but colored trace impurities were observed in some cases. A total of 128 mg. of pure alkaloid was obtained from these fractions. The total amount of pure hunterburnine \( \alpha \)-methochloride recovered from the large extraction I (based on only one half of the aqueous fraction) was 259 mg. or 0.026 \% of dry bark.

2. Short Cut Extractions and Separations

At this stage of the work, the identity of hunterburnine \( \alpha \)-methochloride was not yet recognized. The following pilot extractions were carried out for the main purpose of obtaining more of the alkaloid by greatly simplified methods and taking advantage of its unusually low solubility in most solvents, particularly methanol.

a. Pilot Extraction I

One hundred gram of dried bark (Oahu) was heated to boiling in 500 ml. of 5 \% aqueous ammonium hydroxide for 30 min. After filtration the residue was treated twice more with 500 ml. each of 2 \% ammonium hydroxide for 30 min. at the boiling point and then discarded.

The combined filtrates (1.5 l.) yielded a dark brown glass after evaporation in vacuo. It was triturated with 500 ml. of cold water and a first solid removed by
filtration. Evaporation of the filtrate, boiling of the residue in 500 ml. of methanol, cooling and filtration resulted in a second solid. A third solid formed when the methanolic filtrate was concentrated to 50 ml.

All attempts to recover hunterburnine α-methochloride from these three solids failed.

b. Pilot Extraction II

One hundred gram of dried bark (Oahu) was treated with three portions of boiling chloroform (300, 250, 200 ml.; 45 min. each time) to remove tertiary bases. The residue was then extracted three times with boiling water (400, 300, 300 ml.; 45 min. each time) and discarded. The combined aqueous filtrates (pH 6) were divided into two equal portions of 450 ml.

Picric acid (1.5 g. in 100 ml. of water) was added to one of the portions.\(^{39}\)

The second solution was adjusted to pH 2 with hydrochloric acid, followed by addition of ammonium reineckate (2.0 g. of ammonium reineckate in 40 ml. of 2:1 water-acetic acid).\(^{39}\)

In both cases good precipitates formed which were allowed to coagulate for 5 hr. with frequent stirring. Filtration, washing with water and ether and drying gave the crude picrates (1.40 g.) and reineckates (2.78 g.).

Both solids were dissolved in acetone-methanol-
water (6:2:1) and passed through an anion exchange column, chloride form, using the same solvent mixture for elution. Smooth exchange occurred in both cases, and thin-layer chromatography showed the two crude chloride mixtures to be identical. They were combined and evaporated to dryness. Triturating in 200 ml. of methanol, reducing the volume to 50 ml., cooling and filtering yielded a crude solid. Two recrystallizations from methanol and removal of yellow impurities by activated carbon resulted in 28 mg. of pure hunterburnine $\alpha$-methochloride, representing 0.028% of dry bark.

c. Pilot Extraction III

Eighty gram of dried bark (Oahu) was extracted by treating it four times for 20 min. with 300 ml. of boiling water, 175 ml. of water and two portions of 165 ml. of 10% aqueous acetic acid. A small precipitate formed when the combined filtrates were adjusted to pH 2 with hydrochloric acid. It was removed by filtration and found to contain no alkaloids. Addition of 80 ml. of ammonium reineckate according to Battersby et al.\textsuperscript{41} resulted in a voluminous precipitate which after filtration, washing with water and ether and drying resulted in 3.71 g. of crude reineckates. Mayer's tests indicated that the acidic filtrate still contained alkaloids, and a new precipitate was formed on addition of 80 ml. of Mayer's reagent.\textsuperscript{42} It was
also filtered, washed with water and ether and dried to give 2.74 g. of crude Mayer's complex.

Each of the two derivatives was dissolved in 100 ml. of acetone-methanol-water (6:2:1). Waxy insoluble and non-alkaloidal residues which remained were filtered off and discarded. The filtrates were passed through two columns containing 100 ml. of anion exchange resin, chloride form, using 500 ml. of the same solvent mixture, followed by 200 ml. of water for elution. Evaporation of both eluates to dryness gave brown residues. By treating them with 200 ml. of boiling methanol dark, non-alkaloidal residues which remained were filtered off and discarded. Concentration of both filtrates to 50 ml. gave new brown precipitates. They were filtered off and recrystallizations attempted from methanol. Some activated carbon served again for the removal of colored impurities.

The material derived from the reineckates yielded only a very small amount of hunterburnine \( \alpha \)-methochloride while the crude residue resulting from the Mayer's complex yielded 20 mg. of the pure alkaloid, corresponding to 0.025% of dry bark.

3. Large Extraction II

The following scheme was based on a fourth pilot extraction and is summarized in Fig. 2.

A total of 1.2 kg. of dried and ground bark
Fig. 2. Scheme for Large Extraction II.
(Oahu), treated in three identical batches, was extracted with 3.6 l. of boiling 5% aqueous acetic acid for 1 hr. and the resulting mixture filtered by suction through a bed of filter aid. Two further treatments of the residue, each time in 2.4 l. of water for 1 hr., were required to complete extraction. The pH of the combined filtrates was adjusted to 1.0 with conc. hydrochloric acid and 2.7 l. of Mayer's reagent added under stirring. A resulting voluminous precipitate, which was allowed to coagulate and settle overnight, was filtered by suction with the help of filter aid. The resulting crude Mayer's complex had a light brown color. It was only partially dried in air to avoid possible chemical changes and thoroughly stirred in 1.5 l. of acetone-methanol-water (6:2:1). Filtration through filter aid and washing with 500 ml. of the same solvent mixture gave a non-alkaloidal residue and 2 l. of a clear but very dark reddish-brown solution containing 144 g. of dissolved Mayer's complex as shown by evaporation of an aliquot to dryness.

An anion exchange column was packed with 450 g. of chloride resin (59-65% moisture by weight) in acetone-methanol-water (6:2:1). It took 12 hr. to pass the 2 l. of sample solution, followed by 1.3 l. of the above solvent mixture for washing, through the column. Mayer's tests had now become negative, and the eluate contained 58.3 g. of crude chlorides (found by evaporation of an aliquot to
dryness).

After reducing the volume of the chloride solution (3.3 l.) to 350 ml., the acetone and methanol had evaporated and only water remained as solvent. Water and ammonium hydroxide were added to pH 10 and a volume of 800 ml. A heavy precipitate formed, was filtered, washed and dried (34.0 g.) and found to be rich in tertiary bases. The ammoniacal filtrate (1.0 l.) was extracted in a separatory funnel with 10 portions of chloroform totalling 2.2 l. Evaporation of the chloroform phase gave 1.9 g. of a tan solid rich in tertiary bases, while the aqueous phase furnished 22.0 g. of a dark brown solid rich in quaternary bases.

When the crude quaternary mixture was heated under reflux in 250 ml. of methanol for 30 min., an insoluble non-alkaloidal residue (3.07 g.) remained, was removed by filtration and discarded. Addition of 150 ml. of ethyl acetate to the methanolic filtrate (350 ml.) formed a new non-alkaloidal precipitate (0.84 g.) which was again filtered off. Evaporation of the filtrate gave 18.0 g. of a dark brown solid containing the crude quaternary chlorides.

In spite of the crude appearance of this material its ultraviolet absorption spectrum was found to be identical with that of hunterburnine $\alpha$-methochloride indicating that compounds with the same chromophore were the only or at least predominantly absorbing species in the fraction.
A slurry of 700 g. of Whatman Column Chromedia cellulose powder CF 11 and 6 % water in acetone \(^{40}\) (the acetone employed throughout the operation of this column was of C.P. grade) was poured into a large glass column in small portions. Each portion was allowed to settle for a few minutes and then packed down as uniformly and tightly as possible with a tamping rod. The 700 g. of wet packed cellulose powder occupied a volume of 1.85 l. Impurities from the column were removed by washing with 6 % water in acetone until portions of the eluate left no visible residue on evaporation on a watch glass.

The 18.0 g. of crude quaternary chlorides did not dissolve completely in a mixture of 8 % water in acetone. The material was therefore dissolved in methanol and evaporated to dryness in a rotary evaporator in the presence of 50 g. of diatomaceous earth. The resulting tan solid was ground in a mortar to a fine powder and placed on top of the cellulose column as uniformly as possible.

Elution was started with 12.0 l. of 8 % water in acetone (fractions 1-33) and was completed with 9.0 l. of 20 % water in acetone (fractions 34-47). Each fraction was evaporated to dryness and redissolved in small portions of methanol for storage.

During the collection of samples from the column fractions 22-23 formed light, and fractions 24-27 heavy crystalline precipitates of fine, white needles of hunter-
burnine $\alpha$-methochloride.

Fractions 22-27, on crystallization from methanol, gave a total of 338 mg. of pure hunterburnine $\alpha$-methochloride, corresponding to 0.028 % of dry bark.

Thin-layer chromatography showed that the mother liquors remaining from fractions 24-27 were of sufficient purity for further crystallization. On crystallization and recrystallization from water, they furnished 912 mg. of a compound designated W.J.-3, representing 0.076 % of dry bark.

C. Characterization Studies

1. Hunterburnine $\alpha$-Methochloride

   a. Crystallization

   Hunterburnine $\alpha$-methochloride crystallized best from methanol as fine white needles. Crystallization from methanol-benzene or methanol-ether was more rapid and complete but gave in some cases a slightly tan product. Purity of the material was indicated by thin-layer chromatography on alumina, using varying concentrations of methanol in chloroform as the liquid phase.

   b. Solubility

   The alkaloid was practically insoluble in chloroform, benzene, ether and 5-10 % hydrochloric acid and very sparingly soluble in methanol. Solubility was slightly
higher but still low in water and dilute sulfuric and nitric acids.

c. Alkaloid Tests

Because of the extremely low solubility of hunt­erburnine α-methochloride in hydrochloric acid, conventional alkaloid tests in this medium failed. When 4% sulfuric acid was used as the solvent, strong positive tests were obtained with Mayer's, Dragendorff's, Sonnenschein's and Hager's reagents.

d. Combustion Test

On heating in a porcelain spoon the material turned dark, melted, burned poorly with a dark smoke of unpleasant odor, carbonized and finally combusted without a residue.

e. Halide Test

A halide test with silver nitrate in dilute nitric acid gave a white precipitate which dissolved in an excess of ammonium hydroxide and reappeared in a new excess of nitric acid.

f. Melting Point

The compound did not melt on a Kofler block. It began to darken at 300°. At 360° (limit of the thermometer)
the crystals had turned black-brown but still retained their shape.

In evacuated capillaries, seven different fractions of the alkaloid gave the following remarkably sharp melting points: 322, 322, 320, 322, 322, 324 and 323°. Darkening started at 310° and decomposition occurred on melting.

g. Ultraviolet Absorption Spectra

In methanol (Fig. 3): \( \lambda_{\text{max}} 268 \mu \text{ sh} (\log \epsilon 4.05), 274 (4.08), 302 (3.78), 311 \text{ sh} (3.72); \lambda_{\text{min}} 246 (3.54), 295 (3.76) \).

In water (Fig. 4a): \( \lambda_{\text{max}} 274 \mu (\log \epsilon 4.08), 297 \text{ sh} (3.82), 308 \text{ sh} (3.66); \lambda_{\text{min}} 248 (3.66) \).

No shifts occurred in 1 N aqueous hydrochloric acid.

In 0.25 N aqueous sodium hydroxide (Fig. 4b):
\( \lambda_{\text{max}} 269 \mu (\log \epsilon 4.00), 323 (3.74); \lambda_{\text{min}} 257 (3.98), 295 (3.40) \).

After acidification of the above solution to give 0.25 N sodium chloride and 0.75 N hydrochloric acid:
\( \lambda_{\text{max}} 275 \mu (\log \epsilon 4.03), 296 \text{ sh} (3.83), 309 \text{ sh} (3.62); \lambda_{\text{min}} 249 (3.69) \).

h. Infrared Absorption Spectrum

The infrared spectrum of 2 mg. of hunterburnine
Fig. 3.- Ultraviolet spectrum of hunterburnine α-methochloride in methanol.
Fig. 4.- Ultraviolet spectra of hunterburnine \( \alpha \)-methochloride: a,\( \rightarrow \), in water; b,\( \ldots \), in 0.25 N aqueous sodium hydroxide.
α-methochloride in a disk of 250 mg. of potassium bromide showed the following major absorption bands (Fig. 5):
2.98 μ (sh,s), 3.18 (s), 3.43 (w), 3.48 (w), 3.82 (w), 4.24 (w), 5.34 (w), 6.13 (s), 6.28 (m), 6.37 (w), 6.81 (sh,s), 6.87 (s), 7.19 (m), 7.43 (s), 7.73 (m), 7.83 (w), 8.01 (w), 8.10 (w), 8.17 (w), 8.34 (s), 8.50 (w), 8.64 (s), 8.85 (w), 9.00 (m), 9.15 (m), 9.36 (w), 9.49 (m), 9.68 (s), 9.80 (s), 10.25 (w), 10.47 (s), 10.76 (s), 11.12 (m), 11.24 (w), 11.55 (m), 11.63 (sh,m), 11.84 (w), 12.24 (s), 13.40 (w), 13.86 (m), 14.18 (m), 14.80 (m), 15.24 (m), 15.84 (m).

i. Nuclear Magnetic Resonance Spectrum

The only liquid to dissolve the alkaloid in concentrations sufficiently high for n.m.r. spectroscopy was trifluoroacetic acid. A blank run of the pure solvent revealed that it contained contaminants giving rise to peaks at delta 0.6, 1.0, 4.0 and 7.3-7.4.

The spectrum of hunterburnine α-methochloride in the above acid showed peaks at the following delta values (omitting the solvent impurities): 1.6, 2.2, 3.1, 3.5, 4.6 (water), 5.1, 6.7, 7.0 and 7.1.

j. Elemental and Active Hydrogen Analyses

Found (Bernh.): C, 66.10, 66.20; H, 7.75, 7.62; O, 9.02; Cl, 9.67 %. Calculated for C_{20}H_{27}N_{2}O_{2}Cl: C, 66.19; H, 7.50; O, 8.82; Cl, 9.77 %.
Fig. 5.- Infrared spectrum of hunterburnine $\alpha$-methochloride in a potassium bromide disk.
No active hydrogen could be detected (Bernh.).

k. Hydrogenation

A sample of 54 mg. of hunterburnine α-methochloride was dissolved in 75 ml. of 30% water in ethanol and 21 mg. of platinum dioxide (PtO₂ · x H₂O, 83.8%) was added. Hydrogenation took place by vigorously shaking the system for 4 hr. at room temperature and under a hydrogen pressure of 1.7 atm. Removal of the catalyst and evaporation to dryness furnished 54 mg. of a clear colorless glass.

In an attempt to take an n.m.r. spectrum, the glass dissolved easily in deuterium oxide but precipitated out moments later leaving not sufficient material in solution for measurement. The n.m.r. spectrum in trifluoroacetic acid was essentially identical with the one of the starting material except that the peak of the olefinic protons at delta 5.143 had disappeared. The newly formed ethyl group was indicated but obscured by the impurity peaks of the solvent.

The n.m.r. sample, after crystallization from methanol and recrystallization from water, gave 6 mg. of coarse white needles, m.p. 317-318, 317-318° (Kofler block). Darkening started at 270°, and the compound decomposed on melting.

In order to obtain more material, a second batch (55 mg.) of alkaloid was hydrogenated as described above.
Crystallization from water furnished 31 mg. of off-white needles and rosettes, m.p. 315, 315, 314-315° (Kofler block) with darkening and decomposition as before. Different thin-layer chromatography tests showed the two reduced materials to be pure and identical.

The following ultraviolet spectra were obtained with the hydrogenated product.

In methanol, containing a small amount of water:
\[ \lambda_{\text{max}} 273 \mu \text{ (log } \epsilon \text{ 4.05), 300 (3.72), 308 sh (3.65);} \]
\[ \lambda_{\text{min}} 245 (3.41), 295 (3.71). \]

In 0.2 N methanolic (some water) sodium hydroxide:
\[ \lambda_{\text{max}} 269 \mu \text{ (log } \epsilon \text{ 4.07), 324 (3.79); } \lambda_{\text{min}} 255 (3.95), 294 (3.43). \]

After acidification of the above solution to give 0.2 N sodium chloride and 0.5 N hydrochloric acid in methanol and some water: \[ \lambda_{\text{max}} 273 \mu \text{ (log } \epsilon \text{ 4.05), 300 sh (3.80), 308 sh (3.73); } \lambda_{\text{min}} 248 (3.94). \]

The infrared spectrum of 2 mg. of the hydrogenated material in a thin film of 100 mg. of potassium bromide shows the following major absorption bands (Fig. 6):
3.22 μ (s), 3.40-3.50 (broad, s), 4.30 (w), 5.43 (w), 5.84 (w), 5.94 (w), 6.16 (m), 6.32 (m), 6.40 (m), 6.83 (s), 6.93 (s), 7.25 (s), 7.45 (s), 7.73 (m), 8.12 (m), 8.38 (s), 8.50 (w), 8.67 (s), 9.07 (m), 9.55 (s), 9.65 (s), 9.95 (s), 10.23 (m), 10.36 (m), 10.54 (m), 10.81 (m), 11.17 (m), 11.40 (w), 11.55 (m), 11.78 (m), 12.39 (s), 12.96 (w),
Fig. 6. - Infrared spectrum of dihydrohunternburnine $\alpha$-methochloride in a potassium bromide disk.
13.46 (m), 14.18 (m), 14.74 (m), 15.14 (m), 15.84 (m).

An elemental analysis was obtained for the hydrogenated sample. Found (Berkeley): C, 65.53, 65.73; H, 8.27, 8.42; N, 7.30, 7.58%. Calculated for C_{20}H_{29}N_{2}O_{2}Cl: C, 65.83; H, 8.00; N, 7.67%.

1. Optical Rotation

The readings were taken in a 2 dm. tube of ca. 4 ml. capacity.

A total of 20 blank and 20 sample readings was taken and the sample tube was reversed after 10 observations.

Two solvent pairs were employed. They represent percent compositions which dissolve a maximum of the alkaloid as established by preliminary tests.

For the first measurement 25 mg. of alkaloid was dissolved in 27.5% water in methanol to give 10 ml. of solution, $\alpha + 0.0505$. $[\alpha]^{21}_D + 10.1 \text{ (water-methanol)}$.

For the second measurement, 60 mg. of alkaloid was dissolved in 40% water in pyridine to give 10 ml. of solution, $\alpha + 0.529$. $[\alpha]^{22}_D + 44.1 \text{ (water-pyridine)}$.

m. The Free Base

An anion exchange column, packed in water with 32 ml. of chloride resin, was washed with 1 N aqueous sodium hydroxide (1 l.) until chloride tests with silver
nitrate became negative, then with water to neutral pH and finally with 27.5% water in methanol. A sample of 123 mg. of hunterburnine α-methochloride, dissolved in 100 ml. of 27.5% water in methanol, was slowly passed through the column followed by washing with the same solvent mixture until Mayer's tests were no longer positive. Evaporation of the eluate in a rotary evaporator furnished 112 mg. of the free base as a dark brown glass. It was hygroscopic and sensitive to air oxidation and therefore stored under argon or in an evacuated desiccator.

The free base could not be crystallized. It melted in the amorphous form at 199-205, 200-204, 198-204 and 200-205° (Kofler block).

The following ultraviolet absorption spectra of the free base were recorded.

In methanol: \( \lambda_{\text{max}} 267 \) μm (log ε 3.98), 274 (4.01), 302 (3.75), 312 sh (3.70); \( \lambda_{\text{min}} 247 \) (3.64), 296 (3.73).

In water: \( \lambda_{\text{max}} 274 \) μm (log ε 4.01), 298 sh (3.80), 305 sh (3.71); \( \lambda_{\text{min}} 248 \) (3.70).

In 0.5 N aqueous sodium hydroxide: \( \lambda_{\text{max}} 266 \) μm (log ε 4.02), 321 (3.80); \( \lambda_{\text{min}} 295 \) (3.62).

After acidification of the above sample to give 0.5 N sodium chloride and 1.5 N hydrochloric acid: \( \lambda_{\text{max}} 276 \) μm (log ε 4.00), 296 sh (3.87), 309 sh (3.70); \( \lambda_{\text{min}} 250 \) (3.78).
The free base was readily soluble in methanol or water, and the n.m.r. spectrum in deuterium oxide gave the following delta values: 1.4, 2.0, 3.0, 3.5, 5.2, 6.6-6.7 and 7.1-7.2 (Fig. 7).

n. Pyrolysis of the Free Base

Pyrolysis of the compound (5 mg.) was carried out in a sublimator at 0.05 mm. pressure. Reaction began at 240°. Thin-layer chromatography showed the orange-yellow deposit on the cold finger to be a mixture of several alkaloidal and non-alkaloidal components, and no further investigation was attempted.

o. Acetylation of the Free Base

An acetylation was carried out in an argon atmosphere. The free base (40 mg.) was dissolved in 2 ml. of pyridine and 1 ml. of acetic anhydride and boiled gently under refluxing for 4 hr. The product could not be crystallized and gave a dark brown glass on evaporation in a rotary evaporator and drying in an evacuated desiccator. Examination by n.m.r. suggested contamination by acetate ions, and the highly hygroscopic material was converted to the chloride by ion exchange in water-methanol (1:1).

The n.m.r. spectrum of the acetylated chloride indicated formation of a diacetate and exhibits peaks at the following delta values (Fig. 8): 2.0, 2.3, 3.2, 4.0,
Fig. 7.- Nuclear magnetic resonance spectrum of hunterburnine 
$\alpha$-methohydroxide in deuterium oxide.
Fig. 8.- Nuclear magnetic resonance spectrum of hunterburnine α-methochloride diacetate in deuterium oxide.
5.2, 6.8 and 7.1.

Ultraviolet absorption spectra of the acetylated chloride:

In water: $\lambda_{\text{max}}$ 274 $\mu$m (log $\varepsilon$ 3.94), 282 (3.93), 292 sh (3.85); $\lambda_{\text{min}}$ 249 (3.72).

This spectrum did not change on acidification with hydrochloric acid up to 3.0 N.

In 1.0 N aqueous ammonium hydroxide: $\lambda_{\text{max}}$ 267 $\mu$m (log $\varepsilon$ 4.07), 320 (3.81); $\lambda_{\text{min}}$ 298 (3.74).

After acidification of the above sample to give 1.0 N ammonium chloride and 2.0 N hydrochloric acid: $\lambda_{\text{max}}$ 275 $\mu$m (log $\varepsilon$ 4.03), 309 sh (3.77); $\lambda_{\text{min}}$ 251 (3.93).

In an attempt to hydrolize the material, the entire sample of acetylated chloride was dissolved in 8 ml. of 1.0 N aqueous ammonium hydroxide and evaporated to dryness in a rotary evaporator at 40°. The residue was dissolved in 5 ml. of water, acidified with 2 drops of 5% hydrochloric acid and evaporated to dryness as above. A small column of anion exchange resin (chloride form) was converted to the hydroxide form with 1.0 N sodium hydroxide and the hydrolized sample passed through to remove acetate ions, using water as a solvent and eluant.

The n.m.r. spectrum of the product revealed disappearance of both acetyl peaks. Anion exchange with chloride resin in water and evaporation of the eluate to dryness furnished a material almost insoluble in water,
indicating completion of the cycle by reconversion into
hunterburnine α-methochloride, a fact which could be
confirmed by infrared spectroscopy.

p. Hydrogenation of the Free Base

The remainder of the free base (40 mg.) was
hydrogenated by dissolving it in 15 ml. of methanol, adding
10 mg. of platinum dioxide catalyst and magnetically stir­
ing the mixture at room temperature under a slight over­
pressure of hydrogen for 2 hr. Removal of the catalyst by
filtration, evaporation to dryness and n.m.r. spectroscopy
of the residue in deuterium oxide showed that smooth
hydrogenation of the exocyclic double bond had occurred.
The recorded delta values were 0.8, 1.3, 2.2, 3.2, 3.6,
6.9 and 7.2-7.4 (Fig. 9).

Anion exchange of the hydrogenated product with
chloride resin in water and evaporation of the eluate to
dryness gave the hydrogenated hunterburnine α-methochlor­
ide, like the parent compound a material of unusually low
general solubility.

q. Confirmation of Identity

A sample of the alkaloid described in the fore­
going section was sent to Ciba Corporation, Summit, New
Jersey, where identity with hunterburnine α-methochloride
was confirmed by parallel infrared spectroscopy.
Fig. 9.- Nuclear magnetic resonance spectrum of dihydrohunterburnine \( \alpha \)-methohydroxide in deuterium oxide.
2. W.J.-3

a. Crystallization

The new alkaloid could be crystallized and re-crystallized from water or methanol-benzene, yielding fine white crystals of rhombic shape.

b. Purity Tests

Purity of the compound was demonstrated by eighteen thin-layer chromatography tests on alumina with four different percentages of methanol in chloroform (20, 25, 30, 40 %) as the liquid phase.

A 90 mg. sample was subjected to fractional crystallization, yielding three successive crops, 42 mg. from water, 10 mg. from water and 19 mg. from methanol-benzene. All three fractions were identical as shown by thin-layer chromatography, infrared and n.m.r. spectroscopy.

c. Melting Points

In the presence of air, the compound began to darken at 235° and showed the following sharp melting points, always under decomposition:

Fisher-Johns Apparatus, 262-264, 263-265°;
Kofler Block, 261-262, 260-261°.

When repeated in evacuated capillaries, four fractions of the alkaloid gave the following sharp melting

d. Solubility

The compound is readily soluble in methanol, less so in water and dilute mineral acids. It is sparingly soluble in chloroform and insoluble in the less polar organic solvents such as ether or benzene.

e. Ultraviolet Absorption Spectra

In methanol (Fig. 10): \( \lambda_{\text{max}} \) 267 \( \mu \) sh (log \( \varepsilon \) 3.87), 275 (3.92), 299 (3.65), 309 sh (3.58); \( \lambda_{\text{min}} \) 246 (3.35), 296 (3.64).

In water (Figs. 11a, 12a): \( \lambda_{\text{max}} \) 275 \( \mu \) (log \( \varepsilon \) 3.92), 295 sh (3.75), 306 sh (3.58); \( \lambda_{\text{min}} \) 248 (3.50).

In 0.25 N aqueous sodium hydroxide after standing in air for 15 min.: \( \lambda_{\text{max}} \) 269 \( \mu \) (log \( \varepsilon \) 3.88), 323 (3.64); \( \lambda_{\text{min}} \) 296 (3.38).

In 0.25 N aqueous sodium hydroxide after standing under argon for 16 hr. (Fig. 11b): \( \lambda_{\text{max}} \) 268 \( \mu \) (log \( \varepsilon \) 3.71), 321 (3.47); \( \lambda_{\text{min}} \) 296 (3.30).

In 0.25 N aqueous sodium hydroxide after standing in air for 16 hr. (Fig. 12b): Only a very shallow maximum at 316 \( \mu \) (log \( \varepsilon \) 3.75).

In 0.75 N hydrochloric acid: \( \lambda_{\text{max}} \) 275 \( \mu \) (log \( \varepsilon \) 3.90), 295 sh (3.74), 306 sh (3.56); \( \lambda_{\text{min}} \) 249 (3.53).
Fig. 10.- Ultraviolet spectrum of W.J.-3 in methanol.
Fig. 11.- Ultraviolet spectra of W.J.-3: a,----, in water; b,····, after 16 hr. in base under argon; c,----, after 16 hr. in base under argon and reacidification.

Fig. 12.- Ultraviolet spectra of W.J.-3: a,----, in water; b,····, after 16 hr. in base and air; c,----, after 16 hr. in base and air and reacidification.
After standing in 0.25 N aqueous sodium hydroxide under argon for 16 hr. and reacidification to 0.25 N sodium chloride and 0.75 N hydrochloric acid (Fig. 11c): $\lambda_{\text{max}}$ 275 $\mu$m (log $\varepsilon$ 3.90), 295 sh (3.71), 306 sh (3.53); $\lambda_{\text{min}}$ 248 (3.49).

After standing in 0.25 N aqueous sodium hydroxide in air for 16 hr. and reacidification to 0.25 N sodium chloride and 0.75 N hydrochloric acid (Fig. 12c): Only a very shallow maximum at 286 $\mu$m (log $\varepsilon$ 3.93).

g. Infrared Absorption Spectrum

The infrared spectrum of 1.8 mg. of sample in a thin solid film of 100 mg. of potassium bromide showed the following major absorption bands (Fig. 13): 3.00-3.20 $\mu$m (s, broad), 3.38 (s), 3.66 (w), 3.82 (w), 4.24 (w), 6.16 (m), 6.28 (m), 6.39 (w), 6.86 (s), 7.20 (w), 7.33 (w), 7.48 (m), 8.17 (s), 8.38 (s), 8.68 (w), 8.84 (w), 8.98 (m), 9.16 (w), 9.37 (m), 9.60 (m), 9.80 (w), 10.08 (m), 10.40 (w), 10.70 (m), 11.00 (w), 11.22 (m), 11.86 (w), 12.12 (m), 12.43 (m), 13.68 (w), 14.10 (w), 14.54 (s), 15.60-15.90 (w, broad).

g. Nuclear Magnetic Resonance Spectrum

The n.m.r. spectrum in deuterium oxide (Fig. 14) showed peaks at the following delta values: 0.9, 1.4, 2.6, 2.9, 3.7, 6.9 and 7.4.
Fig. 13.- Infrared spectrum of W.J.-3 in a potassium bromide disk.
Fig. 14. - Nuclear magnetic resonance spectrum of W.J.-3 in deuterium oxide.
h. Mass Spectrum

A simplified reproduction of the mass spectrum of the quaternary base is given in Fig. 15. Only the 31 most prominent peaks (higher than 35 mm.) are shown. They occur at the m/e values 10 (70 mm. height), 15 (42), 17 (143), 18 (152), 28 (60), 36 (90), 41 (70), 42 (49), 43 (43), 44 (47), 50 (103), 52 (36), 55 (55), 58 (59), 91 (102), 106 (54), 170 (46), 172 (49), 184 (41), 185 (64), 186 (74), 199 (38), 200 (39), 241 (44), 267 (95), 283 (72), 312 (49), 313 (157), 314 (150), 327 (45) and 328 (62).

i. Halide Test

Positive halide tests were obtained with silver nitrate in dilute nitric acid.

j. Derivatives and Elemental Analyses

Three derivatives were prepared in order to supplement the analytical data for the original material.

Perchlorate.- Forty milligram of alkaloid was dissolved in 3 ml. of water, and 0.25 ml. of 20 % perchloric acid added at 50°. A white precipitate formed immediately, was well stirred and allowed to settle. It was recrystallized first from 3 ml. and then again from 2 ml. of hot water. Yield, 20 mg. of well-shaped colorless rosettes, m.p. (Kofler block, four times) 250-251° (dec.). For analysis it was ground to a fine powder and dried for 4 hr.
Fig. 15a.– Simplified mass spectrum of W.J.-3.

The peaks at low m/e values.
Fig. 15b. - Simplified mass spectrum of W.J.-3.

The peaks at high m/e values.
at 110° and 0.02 mm. pressure.

Bromide and Iodide. - Two columns were packed with 10 ml. each of anion exchange resin, chloride form. They were treated with 1.0 N aqueous sodium hydroxide until chloride tests became negative and then washed with water to neutral reaction. Three hundred milliliter portions of 1.0 N aqueous hydrobromic and hydroiodic acids, respectively, were passed through the columns followed by washing with water until the reaction became neutral and halide tests negative.

Two samples of 25 mg. each of alkaloid were dissolved in 25 ml. of methanol-water (2:1) and then passed through the columns during 2 hr. using the same solvent mixture for washing until Mayer's tests became negative. Yields of crudes after evaporation, 27 mg. of bromide and 30 mg. of iodide. The derivatives were crystallized and recrystallized from methanol-benzene and dried. The bromide crystallized as white, the iodide as slightly tan-colored rosettes. Yields after grinding to a fine powder and re-drying for 3 hr. at 100° and 0.05 mm. pressure followed by 15 hr. in an evacuated desiccator, 24 mg. of bromide and 20 mg. of iodide.

Melting points (Kofler block): Bromide, 274-275, 273-274° (dec.); Iodide, 259-260, 258-259° (dec.).

Thin-layer chromatography tests of the bromide and iodide on alumina gave Rf values of 0.10 with 10 %
methanol in chloroform and 0.55 with 30% methanol in chloroform.

All the analytical results are shown in Table II, together with the calculated values for the most likely formulas.

**TABLE II**

**ELEMENTAL ANALYSES OF W.J.-3 AND DERIVATIVES**

<table>
<thead>
<tr>
<th>Compound analyzed</th>
<th>Element</th>
<th>% Found</th>
<th>% Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original material</td>
<td>(Berkeley)</td>
<td>C 66.09, 66.01</td>
<td>65.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H 8.44, 8.21</td>
<td>8.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N 7.28, 7.21</td>
<td>7.67</td>
</tr>
<tr>
<td>Per-chlorate</td>
<td>(Berkeley)</td>
<td>C 55.80, 55.77</td>
<td>56.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H 7.18, 6.93</td>
<td>6.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N 6.45, 6.39</td>
<td>6.53</td>
</tr>
<tr>
<td>Bromide</td>
<td>(Berkeley)</td>
<td>C 59.2, 59.3</td>
<td>58.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H 7.3, 7.4</td>
<td>7.13</td>
</tr>
<tr>
<td>Iodide</td>
<td>(Berkeley)</td>
<td>C 52.3, 52.5</td>
<td>52.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H 6.5, 6.7</td>
<td>6.40</td>
</tr>
</tbody>
</table>

k. Optical Rotation

The readings were taken in a 1 dm. microtube of slightly less than 1 ml. capacity. Twenty milligram of sample was dissolved in methanol to give 1 ml. of solution.
Twenty blank and sample readings were taken, reversing the sample tube after 10 observations, $\alpha + 1.705$, $[\alpha]_D^{23} + 85.3$ (methanol).

1. Acetylations

**With Acetic Anhydride-Pyridine.**—A mixture of 4 ml. of pyridine, 2 ml. of acetic anhydride and 40 mg. of the alkaloid was refluxed under argon for 5 hr. Some initially insoluble material had dissolved after 2 hr. A brown glass resulted on evaporation. It was dissolved in water and passed through a small column of anion exchange resin, chloride form, to remove an excess of acetate ions. Weight after thorough drying, 53 mg.

The n.m.r. spectrum in deuterium oxide (Fig. 16) exhibited two new peaks at delta values 2.1-2.2 and 2.4, suggesting formation of a diacetate. Thin-layer chromatography showed the product to be a mixture of several compounds. It was therefore purified by passing it through a small alumina column, using 10% of methanol in chloroform as the solvent. Two major fractions of 13 and 8 mg. dry weight, respectively, were obtained. The n.m.r. spectra in deuterium oxide and deuterochloroform showed again the peaks at delta 2.1-2.2 and 2.4, confirming formation of a diacetate.

**With Ketene.**—Thirty-eight milligram of alkaloid in 25 ml. of chloroform was magnetically stirred at room
Fig. 16. - Nuclear magnetic resonance spectrum of W.J.-3 diacetate in deuterium oxide.
temperature for 4 hr. while ketene was admitted from a ketene generator for 5 min. periods at half hr. intervals. Initially insoluble solid material had dissolved after 2 hr., and the solution looked dark brown and clear at the end of the run. It was evaporated to a dark tar of pungent odor. This residue was separated into two portions by repeated shaking with a water-benzene mixture in a separatory funnel. The dark brown benzene fraction was discarded while the faintly yellow aqueous portion yielded 42 mg. of a yellow glass on evaporation. The n.m.r. spectrum in deuterium oxide was identical with the one of the product obtained by acetylation with acetic anhydride-pyridine and again suggested formation of a diacetate. Numerous attempts to crystallize the compound failed.

m. Methylation Attempts

With Diazomethane.- In order to prepare a phenolic methyl ether, 38 mg. of alkaloid was dissolved in 8 ml. of methanol. Diazomethane (0.25 mM./ml. wet ether) was added dropwise at room temperature until a yellow color persisted. After standing for 30 min., the solution was taken to dryness, yielding 40 mg. of a yellow glass. The n.m.r. spectrum in deuterium oxide showed mostly unreacted starting material. Only a very small peak could be found at delta 3.8-3.9, indicating the presence of a small quantity of the desired methyl ether.
With Dimethyl Sulfate. The following work was done in an argon atmosphere. An excess of aqueous sodium hydroxide was added to the 40 mg. of material left from the previous methylation attempt. After addition of dimethyl sulfate until the mixture became acidic, basicity was restored by addition of more sodium hydroxide. The mixture was refluxed for 3 hr., during which time the pH of the reaction was changed from basic to acidic and back three times. It was finally kept in basic medium for 30 min., acidified with hydrochloric acid and extracted with seven portions of chloroform. On evaporation the chloroform fraction yielded 34 mg. of a yellow glass.

The n.m.r. spectrum in deuterium oxide showed two new peaks between delta 3.7 and 3.9.

Thin-layer chromatography tests indicated the presence of at least six components, four of which gave positive alkaloid tests.

n. Pyrolysis

A pyrolysis was carried out with 100 mg. of the alkaloid in a sublimator at 0.07 mm. pressure. Melting and sublimation began at 288-289°. The reaction was kept at 290-295° for 30 min., then at 300-305° for 15 min. and finally at 310° for 15 min. The dark residue was discarded, while 78 mg. of a yellow glass could be recovered from the cold finger. Thin-layer chromatography tests of this
material showed four strong and several weak alkaloid spots. The ultraviolet spectrum of the mixture in methanol-water (4:1) was identical with the one of the original alkaloid. Addition of base caused a bathochromic shift of the 274 μm peak to 280 μm which was reversed on reacidification with appearance of a new peak at 254 μm.

The mixture was passed through a column of neutral alumina, using 2-20% methanol in chloroform as the liquid phase. Thirteen fractions were collected and examined by thin-layer chromatography and ultraviolet spectroscopy. Fractions 1-8 showed the ultraviolet spectrum of the original alkaloid. Fractions 9 and 10 had, in addition, a new peak at 405 μm while fractions 12 and 13 showed a new peak at 373 μm in addition to the original spectrum.

Numerous attempts to examine several of these fractions by n.m.r. spectroscopy in deuteriated chloroform, acetone, liquid sulfur dioxide or trifluoroacetic acid did not yield any useful spectra. Crystallizations and infrared spectroscopy were also unsuccessful. Only the n.m.r. spectrum of the combined fractions 5-7 in trifluoroacetic acid was sufficiently clear to show the absence of the N-methyl peak, suggesting a conventional pyrolysis.

A. Aromatization

A mixture of 50 mg. of alkaloid and 500 mg. of selenium powder was heated in a test tube in an argon
stream to 280-305° for 30 min. The black residue was leached with methanol and filtered. On evaporation, the filtrate yielded 39 mg. of a yellow-brown glass. Thin-layer chromatography indicated the presence of two major components giving positive alkaloid tests and several trace impurities. Separation was carried out by column chromatography with neutral alumina as the adsorbent and 25-30% methanol in chloroform as the mobile phase. A total of thirteen fractions was collected. Thin-layer chromatography on alumina with 30% methanol in chloroform as the liquid phase gave the following results: Fractions 1-3, double spot of Rf 0.80-0.90; fractions 9-13, single spot of Rf 0.50.

Weights after combining the fractions and evaporation:
Fractions 1-3, 14 mg.; fractions 9-13, 23 mg.

Since all attempts of crystallization failed, both materials were sublimed at a pressure of 0.05-0.08 mm. Fractions 1-3 were kept at 210° for 60 min. and yielded 9 mg. of a yellow material on the cold finger. Fractions 9-13 were kept at 300° for 90 min. and gave 16 mg. of a yellow material on the cold finger. Both cold finger materials were investigated qualitatively by ultraviolet spectroscopy at different pH values in methanol. The prominent maxima are listed in Table III. Since the neutral spectrum of fractions 1-3 showed also a minimum at 272 μm, it is in full agreement with the data reported for alstyrine.32,46,47

Although all attempts to crystallize fractions
TABLE III
ULTRAVIOLET SPECTRA OF AROMATIZATION PRODUCTS OF W.J.-3

<table>
<thead>
<tr>
<th>Medium</th>
<th>Cold finger material 1-3 (μl)</th>
<th>Cold finger material 9-13 (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral</td>
<td>328</td>
<td>232, 356</td>
</tr>
<tr>
<td>0.1 N NaOH</td>
<td>329</td>
<td>243, 377</td>
</tr>
<tr>
<td>Reacidified to 0.1 N NaCl and 0.4 N HCl</td>
<td>296, 375</td>
<td>233, 356</td>
</tr>
<tr>
<td>Directly acidified, 0.5 N HCl</td>
<td>299, 377</td>
<td>232, 356</td>
</tr>
</tbody>
</table>

1-3 failed, fractions 9-13 yielded 6 mg. of fine yellow needles from methanol-benzene-iso-octane. However, no melting point could be observed. When heated on a Kofler block the crystals began to darken at 200°, but remained solid and were black at 350°.

The infrared spectrum of 2 mg. of the needles in a thin solid film of 100 mg. of potassium bromide (Fig. 17) showed the following major absorption bands: 3.00-3.50 μ (s, broad), 6.10 (s), 6.18 (s), 6.34 (s), 6.60 (m), 6.82 (s), 7.14 (s), 7.38 (s), 8.14 (s), 8.46 (s), 8.73 (m), 8.88 (w), 9.19 (m), 9.37 (m), 10.31 (m), 10.51 (w), 11.30 (w), 11.47 (m), 12.17 (s), 12.99 (w), 13.91 (m), 14.92 (s).

p. Comparison with Authentic Samples

Two authentic samples were obtained from the
Fig. 17. - Infrared spectrum (potassium bromide disk) of the material obtained after selenium aromatization of W.J.-3 and column chromatography (fractions 9-13).
Ciba Corporation, Summit, New Jersey: Eight milligram of hunterburnine $\beta$-methochloride and 54 mg. of huntrabrine methochloride. 40

Hunterburnine $\beta$-Methochloride.- The entire sample of hunterburnine $\beta$-methochloride (8 mg.) was dissolved in 95% ethanol, 6 mg. of platinum dioxide catalyst ($\text{PtO}_2 \cdot x \text{H}_2\text{O}, 83.8\%$) was added and the mixture was hydrogenated at room temperature under stirring for 2 hr. Filtration of the catalyst and evaporation of the filtrate to dryness yielded 9 mg. of a colorless glass. Nuclear magnetic resonance spectra in deuterium oxide showed that smooth hydrogenation had occurred. A C-methyl peak could be found while the peak for the olefinic protons of the vinyl group had disappeared. Crystallization from methanol-benzene furnished 7 mg. of fine white needles. The infrared spectrum of 2 mg. of the product in a thin film of 100 mg. of potassium bromide was similar to, but definitely not identical with that of W.J.-3.

Huntrabrine Methochloride.- A sample of 32 mg. of huntrabrine methochloride was dissolved in water and 12 mg. of platinum dioxide catalyst was added. In order to minimize an Emde degradation, it was attempted to hydrogenate the material until only one mole of hydrogen gas (4.4 ml.) was consumed. This appeared to be the case after stirring the mixture at room temperature for 30 min. although the readings of the gas volume were unreliable
because of temperature fluctuations of the system. Filtra-
tion and evaporation of the filtrate gave 31 mg. of a yellow
glass. Thin-layer chromatography on alumina with 15 % meth-
anol in chloroform produced two spots of $R_f$ values of 0.10
and 0.70. With 25 % methanol in chloroform, the $R_f$ values
were 0.30 and 0.75. The $R_f$ value of original huntrabrine
methochloride was also 0.30. Attempts to separate the two
products by triturating and boiling in benzene and chloro-
form failed. They could, however, be separated by column
chromatography using 12 g. of Woelm aluminum oxide, neutral,
activity grade I. The column was packed in chloroform and
the sample (31 mg.) added in a mixture of 5 % methanol in
chloroform. Elution started with the same solvent mixture,
was continued with 15 % and completed with 30 % methanol
in chloroform. Ten samples of 30 ml. each were collected.
Thin-layer chromatography on alumina with 25 % methanol
in chloroform showed that fractions 1-2 gave spots of an
$R_f$ value of 0.30 and fractions 4-6 spots of an $R_f$ value
of 0.30. After combining and evaporation to dryness frac-
tions 1-2 yielded 18 mg. of a yellow glass while fractions
4-6 furnished 6 mg. of a very faintly yellow glass.
Fractions 1-2 gave a negative halide test and must be the
tertiary base resulting from an Emde degradation. Fractions
4-6 gave 4 mg. of very poor and slightly tan-colored
crystals from methanol-benzene. Their infrared spectrum
of 2 mg. of sample in a thin film of 100 mg. of potassium
bromide was very similar to, but still not identical with the one of W.J.-3.

The hydrogenation of huntrabrine methochloride was repeated with the remainder of the authentic material, this time in a well-controlled constant-temperature bath of $24^\circ$ and with a less active catalyst. The sample (18 mg.) was dissolved in water and 10 mg. of 5% palladium on charcoal was added. After 25 min. of stirring, 1.2 ml. of hydrogen gas, corresponding to an uptake of one mole, was consumed and the reaction was stopped. A faintly greenish-yellow glass (18 mg.) resulted after filtration and evaporation. Thin-layer chromatography on alumina with 30% methanol in chloroform gave two spots of equal intensity and $R_f$ values of 0.50 and 0.90. The products were separated by column chromatography as before, giving 8 mg. of the tertiary base as a yellow glass and 8 mg. of the quaternary base as a very faintly yellow glass. The obviously not quite pure quaternary material could be recrystallized from 1-propanol-chloroform and furnished 4.5 mg. of poor and tan-colored crystals. Their infrared spectrum in potassium bromide was again quite similar to, but not identical with that of W.J.-3. An n.m.r. spectrum of 2.5 mg. of the crystals in deuterium oxide, using a microcell, showed that the quaternary base was unreacted starting material. The ethylidene peaks could be clearly distinguished while no ordinary C-methyl peak was present.
D. Yellow Alkaloid III

A crude sample (530 mg.) rich in yellow alkaloid III was available from previous work.\textsuperscript{31,48} According to thin-layer chromatography it still contained substantial amounts of alkaloidal and non-alkaloidal impurities.

Preliminary attempts of purification by direct crystallization from methanol-benzene or by chromatography on alumina with chloroform-methanol (9:1) as the solvent were not successful. The following treatment of two larger batches of the crude material was based on further tests involving separation by different solvents.

A sample of 100 mg. was extracted with benzene in a Soxhlet apparatus for 24 hr. The benzene fraction contained most of the alkaloidal and non-alkaloidal impurities and was discarded. Crystallization and recrystallization of the thimble residue from methanol-benzene furnished a first crop of 6 mg. of large yellow needles, m.p. (Kofler block) 285-292\textdegree (dec.) and a second crop of 35 mg. of small yellow needles, m.p. 278-285\textdegree (dec.); lit.\textsuperscript{31,48} m.p. 289-296, 280-287\textdegree (dec.).

A second sample (355 mg.) of the crude material was extracted with benzene in a Soxhlet apparatus for 2 d. Crystallization and recrystallization of the residue from methanol-benzene gave 155 mg. of small yellow needles, m.p. (Kofler block) 275-280\textdegree (dec.). Purity of the various crops was demonstrated by thin-layer chromatography.
Yellow alkaloid III was very soluble in water or methanol. Solubility in dilute nitric or hydrochloric acids was lower. A solution of the compound in a small quantity of water formed a precipitate on addition of nitric acid. The crystals were insoluble in benzene. Ellipticine\textsuperscript{5} does not dissolve in water.

Chloride tests of different fractions of yellow alkaloid III with silver nitrate in dilute nitric acid were positive.

The ultraviolet absorption maxima of the material in ethanol, 0.1 N hydrochloric acid in ethanol and of ellipticine in 0.1 N hydrochloric acid in ethanol\textsuperscript{5} are shown in Table IV.

The two infrared absorption maxima reported for ellipticine\textsuperscript{5} at 2.86 and 6.23 $\mu$ were also shown by yellow alkaloid III.

The material sublimed easily at 170-175° and 0.2 mm. pressure, leaving a small dark residue.

Elemental analyses were obtained from two sources. Found (Zimm.): C, 70.15; H, 5.38; N, 9.15 %.

Found (Bernh.): C, 71.35, 71.12; H, 5.86, 5.53; N, 9.50; Cl, 13.39 %.

Calculated for $\text{C}_{17}\text{H}_{15}\text{N}_{2}\text{Cl}$: C, 72.21; H, 5.34; N, 9.90; Cl, 12.55 %.

In an attempt to prepare the free base, 9 mg. of yellow alkaloid III was dissolved in a small volume of water and ammonium hydroxide added to a pH above 10.
TABLE IV
ULTRAVIOLET SPECTRA OF YELLOW ALKALOID III IN ETHANOL AND OF YELLOW ALKALOID III AND ELLIPTICINE IN ACID

<table>
<thead>
<tr>
<th>Yellow alkaloid III in ethanol</th>
<th>Yellow alkaloid III in 0.1 N HCl in ethanol</th>
<th>Ellipticine in 0.1 N HCl in ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>(λ max in μm)</td>
<td>(λ max in μm)</td>
<td>(λ max in μm)</td>
</tr>
<tr>
<td>239</td>
<td>240</td>
<td>241</td>
</tr>
<tr>
<td>243</td>
<td>249</td>
<td>249</td>
</tr>
<tr>
<td>249</td>
<td>255 sh</td>
<td>271 sh</td>
</tr>
<tr>
<td>255 sh</td>
<td>271 sh</td>
<td></td>
</tr>
<tr>
<td>261 sh</td>
<td>282 sh</td>
<td></td>
</tr>
<tr>
<td>276 sh</td>
<td>307</td>
<td>307</td>
</tr>
<tr>
<td>287</td>
<td>356</td>
<td>335 (?)</td>
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<tr>
<td>295</td>
<td>369 sh</td>
<td></td>
</tr>
<tr>
<td>333</td>
<td>369 sh</td>
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<tr>
<td>355</td>
<td>403</td>
<td></td>
</tr>
<tr>
<td>369 sh</td>
<td>427</td>
<td></td>
</tr>
<tr>
<td>430 sh</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A yellow precipitate formed, was filtered off, washed and dried. On crystallization from methanol, it gave a first crop (4 mg.) of the free base as yellow needles and rosettes, m.p. (Kofler block) 304-308, 304-312° (dec.) and a second crop (1 mg.) of small yellow needles, m.p. 304-308, 304-309° (dec.). The reported melting point of ellipticine is 311-315° (dec.). Thin-layer chromatography showed the free base to be a pure compound.

The ultraviolet absorption maxima of the free base and of ellipticine, both in ethanol, are shown in Table V.
TABLE V
ULTRAVIOLET SPECTRA OF THE FREE BASE OF YELLOW ALKALOID III AND OF ELLIPTICINE IN ETHANOL

<table>
<thead>
<tr>
<th>Free base</th>
<th>Ellipticine</th>
</tr>
</thead>
<tbody>
<tr>
<td>(λ&lt;sub&gt;max&lt;/sub&gt; in μ)</td>
<td>(λ&lt;sub&gt;max&lt;/sub&gt; in μ)</td>
</tr>
<tr>
<td>(Not on scale)</td>
<td>227-234 sh</td>
</tr>
<tr>
<td>239</td>
<td>238</td>
</tr>
<tr>
<td>246 sh</td>
<td>245 sh</td>
</tr>
<tr>
<td>268 sh</td>
<td>276</td>
</tr>
<tr>
<td>276</td>
<td>287</td>
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<tr>
<td>286</td>
<td>295</td>
</tr>
<tr>
<td>295</td>
<td>317 sh</td>
</tr>
<tr>
<td>317 sh</td>
<td>318-322 sh</td>
</tr>
<tr>
<td>333</td>
<td>333</td>
</tr>
<tr>
<td>347 sh</td>
<td>347-347 sh</td>
</tr>
<tr>
<td>382</td>
<td>384</td>
</tr>
<tr>
<td>399</td>
<td>401</td>
</tr>
</tbody>
</table>

The corresponding minima were also in perfect agreement.

E. Methoxyellipticine

A crude sample (400 mg.) rich in ellipticine and methoxyellipticine<sup>5</sup> was available from earlier work.<sup>31,48</sup> Separation of these two alkaloids was extremely difficult and extensive preliminary tests were performed until the following chromatographic system was found.

A sample of the crude mixture (250 mg.) was dissolved in methanol and evaporated to dryness in a rotary evaporator in the presence of 2.5 g. of silica gel G for thin-layer chromatography. A column was packed in ethyl
acetate with 25 g. of the same type of silica gel and the powdery sample was placed on top. Ethyl acetate (2.5 l.) was the only solvent used for elution and a total of 108 fractions were collected at 50 min. intervals. According to thin-layer chromatography four groups of fractions were combined and evaporated to dryness. Crystallization and recrystallization from ethyl acetate furnished the following four different fractions, all of them in the form of fine yellow needles.

**Ellipticine** (22 mg.). - Pure ellipticine, m.p. (Kofler block) 310-315° (dec.).

**Mixture of Ellipticine-Methoxyellipticine** (9 mg.). - Almost pure methoxyellipticine, containing a very small amount of ellipticine, m.p. 278-283° (dec.).

**Methoxyellipticine I** (15 mg.). - Methoxyellipticine with a very small trace of ellipticine, m.p. 278-283° (dec.).

**Methoxyellipticine II** (11 mg.). - Pure methoxyellipticine without any trace of ellipticine, m.p. 279-284° (dec.).

The reported melting points of ellipticine and methoxyellipticine are 311-315° (dec.) and 280-285° (dec.), respectively.  

Identity and purity of the fractions were confirmed by thin-layer chromatography comparisons with authentic material left from previous work.
Commercial samples of 5-methoxyindole and 6-methoxyindole were obtained from K and K Laboratories, Inc. Melting points (Kofler block) were in agreement with reported values: 5-Methoxyindole, m.p. 55\(^\circ\)C; lit.\(^{49}\) m.p. 55\(^\circ\)C; 6-methoxyindole, m.p. 89-90\(^\circ\)C; lit.\(^{50}\) m.p. 91-92\(^\circ\)C.

The ultraviolet absorption spectra of the following compounds in ethanol were recorded and are reproduced in Fig. 18: 5-Methoxyindole, \(\lambda_{\text{max}}\) 271 \(\mu\)m (log \(\varepsilon\) 4.00), 294 (3.78), 306 sh (3.64); \(\lambda_{\text{min}}\) 243 (3.37), 291 (3.77); 6-methoxyindole, \(\lambda_{\text{max}}\) 262 \(\mu\)m sh (log \(\varepsilon\) 3.71), 267 (3.73), 292 (3.85), 301 sh (3.72); \(\lambda_{\text{min}}\) 244 (3.52), 276 (3.67); methoxyellipticine, \(\lambda_{\text{max}}\) 246 \(\mu\)m (log \(\varepsilon\) 4.41), 276 (4.67), 292 (4.75), 307 sh (4.55), 337 (3.81), 354 (3.57), 402 (3.60), 417 sh (3.59); \(\lambda_{\text{min}}\) 221 (4.09), 253 (4.33), 281 (4.65), 327 (3.70), 348 (3.55), 365 (3.33). Perfect agreement existed between the latter spectrum and the one reported for methoxyellipticine.\(^{5}\)

The maxima of highest intensity for the three compounds occurred at: 5-Methoxyindole, 271; 6-methoxyindole, 292; methoxyellipticine, 292 \(\mu\)m.

Fluorescence spectra of the same samples in ethanol were also recorded and are shown in Fig. 19. The work involved the following detailed steps.

5-Methoxyindole. - Activating wavelength set at 271 \(\mu\)m (most intense ultraviolet maximum). Resulting fluorescence peak, 330 \(\mu\)m. Fluorescence wavelength set at
Fig. 18. - Ultraviolet spectra in 95% ethanol of:
a, ..., 5-methoxyindole; b, ---, 6-methoxyindole;
c, ---, methoxyellipticine.
Fig. 19. - Fluorescence spectra in 95% ethanol of: a, ·······, 5-methoxyindole; b, ----, 6-methoxyindole; c, -----, methoxyellipticine.
330 μ. Maxima in the resulting activation spectrum, 226, 276, 293, 330 μ.

6-Methoxyindole.- Activating wavelength set at 292 μ (most intense ultraviolet maximum). Resulting fluorescence peak, 336 μ. Fluorescence wavelength set at 336 μ. Maxima in the resulting activation spectrum, 226, 292, 335 μ.

Methoxyellipticine.- Activation wavelength set at 292 μ (most intense ultraviolet maximum). Resulting fluorescence peak, 472 μ. Fluorescence wavelength set at 472 μ. Maxima in the resulting activation spectrum, 292, 335, 402 μ.

Two derivatives of methoxyellipticine were prepared.

Hydrochloride.- One milligram of methoxyellipticine was dissolved in a small amount of 95 % ethanol and 0.1 ml. of 5 % aqueous hydrochloric acid added. The crude hydrochloride was filtered off and gave on recrystallization from ethanol-benzene fine orange needles, m.p. (Kofler block) 288-290, 285-290° (dec.). A gradual crystal transformation occurred between 255 and 265°.

Picrate.- One milligram of methoxyellipticine was dissolved in a few drops of 95 % ethanol and 2 mg. of picric acid, also dissolved in 95 % ethanol, was added. Filtration of the crude picrate and recrystallization from 15 % water in acetone furnished fine orange needles, m.p.
(Kofler block) 273-275, 273-275° (dec.).
III. DISCUSSION OF RESULTS

A. Important Experimental Aspects

1. Carbon and Cellulose Chromatography Columns

During the preliminary investigations leading to "Large Extraction I" it soon became obvious that the "aqueous fraction" was the most promising material for the isolation of further alkaloids. This fraction consisted mainly of sugars, undetermined colored matter and water-soluble quaternary bases. A quantity of sugar was obtained as white crystals but not investigated further.

In order to separate these components the choice of carbon as a medium for column chromatography was based on several considerations. Commercial sugar is refined by passing an aqueous solution of the crude through carbon towers. Undesirable impurities are adsorbed while sucrose is eluted with the water.

When crude alkaloid samples were recrystallized using activated carbon for the removal of color, unusually high losses of the alkaloids suggested that they were strongly adsorbed on carbon. Separation of alkaloids on activated carbon was also reported by the Ciba group. Operation of the carbon column, containing 37.5 % of diatomaceous earth to permit sufficient flow, confirmed that the indicated phenomena led indeed to an efficient separation. With water as the eluant sugars appeared first, and were followed by alkaloids. Continuation of elution
with methanol enhanced removal of alkaloids but also of colored matter.

Cellulose powder chromatography as a means of separating complex mixtures of water-soluble chlorides of quaternary bases from calabash curare or other sources has gained wide reputation during recent years. It could be most successfully applied in this present work using simple solvent systems of 8-20% water in acetone for elution.

2. Short Cut Extractions and Separations

"Large Extraction I" had, in a somewhat elaborate way, led to the isolation of hunterburnine α-methochloride. The identity of the alkaloid remained uncertain for a considerable length of time, and attempts were made to isolate more of the compound by simplified techniques. It was known that the alkaloid dissolved fairly well in water but was almost insoluble in methanol.

"Pilot Extraction I" served to establish how far the concept of simplification could be extended. The plant material was extracted in water containing some ammonia to suppress extraction of tertiary bases. Attempts to crystallize hunterburnine α-methochloride directly from the crude extract out of either aqueous or methanolic media failed. Sugars and other impurities appeared to act as crystallization inhibitors, and the need for additional
separation was obvious. Although such a separation had been accomplished by carbon chromatography, exploration of still better ways remained desirable.

Examination of the literature revealed that separation of alkaloids from crude mixtures had been performed in various ways, e.g. by precipitation as picrates,\textsuperscript{39,40} Reineckates\textsuperscript{39,41,51} and Mayer's complex.\textsuperscript{35} All of these precipitates must subsequently be converted into the corresponding crude chlorides. In the case of the picrates this was accomplished by anion exchange through chloride resin.\textsuperscript{39,40} Conversion of the Reineckates took place by the classical but laborious method described by Kapfhammer and Bischoff,\textsuperscript{52} and a similarly involved procedure was suggested for the Mayer's complex.\textsuperscript{53}

It appeared attractive to conduct a systematic study by testing the performance of the three precipitating agents when applied to crude aqueous extracts of \textit{Ochrosia sandwicensis} bark. Furthermore, it seemed even more interesting and important to investigate the possibility of extending the use of chloride anion exchange resin for the decomposition of Reinecke's and Mayer's precipitates.

All three reagents furnished satisfactory precipitates from crude and acidic aqueous plant extracts. Efficiency and quality of performance were, however, not uniform. Precipitation with picric acid was least complete as indicated by strong Mayer's tests of the filtrate, and
filtration of the solid was difficult. Mayer's reagent, on the other hand, led to the most complete precipitation; the solid coagulated rapidly and well and was easily filtered off. Reinecke's reagent occupied an intermediate position between the two.

Smooth conversion of the precipitates to chlorides by anion exchange was possible in all three cases. Differences in performance were again observed. Picrates exchanged most easily and required least excess of resin. Conversion of the Reineckates was slowest and required a substantial excess of resin, while results with Mayer's complex fell between the other two.

Integration of the most efficient steps led to the design of "Large Extraction II."

It must be noted that this novel scheme was no longer aimed at the isolation of a specific alkaloid with unusual solubility properties. The general utility of the approach should be strongly emphasized and deserves some further discussion.

Use of water (or dilute aqueous acetic acid) for the extraction of plant material has never enjoyed much popularity. It is, of course, true that such a method must be avoided when compounds to be extracted undergo chemical changes in the presence of weak acid and possibly atmospheric oxygen at the relatively high temperature during the short boiling periods in the solvent. More
significant is the fact that most extraction schemes involve as the next step evaporation of large quantities of solvent, a laborious task in the case of water. Such reasoning is no longer valid. Although it was observed that the need for a wasteful excess of Mayer's reagent for complete precipitation increases with dilution of the crude extract, no difficulties arose under the conditions of this work. The large amount of water was discarded after filtration.

Treatment of a crude fraction with Mayer's reagent leads to coprecipitation of substantial quantities of non-alkaloidal material. When the filtered Mayer's complex was digested in acetone-methanol-water (6:2:1), some waxy, non-alkaloidal material remained insoluble. Its removal by filtration constituted a purification of the Mayer's compound to be processed by ion exchange.

Crude separation of the mixed chlorides into tertiary and quaternary bases was easily accomplished by ammonium hydroxide precipitation and chloroform extraction.

The described scheme should be of particular utility for workers carrying out plant collection in remote areas where the supply of chemicals is limited and sophisticated laboratory facilities are not available. Crude alkaloid mixtures could be rapidly separated from the plant material and prepared for shipment to parent laboratories.
B. Hunterburnine \( \alpha \)-Methochloride

The alkaloid was isolated under conditions suggesting it to be a quaternary base. It was obtained from the aqueous fraction in which it was soluble and from which it could not be extracted with chloroform even at a basic pH. A positive halide test and the fact that it could be converted into the free base by hydroxyl-type anion exchange resin constituted further proof of its quaternary nature. The free base and its derivatives did not crystallize and were found to be hygroscopic. Because of their considerably higher solubility they were in most cases more suitable for examination by n.m.r. spectroscopy than were the corresponding chlorides.

The ultraviolet absorption spectrum of the alkaloid (Figs. 3, 4a) was typical of the 2,3-disubstituted-5-alkoxy or -hydroxyindole chromophore. Addition of base caused a strong bathochromic shift (Fig. 4b) indicating that the 5-substituent was an easily ionizable phenolic hydroxyl rather than an alkoxy group. Reacidification restored the neutral spectrum which was not affected by an excess of acid. Exactly the same spectral phenomena were observed with the free base.

Further valuable information was derived from the n.m.r. spectrum, particularly the clearer one of the free base (Fig. 7). It confirmed the distribution of the aromatic protons of the indole system, suggested the
presence of an olefinic double bond, a hydroxymethyl and a quaternary N-methyl group but also showed the absence of a C-methyl group.

Elemental analysis permitted calculation of the best fitting formula $\text{C}_{20}\text{H}_{27}\text{N}_{2}\text{O}_{2}\text{Cl}$.

Both the chloride and the free base could be readily hydrogenated. The hydrogenated chloride had the formula $\text{C}_{20}\text{H}_{29}\text{N}_{2}\text{O}_{2}\text{Cl}$ while a better n.m.r. spectrum was again obtained with the hydrogenated free base (Fig. 9). Peaks for the olefinic protons could no longer be observed, while two new peaks typical for an ethyl group had appeared, thus suggesting that the original material possessed a vinyl group. No changes of the ultraviolet spectra in neutral, basic or acidic media took place after hydrogenation, indicating that the double bond in the original compound was not in conjugation with the 5-hydroxyindole chromophore.

Acetylation of the free base furnished a diacetate as deduced from the n.m.r. spectrum (Fig. 8). Although the product was stable in acid, both acetate groups were easily hydrolized in dilute ammonium hydroxide. Reconversion of the hydrolized material to the chloride resulted in the original alkaloid.

Sufficient information was now available for an examination of the literature, and the striking similarity of the compound with hunterburnine $\alpha$-methochloride became
Recognition of identity was delayed by the fact that the alkaloid did not melt on a Kofler block even at 360°. When melting points were finally taken in evacuated capillaries as suggested by the Ciba publication, values of 322-324° were found, now lower than the reported one of 335°.

Comparison of the alkaloid with authentic hunterburnine α-methochloride by parallel infrared spectroscopy carried out by the Ciba workers showed both materials to be perfectly identical in every detail.

Hunterburnine α-methochloride possesses structure VI which is in full agreement with the accumulated and presented physical and chemical evidence. The numbering system is based on a postulated biogenetic formation of this molecule from the corynantheol skeleton.
C. W.J.-3

This new alkaloid, tentatively referred to as W.J.-3, appeared in the separation scheme together with hunterburnine \( \alpha \)-methochloride suggesting that it was also a quaternary base. A drastic difference of the solubility of the two bases in methanol was, however, noted. Hunterburnine \( \alpha \)-methochloride was almost insoluble in methanol while the opposite was the case with W.J.-3. The infrared absorption spectra of both alkaloids (Fig. 13) were similar, and W.J.-3 also gave a positive test for halide ion confirming its quaternary nature.

The ultraviolet absorption spectra of the two bases in neutral methanol (Fig. 10) or water (Figs. 11a, 12a) were identical. The same strong bathochromic shift occurred on addition of base, again suggesting the presence of the 2,3-disubstituted-5-hydroxyindole moiety. One striking difference between the two compounds did now, however, become evident. When a sample of W.J.-3 in basic medium was exposed to the atmosphere, its ultraviolet spectrum deteriorated rapidly (Fig. 12b). Reacidification did not at all result in the original neutral spectrum but in a rather meaningless curve with very broad and diffuse maxima (Fig. 12c). If the sample in basic medium was kept under argon, the spectrum (Fig. 11b) was identical with the one of hunterburnine \( \alpha \)-methochloride in base and did not change even on prolonged standing. Reacidification after 16 hr. restored
the original neutral spectrum which did not change with excess acid (Fig. 11c). Such a deterioration of an alkaloid and its ultraviolet spectrum is not at all unfamiliar and was reported with other compounds containing the indole moiety oxygenated in the 5-position.\textsuperscript{28,54} Since in the present case participation of oxygen was required, it is reasonable to assume that the first step in the chemical transformation is the oxidation of the hydroxyindole to the quinonimine as shown below.

\[ \text{HO} \quad \text{[O]} \quad \text{O} \]

The n.m.r. spectrum (Fig. 14) was best examined together with the one of the hydrogenated free base of hunterburnine $\alpha$-methochloride (Fig. 9). Peaks at delta 0.9 and 1.4 suggested again an ethyl group while the signal at 3.7 was typical for hydroxymethyl. Absorption around delta 6.9 represented the protons in the 4- and 6-position and absorption around delta 7.4 the proton in the 7-position of the 5-hydroxyindole system. Of particular interest was the widely split (20 c.p.s.) quaternary N-methyl peak at delta 2.6-2.9. The low delta value suggested that this methyl group was in an axial position and part of a trans-fused quaternized quinolizidine system.\textsuperscript{55} Integration was
difficult due to superimposed broad absorption of undetermined nature in the same region, but the best attempts showed that the peaks at 2.6-2.9 added up to a total of three protons. Such drastic splitting of peaks has been observed before, e.g. during work on geissoschizoline, and indicates that the molecule is capable of undergoing a rotational transformation which places the absorbing group into different steric or polar environments.

Various elemental analyses of the methochloride and its derivatives were performed (see Table II), and the best fitting formula for W.J.-3 was $\text{C}_{20}\text{H}_{29}\text{N}_{2}\text{O}_{2}\text{Cl}$. The molecular weight calculated from this formula was 364.9 while the one deduced from the mass spectrum (Fig. 15) was 364.0. Since the slight discrepancy of 0.9 mass units is readily eliminated by the necessary isotopic corrections, the data agree.

Acetylation of W.J.-3 gave a diacetate. The n.m.r. spectrum of the product exhibited new peaks at delta 2.1-2.2, 2.4 and 4.1 (Fig. 16). It should be recalled that the n.m.r. spectrum of the acetylated hunterburnine $\alpha$-methochloride (Fig. 8) showed the same set of new peaks confirming the similarity of the two alkaloids and their functional groups. The derivatives were most likely the 0,0-diacetates. The phenolic hydroxyl group should be acetylated readily and give rise to the peak at delta 2.4. Although the peak at delta 2.1-2.2 could a priori be
assigned to the acetylated indole nitrogen or the acetylated aliphatic hydroxymethyl group, the latter case was far more probable. In the first report on hunterburnine methochloride the molecule was stated to contain an aliphatic hydroxyl group which is readily acetylated. 37

Djerassi et al., during their work on dihydrocorynanthe-
ol, 57 prepared the acetate of the compound and showed by mass spectrometry that the group involved was the \( \beta \)-hydroxyethyl side chain and not the \( \beta \)-carboline portion of the molecule. They also observed, as was the case in the present work, that the n.m.r. peak around delta 4.1 was typical of the methylene protons in \( -C-\text{CH}_2-0-\text{CO-R} \) and that a split C-methyl peak at delta 0.6-1.0 was part of an ethyl side chain.

Aromatization with selenium powder resulted in two main fractions which could be separated by chromatography on alumina. The faster migrating material was according to thin-layer chromatography a mixture of two very closely related compounds which could neither be separated nor crystallized and gave the typical ultraviolet absorption spectrum of a 2-pyridylindole so that one of the components may well be alstyrine of structure VII.
Base did not affect the spectrum but acid caused a shift. The slower-migrating fraction was a single compound and could be crystallized. Its main absorption maximum in the ultraviolet occurred now at a longer wavelength and underwent a further drastic bathochromic shift on addition of base, which reversed back to the original on reacidification. The infrared spectrum was very similar to, but not identical with the one reported for alstyrine.\(^{58}\) The aromatization product showed a broader absorption in the 3.00-3.50 \(\mu\) region than does alstyrine. It may then be concluded that the chromatographic fractions differ mainly in that the slower-migrating one has retained the phenolic hydroxyl group of the indole system. All evidence resulting from aromatization suggests strongly that W.J.-3 belongs to the corynantheine-alstonine group of alkaloids.\(^{32,59}\)

At this point it became desirable to compare W.J.-3 with similar alkaloids reported in the literature. Identity with the dihydro-derivative of hunterburnine \(\alpha\)-methochloride could be ruled out as the latter base had been repeatedly hydrogenated during the present work and resulted in an obviously different product. The melting point of W.J.-3 was 288-289\(^{\circ}\) (evacuated capillary) and the optical rotation \([\alpha]_D + 85.3\) (methanol).

\[
\text{Hunterburnine } \beta\text{-methochloride }^{40} \text{ melts at } 307-308^\circ \text{ (evacuated capillary) and shows a rotation } \left[\alpha\right]_D + 105\text{ (water-methanol). }^{40}
\]

Hydrogenation of an authentic sample
furnished again a non-identical product, as shown by the clearly different infrared spectra.

Huntrabrine methochloride,\textsuperscript{40} m.p. 285-287° (evacuated capillary), and of structure VIII has a rotation \([\alpha]_D^\circ + 54^\circ\) (water).

\[
\text{VIII}
\]

The stereochemistry of huntrabrine methochloride was not reported. Attempts to hydrogenate the ethylidene double bond of an authentic sample failed, and any uptake of hydrogen resulted immediately in an Emde degradation. The infrared absorption spectrum of the remaining unreacted and somewhat impure starting material was very similar to, although not identical with that of W.J.-3.

All the data and analogies presented thus far are in support of structure IX for the new base.
If one disregards for the moment the stereochemistry, the compound can be named 10-hydroxydihydrocorynantheol methochloride, a base not yet described in the literature.

Djerassi et al. recorded the mass spectrum of dihydrocorynantheol and reported strong peaks at m/e 156, 169, 170, 184 and 297. If it is assumed that W.J.-3 first loses methyl chlorides when heated in the instrument, its spectrum should exhibit the same peaks plus 16 units to account for the 10-hydroxyl. The resulting peaks of m/e 172, 185, 186, 200 and 313 could indeed all be found in the spectrum of W.J.-3 (Fig. 15). Compounds with an opened E-ring tend to lose the C-15 substituent easily. In the present case, it would be the β-hydroxyethyl group of m/e 45, and the original spectrum contained this peak although of only 12 mm. height so that it is not shown in the simplified Fig. 15. Such an entity would be expected to be of low stability. The corresponding remainder of the molecule, after loss of methyl chloride and hydroxyethyl, has an m/e value of 269, and this peak was also present and of 34 mm. height.

Assuming that structure IX was correctly assigned for W.J.-3, the stereochemistry of the compound must now be discussed. Although the position of the quaternary N-methyl peak in the n.m.r. spectrum already suggested a C/D trans ring juncture, this point may be further inves-
tigated by examination of the infrared spectrum. Several reports in the literature indicate that the spectrum for a C/D trans system is more complex in the 3.4-3.7 \( \mu \) region than for the corresponding cis case.\textsuperscript{61-63} The validity of this statement was tested on the various infrared spectra recorded during the present work. Dihydrohunterburnine \( \beta \)-methochloride, known to be a C/D trans compound, exhibited a relatively small but clearly discernible peak between 3.6 and 3.7 \( \mu \) while hunterburnine \( \alpha \)-methochloride (Fig. 5) and its dihydro-derivative (Fig. 6), both members of the C/D cis group, showed only very weak shoulders in that region. Huntrabrine methochloride, the stereochemistry of which was not reported,\textsuperscript{40} gave the 3.6-3.7 \( \mu \) peak in perfect clarity, and the same was the case with W.J.-3 (Fig. 13). Both compounds should, then, contain the C/D trans system, and their close relationship was thereby further confirmed.

Wenkert's C-15 rule\textsuperscript{64} places the hydrogen atom at C-15 into the \( \alpha \)- and, consequently, the \( \beta \)-hydroxyethyl group into the \( \beta \)-position.

The latter fact suggests assignment of structure X and the name \( \Delta^{19,20} \)-dehydro-10-hydroxydihydrocorynantheol methochloride for huntrabrine methochloride.

Much useful information applicable to the present problem was obtained from the published work on melinonine B. Structure XI was assigned to melinonine B and
Melinonine B chloride melts at 311° (capillary) and has a rotation of $[\alpha]_D - 15$ (water-methanol). Dihydromelaninone B chloride melts at 295-296° (capillary) and has a rotation of $[\alpha]_D - 14$ (water-methanol). The only stereochemistry reported for the two compounds is a C/D cis ring juncture, a fact confirmed by the absence of the 3.6-3.7 μ peak in the infrared spectra. Both infrared spectra look quite different from the one of W.J.-3.

Further interesting data may be derived from the same publication. Dihydrocorynantheol of struc-
ture XIII melts at 182-183° and shows a rotation $[\alpha]_D - 37$ (pyridine). Corynantheidol has structure XIV, a melting point of 183-186° and a rotation $[\alpha]_D - 99$ (pyridine).

Both compounds were converted into their methochlorides. Dihydrocorynantheol methochloride of structure XV melts at 272-273° (capillary) and shows a rotation $[\alpha]_D + 63$ (water-methanol). Corynantheidol methochloride has structure XVI, a melting point of 223-225° (capillary) and a rotation $[\alpha]_D - 43$ (water-methanol).

Both compounds are clearly different from
dihydromelinonine B chloride.

It must be noted that the Ciba group reported a melting point of 296-297° (evacuated capillary) and a rotation $[\alpha]_D + 101$ (medium not stated) for dihydrocorynantheol methochloride. 40

As seen in Table VI, the best criterion for the present argument is the optical rotation. Only one of the mentioned methochlorides (dihydrocorynantheol methochloride) shows a positive value, and the magnitude is quite comparable to the one for W.J.-3.

### TABLE VI

MELTING POINTS AND OPTICAL ROTATIONS OF W.J.-3 AND RELATED COMPOUNDS

<table>
<thead>
<tr>
<th>Compound</th>
<th>M.p.°</th>
<th>$[\alpha]_D$</th>
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<tbody>
<tr>
<td>W.J.-3</td>
<td>288-289 (C)</td>
<td>+ 85 (M)</td>
</tr>
<tr>
<td></td>
<td>261-262 (K)</td>
<td></td>
</tr>
<tr>
<td>Dihydrocorynantheol methochloride</td>
<td>272-273 (C)</td>
<td>+ 63 (WM)</td>
</tr>
<tr>
<td></td>
<td>296-297 (C)</td>
<td>+ 101 (?)</td>
</tr>
<tr>
<td>Corynantheidol methochloride</td>
<td>223-225 (C)</td>
<td>- 43 (WM)</td>
</tr>
<tr>
<td>Melinonine B chloride</td>
<td>311 (C)</td>
<td>- 15 (WM)</td>
</tr>
<tr>
<td>Dihydromelinonine B chloride</td>
<td>295-296 (C)</td>
<td>- 14 (WM)</td>
</tr>
<tr>
<td>W.J.-3 methiodide</td>
<td>258-260 (K)</td>
<td>?</td>
</tr>
<tr>
<td>10-Methoxydihydrocorynantheol methiodide</td>
<td>263-266 (?)</td>
<td>?</td>
</tr>
</tbody>
</table>

C, evacuated capillary; K, Kofler block; M, methanol; W, water; ?, not reported.
All the available evidence suggests that W.J.-3 is 10-hydroxydihydrocorynantheol methochloride and possesses structure XVII.

The earlier mentioned splitting of the quaternary N-methyl peak in the n.m.r. spectrum may now be explained by the free rotation of the \(\beta\)-hydroxyethyl group as such a phenomenon should create different environments for the absorbing protons. A chair-boat equilibrium of ring D may be mentioned as a further but rather unlikely possibility.

Dastoor and Schmid\textsuperscript{65} reported on a compound they believed to be 11-methoxydihydrocorynantheol although they were uncertain about its stereochemistry. The nomenclature used by these workers does obviously not conform with that used here. Inspection of the ultraviolet absorption data shows clearly that its name should be 10-methoxydihydrocorynantheol. This error has also been recognized and corrected by other workers.\textsuperscript{66} The methiodide of the
base melted at 263-266° while W.J.-3 gave a methiodide of melting point 258-260° (Kofler block). The ultraviolet spectrum is in agreement with W.J.-3 although no base shift can be observed because of the methoxyl group rather than a hydroxyl group in the 10-position. The relatively few reported infrared absorption bands may all be found in the spectrum of W.J.-3 except, as would be expected, the bands for the Ar-O-C- system at 8.28 μ.

Work on W.J.-3 was discontinued at this stage, mainly because of the unusually high sensitivity of the molecule. Its rapid decomposition in base and air was described earlier.

Attempted methylation resulted in at least six different products. Rather similar difficulties were encountered by Doy and Moore during their work on powerine, an alkaloid also oxygenated in the 5-position of the indole moiety.

Pyrolysis resulted in a complex mixture of products, a phenomenon familiar from the reported work on melinonine B.

The unusually large number of peaks in the original mass spectrum served as a further illustration of the pronounced tendency of the molecule to disintegrate.

Since W.J.-3 crystallizes relatively well, the most promising avenue of approach to prove the postulated structure appears to be X-ray crystallographic analysis.
D. Yellow Alkaloid III

When the present investigation of yellow alkaloid III was started, the following elemental analysis was available from previous work: $^{48}$ C, 71.88; H, 5.78; N, 9.07; O, 0.00; P, 0.00%. The difference between the sum of these figures and 100% is 13.27%. Spectral information suggested a close relationship to the $C_{17}$-alkaloid ellipticine.\footnote{5} If it was assumed that yellow alkaloid III also contained 17 carbon atoms, the missing 13.27% were calculated to represent a unit of mass number 37.7. Although this result should immediately suggest involvement of hydrochloric acid, such a line of thinking was initially obscured by the fact that repeated negative halide tests during previous work had been reported.\footnote{48}

An eventual repetition of halide tests on several fractions of the alkaloid gave indeed positive results. The next step, preparation of the free base, followed as a logical consequence and was accomplished without difficulty. The free base was identical with ellipticine in every respect.

Yellow alkaloid III is, then, ellipticine hydrochloride of structure XVIII.
It is not known in what form ellipticine exists in the original plant material. The isolated hydrochloride could well have arisen as an artifact during earlier work when hydrochloric acid was employed as a means of crude separation.\textsuperscript{31,48}

Recognition of the nature of yellow alkaloid III was also somewhat delayed by the results of ultraviolet spectroscopy. Compounds of this type give spectra of unusually high complexity. Although the spectra of yellow alkaloid III and ellipticine were identical in hydrochloric acid, the spectrum of yellow alkaloid III in neutral medium was neither identical with the one of ellipticine in neutral medium nor that of ellipticine in hydrochloric acid. The spectrum represented a composite of the latter two. This may simply indicate that the crystallized samples of yellow alkaloid III were substantially contaminated with ellipticine, a fact which would also account for the unusually wide melting range of the material. It is however also possible, and perhaps more likely, that ellipticine hydrochloride in neutral medium is sufficiently hydrolized to give rise to spectral maxima of the free base and of the quaternary cation.

E. Methoxyellipticine

In their report on a new alkaloid from \textit{Ochrosia oppositifolia}, Buzas \textit{et al.}\textsuperscript{13} point out that the material
might be identical with methoxyellipticine characterized by Goodwin et al., although the available evidence was not sufficient to substantiate such a speculation.

Since methoxyellipticine was also isolated in pure form from _Ochrosia sandwicensis_ during the present work, an attempt was made to clarify the findings of the French workers. The significant data are shown in Table VII.

**TABLE VII**

**COMPARISON OF THE YELLOW BASE FROM _O. OPPOSITIFOLIA_ AND METHOXYELLIPTICINE**

<table>
<thead>
<tr>
<th></th>
<th>Buzas et al.</th>
<th>Present work</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M.p.</strong></td>
<td>282-284°</td>
<td>279-284°</td>
</tr>
<tr>
<td><strong>U. V. maxima</strong></td>
<td>242, 275, 290, 335 μ</td>
<td>246, 276, 292, 337 μ</td>
</tr>
<tr>
<td><strong>Hydrochloride, m.p.</strong></td>
<td>282-290°, crystal transformation at 260°</td>
<td>288-290°, crystal transformation at 255-265°</td>
</tr>
<tr>
<td><strong>Picrate, m.p.</strong></td>
<td>268-272°</td>
<td>273-275°</td>
</tr>
</tbody>
</table>

Both Buzas et al. and Goodwin et al. report the formula C_{18}H_{16}N_{2}O and the presence of one methoxyl group.

Integration of all the evidence establishes that the material isolated by the French group was indeed methoxyellipticine and that _O. oppositifolia_ thereby becomes another _Ochrosia_ species in which the presence
of this alkaloid was detected.

No information was thus far available concerning the position of the methoxyl group in the methoxyellipticine molecule, and attempts were made to clarify this point. It appears reasonable to assume that the choice lies between structures XIX and XX.

![Chemical Structures](attachment:image)

XIX  

XX

The corresponding model compounds 5- and 6-methoxyindole were employed for spectral comparison. Figures 18 and 19 show the ultraviolet absorption and fluorescence spectra, respectively, of the two methoxyindoles and of methoxyellipticine. Only the pertinent maxima are contrasted in Table VIII.

Inspection of the spectral data suggests that methoxyellipticine possesses structure XX.
TABLE VIII
SPECTRAL DATA FOR 5-METHOXYINDOLE, 6-METHOXYINDOLE
AND METHOXYELLIPTICINE

<table>
<thead>
<tr>
<th></th>
<th>5-Methoxyindole (μ)</th>
<th>6-Methoxyindole (μ)</th>
<th>Methoxyellipticine (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most intense U. V. maxima</td>
<td>271</td>
<td>292</td>
<td>292</td>
</tr>
<tr>
<td>Fluorescence maxima</td>
<td>276</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>293</td>
<td>292</td>
<td>292</td>
</tr>
<tr>
<td></td>
<td>330</td>
<td>335</td>
<td>335</td>
</tr>
</tbody>
</table>
IV. CONCLUSIONS AND SUMMARY

Renewed investigation of the bark of Ochrosia sandwicensis did not result in the isolation of new and unidentified tertiary bases. Although thin-layer chromatography suggested the presence of such compounds, they occurred in quantities too small for separation and crystallization. The simultaneous great abundance of the known alkaloids ellipticine and methoxyellipticine had a particularly detrimental effect. These compounds exhibited some, but never a high, solubility in most common solvents and were therefore widely distributed thus causing overlapping during numerous attempts of separation by differential solvent treatment, column chromatography and counter-current work. Whenever thin-layer chromatography indicated that certain fractions contained a new base in reasonable purity, the quantities involved were never sufficient to crystallize even minute amounts. Some success related to the tertiary bases was, however, encountered when it could be shown that the earlier isolated yellow alkaloid III\textsuperscript{31,48} was ellipticine hydrochloride and that the unknown base isolated by Buzas et al. from Ochrosia oppositifolia\textsuperscript{13} was methoxyellipticine. A likely position of the methoxyl group in the latter molecule was derived from spectral analogies.

The isolation of the quaternary base hunterburn-nine $\alpha$-methochloride in quantities corresponding to
0.03% of dry bark must be termed a fortunate event for more than one reason. It was indeed fortunate that the alkaloid was detected at all. Its solubility in dilute hydrochloric acid is so low that conventional Mayer's tests in this medium failed. Even after the material, which did not melt on the Kofler block and which was almost entirely insoluble in methanol, had been crystallized, doubt persisted for some time whether it was an alkaloid.

Hunterburnine \( \alpha \)-methochloride and its \( \beta \)-isomer were only recently described as a new class of indole alkaloids and have thus far only been found in Hunteria eburnea. Structure elucidations were accomplished by X-ray crystallographic analyses by the Glasgow group. The bases represent the first pair of \( N(b) \)-epimeric quaternary alkaloids isolated from a natural source. This type of isomerism had been known. \(^{67,68,69}\) It was fully described by Witkop et al., \(^{68,69}\) who prepared the \( N(b) \)-diastereoisomeric methiodides of the previously known yohimbane. \(^{70}\)

An interesting correlation between C/D ring juncture and solubility in methanol was observed. Hunterburnine \( \alpha \)-methochloride and melinonine B chloride, \(^{58}\) both C/D \textit{cis} compounds, are very sparingly soluble in methanol. Of the epimeric yohimbane methiodides, the higher melting one, which must be assumed to contain the C/D \textit{cis} system, is only sparingly, the other one readily soluble in methanol. \(^{68,69}\) There may well be a further
correlation between the unusually low solubility of hunterburnine α-methochloride, even in rather polar solvents, and the fact that no active hydrogen could be detected in the compound by conventional analysis (Bernh.), although three such units are known to be present.

A significant point of the isolation of hunterburnine α-methochloride from Ochrosia sandwicensis lies in the fact that the physiological activity of the crude alkaloid extract of the plant can now be readily explained, at least in part. Various tests have shown that the base possesses a strong hypotensive activity. The importance of the 5-hydroxyl group in the indole system with respect to physiological activity was only recently reported by Gyermek. He investigated a number of derivatives of N,N-dimethyltryptamine and of N,N-dimethyl-5-hydroxytryptamine, also known as bufotenine. The derivatives of bufotenine were found to be 2.4-25 times more potent in their effects than the corresponding unsubstituted compounds. The hydroxyl group, however, is not the only significant criterion and generalizations must be treated with caution, a point which is illustrated by the observation that hunterburnine β-methochloride did not show any activity.

The new base W.J.-3 was isolated in yields representing 0.08% of dry bark. A tentative structure of the compound was proposed, suggesting it to be
10-hydroxydihydrocorynantheol methochloride. Chemical work on the compound was extremely troublesome because of the unusual sensitivity of the molecule.

The information gained during the work on W.J.-3 made it also possible to deduce the stereochemistry of the related alkaloid huntrabrine methochloride.40

Relatively little can be derived from this work concerning botanical correlations.

The earlier reported statement that Ochrosia sandwicensis is closely related to O. elliptica and O. oppositifolia4 can now be supported by the occurrence of methoxyellipticine in all three of these species.

Isolation of isoreserpiline from the leaves of O. sandwicensis33 confirms that this base is widespread within the genus Ochrosia28 and establishes a link with the genus Rauvolfia.14

When Scheuer and Metzger reported on the isolation of holeinine or N(b)-methylisoreserpilinium chloride from O. sandwicensis,31 they emphasized its similarity with melinonine A from Strychnos melinoniana of the family Loganiaceae.32 If the postulated structure for W.J.-3 is correct, it would be closely related to melinonine B isolated from the same Strychnos species,58 and the mentioned connection with the Loganiaceae would gain further support.

Hunterburnine C(-methochloride and W.J.-3 both
establish a new and interesting link between Ochrosia sandwicensis and the African Hunteria eburnea although no identities have been detected between the tertiary bases thus far found in the two plants. 74

Finally, if W.J.-3 is indeed a derivative of dihydrocorynantheol, the close relationship of the genera Ochrosia and Aspidosperma, already manifested by the occurrence of ellipticine in both of them 23 and the fact that Aspidosperma tuberculatum and Ochrosia sandwicensis have appeared as synonyms in the literature, 10 could again be demonstrated. 57, 65, 66
V. BIBLIOGRAPHY


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