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A CONTRIBUTION TO THE STUDY OF THE ALKALOIDS IN THE
GENUS ZANTHOXYLUM (RUTACEAE)

DISSERTATION

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

By

Albert Tewfik Awad, B.Sc., M.Sc.

* * * * * *

The Ohio State University
1966

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6. The Isolation and Identification of Three Alkaloids from the Bark of \textit{Zanthoxylum elephantiasis} Macf., submitted for publication to \textit{J. Pharm. Sci.}
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INTRODUCTION

Many of the plants belonging to the genus *Zanthoxylum*\(^1\) L. have been used medicinally for a variety of ailments, e.g., stomach disorders and toothache. Some have been used as fish poisons and as insecticides.

In the past, the drug Xanthoxylum or Prickly Ash was an article of American aboriginal medicine called Hantola (1). The Western tribes used principally the bark of the root as a decoction for the treatment of various unrelated ailments such as colic, gonorrhea, and rheumatism. In addition, the bark was chewed for relief of toothache and made into a poultice with bear grease to be applied to malignant ulcers and sores. It was reported to be used in a case of uterine fibroid (2) with benefit and the tincture of the berries was used in cases of typhus fever and typhoid pneumonia (3).

As a matter of fact, Prickly Ash had such a good reputation in old medical practice that it was accepted in the Pharmacopoeia of the United States (U.S.P.) (4) in 1830 and remained an official drug until 1916 in the U.S.P. and until as late as 1942 in the National Formulary (5). The official drug was named Xanthoxylum and consisted of the dried bark of *Z. Americanum* Miller, known in Commerce as Northern

---

\(^1\)Other spellings: *Xanthoxylum* Gmel.; *Zanthoxylon* Walter; *Xanthoxylon* Spreng.; *Zantoxilum* Dukamel and *Zanthoxilon* French et Sav. For abbreviations, Z. or X.
Prickly Ash Bark, or of *Z. Clava-Herculis* Linne, known in commerce as Southern Prickly Ash Bark (family Rutaceae).

Although officially classed as an aromatic bitter, Prickly Ash had been used as a drug for other pronounced physiological actions (6). It was mentioned to be both a diaphoretic and irritant diuretic and was regarded as having emmenagogue properties. The berries were used in the same way and were considered more active.

Chemical investigations on some 30 species of *Z.* have been reported in the literature, and the investigations were concerned with constituents in the stem and root barks, woods, leaves and fruits. The principal compounds, so far isolated, represent a wide variety of chemical constituents such as alkaloids, flavonoids, coumarins, insectical olefinic amides, volatile oils, and terpenes.

The first record dealing with the chemical examination of a species of *Zanthoxylum* dates as early as 1326 when Chevalier and Pelletan (7) discovered in the bark of *Z. Clava-Herculis* L. (*Z. carabaeum* Lam.) in the West Indies the first protoberberine alkaloid, berberine, described by them under the name "zanthopicrite". It was Perrins (8), in 1862, who proved that zanthopicrite and berberine, isolated from *Berberis vulgaris*, were identical.

Staples (9), in 1829, isolated from the bark, Northern Prickly Ash, *Z. Americanum* (Mill), a crystalline substance which he named xanthoxyline. This substance was renamed twice, first "xanthoxylin-N" by Gordon (10), in 1906, and then "xanthoyletin" by Dieterle (11),
in 1931. A study of the structure of xanthoxyletin and two other
coumarins, xanthyletin and allo-xanthoxyletin, isolated from the same
plant were published in a series of five papers by Robertson et al.
(12-16).

Since the chewing of various plant parts, especially of the
bark, of many Z. species was known to produce a persisting burning,
a paralyzing and analgesic sensation on the lips and tongue, and since
extracts from some of the same species were reported to possess
insecticidal activities, a great deal of effort has been made through
the years in the isolation of the principles responsible for such
claimed activities. In 1939 Dieterle (17) was able to isolate from
Z. Clava-Herculis L. a compound related to sesamin to which he gave
the name of asarinine. This was found to have a synergistic effect
with the pyrethrins as an insecticide. In 1942 LaForge et al. (18)
reported the presence of an insecticidal principle in the bark of
Z. Clava-Herculis. The principle was similar to pyrethrin in its
action on house flies; and, as a result, a patent (19) was obtained
on the use of the plant (roots, bark, leaves and fruits) as an in-
secticide. Jacobson (20), in 1948, succeeded in isolating this
principle in a pure crystalline state, in a 21 per cent yield based
on dry weight of plant part and gave it the name herculin. Moreover,
he noticed that a trace of herculin, when placed on the tongue, pro-
duced an intense burning paralyzing effect and reported that herculin
had approximately the same order of toxicity to house flies as the
pyrethrins.
In 1950 the Japanese workers Tuto Aihara et al. (21 - 25) published a series of papers on the isolation of two insecticidal pungent principles from Z. piperitum which they named sanshool I (N-isobutyl-2,4,8-dodecatrienamide) and sanshool II (N-isobutyl-2,4,8,10-dodecatrienamide). They also isolated a third amide sanshoamide (N-isobutyl dodeca-(2,4,8)-2hydroxy-tetraenamide) which, unlike the other two, did not possess a strong hot taste or any local anesthetic action.

Since the structure of herculin, (N-isobutyl dodeca-2,8-dienamide), previously reported by Jacobson in 1948, was later disproved by synthetic evidences, L. Crombie (26) in 1954 reinvestigated the bark of Z. Clara-Herculis in an effort to re-isolate herculin and study its chemistry. He, however, failed to isolate herculin but was able to isolate a new compound, neo-herculin, which he considered to be the major insecticidal principle, on the basis that it was shown to be very toxic to house flies. Chemically, neo-herculin is N-isobutyl dodeca-2,6,8,10-tetraenamide.

Among other amides, fagaramide, the alkaloid amide isolated from two species of Z., was reported (27) in 1955 to possess a synergistic effect with pyrethrin. Likewise, fagarol which was reported (28) to be chemically identical with (+)sesamin was shown to possess the property of increasing the toxic effect of pyrethrin to flies.

Recently, in 1963, Bowden and Ross (29) reported the isolation of a local anesthetic principle from the root bark of Fagara
xanthoxyloides, also known as *Z. senegalense* D.C. The isolated substance was shown to be an amide and when applied to the tongue, caused intense fornication followed by local anesthesia. The structure of this amide is N-isobutyl dec-trans 2, trans 4-dienamide, \( \text{CH}_3(\text{CH}_2)_4(\text{CH} = \text{CH})_2\text{CONHCH}_2\text{CH(CH}_3)_2 \).

In a survey of the poisonous plants of Queensland, Webb (30) in 1948, described the toxic effects of *Z. veneficum*, *Z. brachycanthum* and *Z. torvum*. Moreover, reference was made to several other toxic species in various parts of the world. It was obvious from Webb's survey that although the lethality of the mentioned species of *Z.* was mainly due to the alkaloidal content of the plant, yet it could be also attributable to other constituents such as coumarins, pungent principles, saponins, and even essential oils. In 1950 Gotosh (31) reported that the bitter principle sanshotoxin of *Z. piperitum* produced in mice, at a dose of 4 mg./10g., akinesia, abnormal posture, tremor of muscles, repeated clonic and tonic convulsions, opithotonus, apnea, and death.

\( \alpha \)-Fagarine, one of the major alkaloids isolated by Stuckert (32), in 1925, from the leaves and young twigs of *Fagara coco* Engl. (*Z. coco* Gill) and later found to be identical with \( \alpha \)-allocryptopine (33) isolated from *Z. brachycanthum* and *Z. senegalense*, was subjected to intensive pharmacological study and was used clinically for its anti-fibrillatory activity. \( \alpha \)-Fagarine was found to be nearly five times more active than quinidine in both auricular flutter and
fibrillation (34 - 36). It was also reported to be well tolerated by patients; however, the toxic reactions of α-fagarine, unfortunately, were later found to be not predictable, a fact which led to the discontinuation of its clinical use.

In a literature review of antifibrillatory drugs Di Palma (37), in 1950, concluded that quinidine remained the drug of choice in the therapy of ectopic rhythm of the heart. This brought an end to the use of α-fagarine, although hope still remained that a modification in the structure of α-fagarine might result in a compound possessing the desired activity but with less toxicity.

It is, therefore, evident from the foregoing introduction that the genus Z. possesses species which contain a variety of active constituents including alkaloids having marked pharmacological properties. In this respect the genus Zanthoxylum warrants further phytochemical study.
STATEMENT OF PROBLEM

In the search for new medicinal compounds from natural sources, two crude drug samples, representing stem barks of two different species of Zanthoxylum, family Rutaceae, namely, Z. elephantiasis Macf. and Z. martinicense DC. were collected from plants obtained in Jamaica in the summer of 1962.

In testing the plant material for alkaloid content, a positive test was obtained. A preliminary investigation was then carried out to determine the number of alkaloids present as well as the relative quantitative amounts.

Fortunately, the results of the preliminary investigation were promising and were suggestive of a phytochemical study worthy of consideration. Moreover, a literature survey revealed that the alkaloids of the above mentioned Z. species had not been previously studied. It was, therefore, deemed desirable to make a phytochemical investigation of the stem bark of both Z. elephantiasis Macf. and Z. martinicense DC. growing in Jamaica, with a purpose to isolate, separate, and identify as many as possible of their alkaloidal constituents.
Taxonomy of the Genus Zanthoxylum

Zanthoxylum L, also known as Xanthoxylum Gmel (38) is a genus which belongs to the tribe Zanthoxyleae.

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Rutoideae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family</td>
<td>Rutaceae</td>
</tr>
<tr>
<td>Order</td>
<td>Rutales</td>
</tr>
<tr>
<td>Grade</td>
<td>Polypetalae</td>
</tr>
<tr>
<td>Subphylum</td>
<td>Dicotyledonae</td>
</tr>
<tr>
<td>Phylum</td>
<td>Angiosperm</td>
</tr>
</tbody>
</table>

The generic name Zanthoxylum -- derived from the Greek Xanthos = yellow and xylon = wood -- is orthographically correct when spelled Xanthoxylum. Other spellings are: Zanthoxylon Walter; Xanthoxylon Spreng; Zantoxilum Dakamel and Zanthoxilon French et Sav. (39). However, the original spelling of Linnaeus was Zanthoxylum and the International Code of nomenclature recommends the retention of his name (40).

Zanthoxylum L. is a large genus which consists of about 200 species dispersed mostly over the tropical and subtropical regions of the world. It represents one of the 140 genera constituting the fairly large Rutaceae family. The latter consists of about 1,500 species almost cosmopolitan in distribution.

According to Engler and Prantl's classification (41), the Rutaceae family is divided into seven subfamilies. The bulk of the genera and species are found in the following three main subfamilies,
narily, the Rutoideae, the Aurantioideae, and the Toddalioidae. The Flindersioideae subfamily consists of only two genera and the last three subfamilies are monogeneric. Chart I, page 10, shows the outline of Engler and Prantl's classification of the Rutaceae family, as well as some of the names of the genera in each of the main subfamilies.

Botanical Description of the Genus Zanthoxylum

The botanical description of the genus Zanthoxylum L. has been given in many botanical manuals and floras (43-44). The following are some botanical characteristics of diagnostic value:

1. Trees and shrubs often armed with stout prickles.
2. Leaves alternate pinnate; leaflets opposite or alternate, entire or crenate, often oblique and punctate.
3. Inflorescence: cymose usually paniculate.
4. Flowers: small axillary or terminal, white, pink or greenish, often unisexual.
   Calyx, 3-8 fdl, rarely 0.
   Petals, 3-5 fdl, rarely 0 imbricate or induplicate-valvate.
   Male flowers: 3-5 stamens, hypogynous
   Female flowers: stamens none or scale-like,
   carpels 1-5 oblique one-celled, style sublateral, stigma capitate, ovules 2 in each cell usually collateral.
5. Fruit: 1-5 globose coriaceous or fleshy one-seeded carpels, dehiscing ventrally, endocarp horny, separating or not.
6. Seeds: oblong and compressed or globose, often hanging out of the carpels, hilum broad, testa horny or crustaceous blue or black shining.
7. Embryo: straight or curved, in the axile of a fleshy endosperm with round flat foliaceous cotyledons and a very short radicle. (45)
Chart I

1. Barosma Willd  8. Lunasia* Blanco
2. Dictamnus* L.  9. Melicope* Forst
5. Evodia* Forst  12. Ravenia L.
6. Fagara* L.  13. Ruta* Aubl

5. Dictyolomatoideae
   Dictyoloma A Juss
   1. Citrus* L.
   2. Aegle* Correa
   3. Glycosmis* Correa

2. Aurantioidae

Family
Rutaceae

6. Spatheloideae
   Spathelia L.
   1. Achronychia* Forst
   2. Balfoudendron* Mello
   3. Halfordia* F. Mial
   4. Philodendron* Rupr.
   5. Ptelea L.
   6. Toddalia* Juss
   8. Skimmia* Thumb

7. Rhabdodendroideae
   Rhabdodendron Gilg.

4. Flindersioideae
   1. Flindersia* R. Br.
   2. Chloroxylon* DC.

Diagram of the Engler and Prantl's Classification of the Rutaceae family showing some important genera

*Genera containing alkaloids (42).
The Taxonomic Problem of the Genus Zanthoxylum L.

A review of the literature pertaining to the taxonomy and geographical distribution of the genus Zanthoxylum revealed the fact that a botanical synonymy between the members of the genus Z. and those of the closely related genus Fagara L. has been widely adopted, the majority of Z. species being alternatively named under F. and vice versa.

Linnaeus (46) who established the genus Z. and the genus F. in 1753 and 1759, respectively, has described the former as having only one perianth whorl, the calyx, and he described the latter as having two perianth whorls. Engler (41), however, in 1895 transferred a large number of species from the genus Z. to the genus F., thus creating a botanical nomenclatural confusion which still exists.

In an article pertaining to the discussion of the nomenclature of the genus Zanthoxylum Fosberg2 in 1958 stated the following:

Seldom has failure to ascertain fully the facts in a case led to so much wasted time, efforts, and printers ink as have the efforts to place Fagara L. and "Zanthoxylum Mill" on the list of Nomina Conservanda. Five articles have been published on this and the time of two international nomenclature committees has been taken up with it. . . .

As I see it we have one taxon, Zanthoxylum, proposed by Linnaeus, in 1753-54, with Z. clava-herculis as type. Then in 1759 he described another taxon, Fagara, based on F. pterota. Nomenclaturally, these are two separate and distinct entities (taxa), even though in the opinion of most, if not all botanists, they are to be united taxonomically.

(46)

2 Member of the International Nomenclature Committee for Phanerogam (Falls Church, Virginia).
In his second article entitled "Typification of Zanthoxylum L."

Fosberg, in 1959, concluded the situation regarding the names Zanthoxylum and Fagara as follows:

For those who combine the genera with one and two perianth whorls the correct name is Zanthoxylum L. For those who separate them the tropical genus with two whorls has the correct name Fagara L. (nomen conservandum) with F. pterota L. as type and the temperate genus with one perianth whorl still bears the name Zanthoxylum L. typified by the plant now called Z. fraxineum Willd. (47)

Recently, in 1963, Price (48) in Australia attacked the problem from a pure chemotaxonomic approach. He studied the alkaloid pattern in both genera to see whether some light might be shed on the existing nomenclature problem, and thereby, might help to distinguish between the members of both genera. In conclusion, Price pointed out the need for acquiring more chemical data before any far-reaching solution could be found. However, he drew attention to the fact that the data available to him from twenty species fell into two groups according to whether furoquinoline alkaloids are or are not present.

From what has been said before, it is evident that the only hope, at least at the present time, for the solution of this old and difficult problem, remains in the hands of chemotaxonomists. The taxonomic problem will then be solved when most of the remaining available species of both genera Z. and F. are subjected to chemical study.
Geographical Distribution of the Genus Zanthoxylum L.

Gleason, in 1952 (49), stated that about 50 species of Z. occur in the United States of America. However, he only described the two well-known American species, namely, Z. Americanum Miller and Z. Clava-Herculis L. The former is found in the rocky woods and along the streams from Quebec to Minnesota, south to Virginia, Kentucky, Missouri, and Kansas. On the other hand, Clava-Herculis L. is found in dry soil along the sandy coast from Virginia to Florida and west to Texas and north to Arkansas.

In Australia, Bailey (50) described four Z. species; and in British India, Hooker (45) listed eleven species. In China, Shun-Ching Le (51) divided the members of the genus Z. into two groups according to whether they are trees or small shrubs and climbers. In the former group he described six species and in the latter he mentioned thirty-three. In Japan, published work indicates the presence of at least six different species of Z. In west tropical Africa, Hutchinson and Dalziel (52) listed twelve species under the generic name of Fagara. In South Africa, Palmer (53) described two species of Fagara, and in Kenya, Dale and Greenway (54) in 1961 stated the names of six species under Fagara. A taxonomical survey of the genus Fagara in Argentine was published by Escalante (55) in 1961, indicating the presence of eleven species. Most of them have Z. as synonyms. Notes on the Hawaiian species of Fagara L. were recently published in the American Journal of Botany. In these notes, Sherff (56) described six species
and stated that they were all formerly transferred by Engler from the
genus Z.

In regard to the flora of Jamaica, Fawcett and Rendle (57) described thirteen species of *Zanthoxylum*; moreover, they gave the following relatively simple key for the identification and differentiation among these different species.

**TABLE 1**

**KEY FOR THE IDENTIFICATION AND DIFFERENTIATION OF THE SPECIES OF Z. GROWING IN JAMAICA**

<table>
<thead>
<tr>
<th>Petiole and Rachis of Leaves</th>
<th>Not Winged</th>
<th>More or Less Winged</th>
<th>Grooved but Not Winged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (I)</td>
<td></td>
<td>Group (II)</td>
<td>Group (III)</td>
</tr>
<tr>
<td>1. Z. <em>elephantiasis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Z. <em>martinicense</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Z. <em>flavum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Z. <em>insulare</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Z. <em>Fagara</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Z. <em>spinifex</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Z. <em>rhodoxylon</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Z. <em>trifoliatum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Z. <em>Hart</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Z. <em>jamaicense</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Z. <em>spinosum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Z. <em>negrilense</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. Z. <em>acuminatum</em></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: *Flora of Jamaica* (57)

Further distinction between the members of each group is based on other characteristics such as

1. The shape, size and arrangement of leaflets.
2. The distribution of the pellucid dots (glands) on the lamina.
3. The type of inflorescence.
4. The presence or absence of non-glandular hair and their shape.
5. The number of floral parts 3, 4 or 5.
6. The number of cocci -- the fruit 1, 2 or 3.
For example, the following characters were used to distinguish among the members of group (I), two of which are the subjects of this dissertation, namely, *Z. elephantiasis* Macf. and *Z. martinicense* DC.

1. **Leaflets with many pellucid dots**
   - Twigs, leaves, inflorescence glabrous
   - Twigs, petioles, inflorescence puberulous with simple hairs

2. **Leaflets with very many pellucid dots**
   - Twigs, petioles, rachis of inflorescence with minute star-shaped hairs

3. **Z. flavum**

**Description of Z. elephantiasis Macf.**

*Z. elephantiasis* Macf. also known as *Z. aromaticum* DC.; *F. elephantiasis* Kr. and Urb. is a species of the genus *Zanthoxylum* which is found in Jamaica, Cuba, Hispaniola, Mexico and Costa Rica.

*Z. elephantiasis* Macf. is a tree 15-40 ft. high, with corky conical spiny knobs, if present, only at the base of the trunk. Branches without spines. Leaves with an odd leaflet, 1-2.5 dm. long; leaflets in 5-6 (2-8) pairs, opposite or nearly so, ovate-elliptical or oblong-lanceolate, somewhat acuminate, base more or less unequal-sided, 5-10 cm. long; petiolules 3-6 long.

Panicles with corky wrinkled rachis. Pedicels 1-2.5 mm. long, increasing to 5 mm. in fruit. Sepals 5, imbricate in bud, roundish, leathery, not corky, 1.3-1.8 mm. long; 1.5-2 mm. broad.

Petals 5, 5-6 mm. l., elliptical or elliptical oblong. Male flowers: filaments 5, about as long as the petals; ovary rudimentary.

Fruit cocci with glandular dots forming tubercles, usually 5, obovate or subobliquely or biculate, 6-8 mm. l.; stalks 3-8 mm. l.; diverging star-like from one another. Seeds nearly as long as the cocci. (57)
Description of *Z. martinicense* DC. (Prickly Yellow, Yellow Hercules)

Also known as *Z. foliis oblongo-ovatis* Browne
*Z. Clava-Herculis* SW.
*Z. Caribaeum* Hichc.
*Fagara martinicensis* Lam.

Found in West Indies

Tree 20-40 (-80) ft. high; trunk to 3 ft. and more in diam., with numerous corky conical spiny knobs (to 1.5 cm. long and brown); twigs with or without prickles (2-4 mm. l.).

Leaves 1-3 dm. l., with or without an odd leaflet; petiole, rachis and midrib puberulous, with or without small prickles in the rachis and on the midrib of the leaflets beneath; leaflets variable in number, in 4-7 (-15) pairs (young shoots with more leaflets than those more mature), opposite or alternate, sessile or subsessile, oblong elliptical or oblong-lanceolate or oblanceolate, apex rounded, sometimes obtuse to shortly acuminate, base unequal-sided, 5-10 (3-13) cm. long.

Panicles terminal, rarely lateral. Flowers subsessile. Sepals 5, open-bud, triangular, membraneous, about 0.5 mm. long. Petals 5, 1.5-2 mm. l., elliptical.

Male flowers: filaments 5, about half as long again as the petals.

Fruit: cocci glandular-dotted, obliquely ovate to orbicular about 5 mm. l., seeds about 4 mm. l.

The wood is of a light yellow color and even grain, saws readily straight, is useful in furniture and inside work but does not last when exposed. (57)

Alkaloids in the Genus *Zanthoxylum* L.

The genus *Z.* is one of the 36 genera of the family Rutaceae (total 140 genera) which has received a great deal of chemical study. From a purely chemical viewpoint, *Z.* is a fascinating genus because with respect to its constituents it is probably one of the most versatile and heterogenous genera. This is evident not only in the capacity of producing different chemical constituents such as
alkaloids, flavonoids, coumarins, amides, volatile oils, and terpenes, but also in that its alkaloids may be considered as representing five different structural classes, or at least four, from a biogenetic point of view.

Because of the taxonomical nomenclatural confusion between the members of both genera \( Z \) and \( F \), a survey of the chemistry of \( Z \) would normally be expected to encompass that of \( F \).

The present survey deals essentially with the alkaloids isolated from all the studied species of both genera \( Z \) and \( F \), and includes references and some chemical and physical properties worthy of consideration. The presentation of the data at the end of the survey, in tabular form, was meant to make this part more useful as a reference work.

For the purpose of this survey, the 30 different alkaloids, isolated and fully characterized, can be classified under the following groupings:

1. Furoquinoline alkaloids.
2. Canthinone alkaloids.
3. Quinazolino-carboline alkaloids.
5. Simple aromatic amines and amide alkaloids.

Those alkaloids for which the structure has not been elucidated are listed in Table 3, page 43 under uncharacterized alkaloids.
1. **Furoquinoline Alkaloids**

The furoquinoline alkaloids are found exclusively in the family Rutaceae, as no report of their isolation from outside this family is available. In fact, they are so widespread within the members of the Rutaceae that their occurrence has been reported in 29 of the 36 genera in which alkaloids have been identified (58).

Generally speaking, the alkaloids of this group tend to crystallize easily, and they are colorless except for skimmianine, which is pale yellow.

In the genus *Z.* and *F.*, only the three closely related simple linear tricyclic furoquinoline alkaloids, dictamnine (I), skimmianine (II), and γ-fagarine (III) have been isolated from the investigated species (Table 3, p. 43); skimmianine being the most widespread member of the three.

```
I. R = R' = H  Dictamnine
II  R = R' = OCH₃  Skimmianine
III R = H, R' = OCH₃  γ-Fagarine
```

Important clues to the structural features of this group of alkaloids can be illustrated using dictamnine as a typical example, in the following three different ways.

a) The unusual behavior of the furoquinoline alkaloids toward alkyl halides

Dictamnine (I) is unaffected by treatment with boiling methyl iodide, but when heated with this reagent in a sealed tube at 80° for
four hours, it is converted into an isomeric substance isodictamnine (IV) which contains a methylimino group but no methoxyl (59); moreover, the ultraviolet spectrum will show a carbonyl absorption peak.

b) Demethylation and remethylation

The methoxy group in dictamnine can be demethylated by either acid or alkaline hydrolysis to give nor-dictamnine (V), the latter upon methylation with dimethyl sulfate is converted to isodictamnine (IV), but with diazomethane a mixture of dictamnine and isodictamnine is produced.

c) Hydrogenolysis of the double bond in the furan ring

Instead of oxidation of the double bond in the furan ring to give an acid (VI), an increasing use is being made of hydrogenolysis which has the advantage of requiring less material.

The hydrogenolysis of dictamnine over palladium gives dihydro-
dictamnine, whose ultraviolet spectrum resembles that of 2,4-
dimethoxyquinoline (60). With platinum oxide as catalyst, however, hydrogenolysis occurs with the formation of 3-ethyl-4-methoxy-2-
quinolone (VII), the identity of which was established by methylating
synthetic 3-ethyl-4-hydroxy-2-quinolone with diazomethane, and this confirmed the linearity of the dictamine molecule (61).

![Chemical structures](image)

The spectra of furoquinoline alkaloids, in general, show two maxima, one at 245 μm and the other a very broad complex band in the region 290-330 μm. They absorb at longer wave length than the simple quinoline alkaloids due to the presence of a double bond in the furan ring conjugated with that of the quinoline. In fact, the spectra of the dihydro-furquinolines are quite similar to the quinolines, with few exceptions, which show very high absorbance values not consistent with the substituted quinoline ring system (62).

2. 6-Canthinone Alkaloids

A recent authoritative reference (63) has mentioned this class of alkaloids under the title of "Pentaceras Alkaloids," after the plant *Pentaceras Australis* Hook, Rutaceae, from which the first three members, namely, 6-canthinone (VIII), 5-methoxy-6-canthinone (IX), and 4-thiomethyl-6-canthinone (XI), were isolated (64–66). These alkaloids are also considered 6-carboline alkaloids since the structure of 6-canthinone was readily derived when with permanganate
oxidation it was found to give \( \beta \)-carboline-1-carboxylic acid (XI).

\[
\begin{align*}
\text{VIII} & \quad R = R' = H & 6\text{-canthinone} \\
\text{IX} & \quad (R = \text{OCH}_3, R' = \text{H}) & 5\text{-methoxy-6-carthinone} \\
\text{X} & \quad (R = \text{H}, R' = \text{SCH}_3) & \text{4-thiomethyl-6-carthinone}
\end{align*}
\]

\[
\begin{align*}
\text{XI} & \quad \text{COOH} \\
\text{XII} & \\
\end{align*}
\]

A preferred naming of this group of alkaloids would be 6-carthinone or simply canthinone alkaloids due to the fact that they can be considered as derivatives of the base canthine (XII) and also because they are no longer confined to the genus Pentaceras and, in fact, were found in additional families. There are five closely related canthinones isolated from the plant kingdom, and these are shown in Table 2.
### TABLE 2

**6-CANTHINONE ALKALOIDS ISOLATED FROM PLANTS**

<table>
<thead>
<tr>
<th>Alkaloid</th>
<th>Plant</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 6-Canthinone . . .</td>
<td>from <em>Pentaceras Australis</em></td>
<td>Rutaceae (64)</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>from <em>Z. suberosum</em></td>
<td>Rutaceae (67)</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>from <em>Z. elephantiasis</em></td>
<td>Rutaceae ( )</td>
</tr>
<tr>
<td>2. 5-methoxy-6-canthinone . . .</td>
<td>from <em>Pentaceras Australis</em></td>
<td>Rutaceae (65)</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>from <em>Z. elephantiasis</em></td>
<td>Rutaceae ( )</td>
</tr>
<tr>
<td>3. 4-thiomethyl-6-canthinone . .</td>
<td><em>Pentaceras Australis</em></td>
<td>Rutaceae (66)</td>
</tr>
<tr>
<td>4. 4,5-dimethoxy-6-canthinone . .</td>
<td><em>Picrosma ailanthoides</em></td>
<td>Simaroubaceae (68)</td>
</tr>
<tr>
<td>5. 4-methoxy-6-canthinone . . .</td>
<td><em>Charpentiera obovata</em></td>
<td>Amaranaceae (69)</td>
</tr>
</tbody>
</table>

One of the important features which helps greatly in the identification of this group of alkaloids is that they possess very characteristic ultraviolet spectra which resemble that of the carbazole (XIII) analogues. Moreover, boiling with aqueous ethanolic alkali leads to the opening of the lactam ring with formation of the corresponding cis acid, and the latter under mild aqueous alkaline condition, can be made to reclose to the parent lactam. However, prolonged heating with alkali will result in formation of the trans acid; the latter could not be made to recylize.
3. **Quinazolino-Carboline Alkaloids**

As the name indicates, the quinazolino-carboline alkaloids contain both carboline and quinazoline nuclei and are also known under the name of Indoloquinazoline alkaloids.

The first known representatives of this group were the alkaloids rutaecarpine and evodiamine (XIV) isolated from several species of *Evodia* and *Hortia* (Rutaceae). Recently, rhetsine, identical with evodiamine, was reported in the genus *Zanthoxylum* (70). Therefore, the quinazolino-carboline alkaloids appear to be not only exclusive to the Rutaceae family but are, in fact, restricted to only a few of its genera. From the bark of *Z. rhetsa* (71), the alkaloid rhetsine was isolated, together with another tertiary base rhetsinine which was considered as being identical with the product of the mild permanganate oxidation of evodiamine (XIV), namely, 2-hydroxyevodiamine (XVI). The latter structure was confirmed by degradative experiments and by its synthesis from evodiamine (72) and from 2-keto-1,2,3,4-tetrahydro-α-carboline and methyl N-methylanthranilate according to the method of Pachter and Suld (73). Pachter and Suld disproved the proposal of structure (XV) for rhetsinine and proposed structure (XVI) for it. Their argument was based on the fact that the infrared spectrum of rhetsinine showed a twin peak at 5.94 and 6.00 μ indicating the presence of two conjugated carbonyls in the molecule as in structure (XVI). However, Pachter and Suld disregarded in their argument the presence of an -OH band in the infrared and other important chemical
Figure 1

Quinazolino-Carboline Alkaloids in the Genera *Zanthoxylum* and *Fagara*

XIV Evodiamine or rhetsine

XV Hydroxyevodiamine

XVI Rhetsinine
Figure 1—Continued

Ketonic Form\textsuperscript{a}  
XVI. Rhetsinine

Ammonium Form\textsuperscript{b}

\textsuperscript{a,b} Tautomers of Hydroxyevodiamine
behaviors of rhetsinine, which were referred to in a paper by Chatterjee and Mukherjee, in 1964, when they reported the isolation of the same alkaloid from the stem-bark of *Z. oxyphillum* Edgw (74). The compound isolated from *Z. oxyphillum* showed the same ultraviolet, infrared and X-ray diffraction patterns as rhetsinine isolated from *Z. rhetsa*. The infrared spectrum showed characteristic peaks for bonded -OH (2.92 u) for -NH (3.0 u) and for the two conjugated carbonyls.

Moreover, the yellow base rhetsinine was found to turn red on drying in vacuo. This red compound was found to be the anhydronium base of rhetsinine, i.e., dehydroevodiamine (XVII), which was isolated from the leaves of *Evodia rutascarpa* Hook and Thomas by Nakasato et al. in 1962 (75).

Based on some important chemical behavior and physical data Chatterjee et al. proved that the structure of rhetsinine (XV), as proposed by them earlier, was indeed correct and that the Pachter et al. proposal was not justified. Chatterjee et al. stated that structure (XVI) is only a tautomeric form of the base rhetsinine (XV) which is a carbinol-amine capable of existing in two other
tautomeric forms, viz., carbonyl ketonic form and ammonium form. The latter form has been recognized during the synthesis of rhetsinine.

4. Benzylisoquinoline Alkaloids

Although the ability to synthesize and accumulate benzylisoquinoline alkaloids is considered to be a constant feature in the family Papaveraceae of the order Rhoeadales, and in several families of the order Polycarpicae such as Anonaceae, Aristolochiaceae, Berberidaceae, Lauraceae, Magnoliaceae, Monimiaceae, Nymphaeaceae, and Ranunculaceae, yet representative of such compounds were isolated from the far distant and quite unrelated Rutaceae family which belongs to the order Rutales. From a chemotaxonomic point of view, the presence of benzylisoquinoline alkaloids in the Rutaceae family is not easy to interpret; however, it should be of great value in characterizing and distinguishing this fairly large family from others, such as the Malaceae, Zygophyllaceae, Burseraceae, and Simarubaceae, which are phylogenetically considered closely related to the Rutaceae and are, at least currently, devoid of benzylisoquinoline alkaloids.

The benzylisoquinoline alkaloids can be described under several sub-groupings but those alkaloids which have been isolated from the genus Z. or F. fall into four categories:

A -- Aporphine alkaloids
B -- Protopine alkaloids
C -- Protoberberine alkaloids
D -- Benzophenanthridine alkaloids
Moreover, recently, one simple benzylisoquinoline alkaloid, namely, (+)-tembetarine (N-methyl reticuline) (XV), was isolated in the form of its chloride salt from five species of Fagara growing in Argentine (76). The oxidative condensation of this alkaloid with ferric chloride in aqueous solution following the method of Franck and Schlingloff (77) produced (+)-laurifoline chloride (XIX) in 3 percent yield. The existence of a genetic relationship between (+)-tembetarine and (+)-laurifoline in the plants remains to be tested.

A) Aporphine alkaloids

In the genera Z. and F. six aporphine alkaloids have been isolated, all of which were quaternary alkaloids. During the past ten years Japanese investigators have made substantial contributions in the isolation, characterization, and synthesis of several new aporphine alkaloids, three of which belong to the genus Z.
### Aporphine Alkaloids in the Genera *Zanthoxylum* and *Fagara*

![Chemical structures](image)

<table>
<thead>
<tr>
<th>Alkaloids</th>
<th>1</th>
<th>2</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>XIX Laurifoline</td>
<td>-OH</td>
<td>-OCH₃</td>
<td>-OH</td>
<td>-OCH₃</td>
<td>--</td>
</tr>
<tr>
<td>XX Magnoflorine</td>
<td>-OH</td>
<td>-OCH₃</td>
<td>--</td>
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<td>-OH</td>
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<tr>
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<td>-OH</td>
<td>-OCH₃</td>
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<tr>
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<td>-OCH₃</td>
<td>--</td>
<td>-OCH₃</td>
<td>-OH</td>
</tr>
<tr>
<td>XXIII N-Methyl-corydine</td>
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<td>-OCH₃</td>
<td>--</td>
<td>-OCH₃</td>
<td>-OCH₃</td>
</tr>
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</table>

**Fagara tinguassoiba alkaloid**

<table>
<thead>
<tr>
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<td>XXIVa old structure</td>
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<td>-OH</td>
<td>-OCH₃</td>
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<tr>
<td>XXIVb new structure</td>
<td>-OH</td>
<td>-OCH₃</td>
<td>-OCH₃</td>
<td>-OCH₃</td>
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</tbody>
</table>
Laurifoline (XIX), the first quaternary aporphine to be isolated from nature, was obtained in 1953, by Tomita and Kusuda (78) from Cocculus laurifolius (Menispermaceae). In 1958, Tomita and Ishii (79) reported the isolation of laurifoline from the bark of Z. ailanthoides. Recently, laurifoline was isolated from P. pterota also known as Z. pterota (76) growing in Argentine. The isolation of laurifoline, in this laboratory, from the bark of Z. elephantiasis Macf. marks the fourth occurrence of laurifoline in the plant kingdom and the third in the genus Z.

Magnoflorine (XX), a quaternary alkaloid, is isomeric with laurifoline. It was first isolated in 1954 by Nakano (80) from Magnolia grandifolia and in 1957 by Tomita and Ishii (81) from two species of Z., namely, Z. piperitum (81) and Z. ailanthoides. In 1961, Ishii and Harada (82) isolated magnoflorine from Z. planispium = Z. alata Roxb var. planispium.

Xanthoplanine (XXI) corresponds to the quaternary alkaloid derived from N-methyl laurotetanine (N,N-dimethyl laurotetamine salt). It was isolated together with magnoflorine from Z. planispium (82). The alkaloid has one hydroxyl and 3 methoxyl groups. When treated with methyl iodide and base, it gave 0-methyl xanthoplanine iodide which was identical to the 0,0-dimethyl laurifoline iodide both in melting point and infrared spectrum. This confirmed the 1,2,9,10 substitution pattern in xanthoplanine. The position of the free hydroxyl group in xanthoplanine was settled to be at C-9 by treatment
of xanthoplanine chloride with ethyl iodide. The infrared absorption
spectrum of the resulting ethyl ether was found to be superimposable
with that of 9-O-ethyl-N-methyl laurotetamine methiodide.

N-methyl corydine -- A quaternary alkaloid very recently iso-
lated for the first time by Albonico et al. (76) in 1966 from the
bark of F. nigrescens Fries, growing in Argentina. It is considered
a new phenolic quaternary alkaloid, although its isomer, the N-methyl
isocorydine, has been reported from several plants. N-methyl corydine
(XXIII) fails to give a positive Gibb's Test, whereas its isomer gives
a brilliant blue color with the Gibb's reagent 2,6-dibromoquinone
chlorimide.

N-methyl isocorydine (XXIII) (Menispermine) -- A quaternary
aporphine alkaloid reported in 1954 to be a major alkaloid in the bark
of F. Coco by Comin and Deulofeu (83). It was also found in the bark
of both Z. brachycanthum and Z. veneficum growing in Australia (67).
The iodide of N-methyl isocorydine did not depress the melting point
when admixed with the methiodide salt of isocorydine and also gave an
identical infrared spectrum.

Quaternary aporphine from F. tinguassoiba -- A quaternary
alkaloid isolated by Marion et al. in 1961 (84), and was reported to
possess one phenolic and three methoxyl groups. The phenolic hydroxyl
was assigned the C-2 position (XXIVa). The ultraviolet spectrum of
its chloride salt was reported to be nearly identical with that of
glaucine. O-methylation of the alkaloid iodide gave N-methyl
glaucine iodide, thus proving that the oxygenated positions were at C-1,2,9 and 10.

Recently some revisions in the structural assignments for a number of 1,2,9,10 tetraoxygenated aporphine alkaloids were proposed by Tschersche et al. (85). These revisions included, among other alkaloids, the quaternary base from F. tinguassoiba. For the new proposed structure (XXIVb), the phenolic group was assigned at C-1 instead of C-2 position.

Schamma and Slusarchyk (86) recently synthesized the alkaloid of proposed structure (XXIVb), and they showed that it was completely identical with the quaternary alkaloid of F. tinguassoiba. Therefore, the newly proposed structure has been proved to be correct. In addition, Baarscher and Pachter (87), in one of their reports concerning the n.m.r. study of aporphine alkaloids, confirmed the revised structure of the F. tinguassoiba alkaloid (XXIVb).

It is worth mentioning here that among the structure revisions of certain aporphines proposed by Tschersche et al. (85) was the alkaloid glaucentrine. Shamma and Slusarchyk (86), however, not only disagreed with Tschersche for his newly proposed structure for glaucentrine but also claimed that natural glaucentrine corresponded to the alkaloid corydine and concluded that "all 1,2,9,10-tetraoxygenated aporphines whose structures were elucidated by comparison with samples of natural glaucentrine should now have their structures reconsidered."
B) Protopine alkaloids

The protopine alkaloids form a natural group which are characterized by the presence of a 10-membered N-hetero-ring containing one carbonyl function. They are found predominantly in the Papaveraceae family. (93).

In the Rutaceae, the protopine alkaloids have been found only in the genera Z. and F. Jowett and Pyman in 1913 (88) were the first to isolate a protopine alkaloid from outside the Papaveraceae. From the bark of Z. brachycanthonum, they isolated, among other alkaloids, a small amount of a base which chemically and physiologically was found to be in close agreement with \( \alpha \)- and \( \beta \)-homochelidonine isolated from Chelidonium majus and Sanguinaria canadensis. To this base they gave the name of \( \gamma \)-homochelidonine. However, this naming is no longer tenable and the now accepted name of allocryptopine was suggested by Gadamer (89) to replace homochelidonine. Like protopine, allocryptopine occurs in two allotropic modifications, an \( \alpha \)-form which melts at 160\(^\circ\) and a \( \beta \)-form melting at 170\(^\circ\).

Stuckert, in 1925, reported the isolation of three crystalline alkaloids, from F. coco, which he named \( \alpha \)-, \( \beta \)-, \( \gamma \)-fagarines. The \( \beta \)- and \( \gamma \)-fagarines were identified as furoquinoline alkaloids (113), whereas \( \alpha \)-fagarine behaved differently and its structure remained unidentified for many years. However, when \( \alpha \)-fagarine was reported to be a superior substitute for quinidine in controlling cardiac arrhythmia (35), its structure became of great interest. It
was Redeman et al. (33), in 1949, who reinvestigated the alkaloids of F. coco and proved the identity of \( \alpha \)-fagarine with \( \alpha \)-allocryptopine (XXV). They, moreover, isolated two other related alkaloids, one of which, Fagarine II (XXVI), was shown to be isomeric with \( \alpha \)-fagarine, differing only in the position of the substituents. The structure of the second alkaloid, Fagarine III, is still undetermined.

The ultraviolet spectra of the protopine alkaloids show usually one maximum at 286-293 nm and a shoulder at or near 240 nm. It has been shown that bonding of the type \( \text{CH}_3 \text{N} \ldots \text{CO} \) strongly influences the carbonyl absorption frequency in the infrared spectrum (90) and is expected to influence the ultraviolet pattern of the bases. For structure elucidation the mercuric acetate oxidations in conjunction with ultraviolet spectral studies are of some diagnostic value. The product of oxidation would be the oxyalkaloid, a 13-keto derivative (XXVII), the ultraviolet of which would shift to a longer wave length (91).

C) Protoberberine alkaloids

This group of alkaloids is represented in the genus \( Z. \) by a very few, but well-known alkaloids, namely berberine (XXVIII), tetrahydroberberine (XXIX) or canadine, and 1-\( \alpha \)-N-methyl canadine chloride (XXX). In fact, the first protoberberine alkaloid isolated from a natural source was berberine which was found in the bark of \( Z. \) clava-herculis L. Nowadays, berberine is considered to be one of the most widespread alkaloids in the plant kingdom; its presence has been
Figure 3
Protopine Alkaloids in the Genera Zanthoxylum and Pagarar

XXV $\alpha$-Allocryptopine (\(\alpha\)-Fagarine)

XXVI Fagarine II

XXVII 13-keto-protopine derivative (Oxyprotopine)
Figure 4
Protoberberine Alkaloids in the Genera
*Zanthoxylum* and *Fagara*

XXVIII Berberine

XXIX Canadine

XXX N-methyl-canadine chloride
recorded in 89 species, representing 26 genera from 7 families (92). In the genus Z., berberine was reported to be present in nine species (see Table 3).

Canadine was isolated from Z. brachyspermum and Z. venenatum and occurred with the quaternary salt 1-α-N-methyl canadine chloride in the former species (88). Canadine is very easily oxidized, even by atmospheric oxygen, to give berberine, a fact, which put some doubt about berberine existing as a natural constituent in plants which synthesize canadine. However, there are many occurrences of berberine unaccompanied by canadine and vice versa, particularly, in plants of the Berberidaceae and Menispermaceae.

The characterization of 1-α-N-methyl canadine chloride (XXX) isolated by Jowett and Pyman (88) was done by direct comparison with an authentic sample prepared from the 1-canadine base. Fortunately, the prepared stereoisomers α- and β-methochloride salts were easily separated due to their different solubility behavior in water. The β-form, being sparingly soluble in water, was obtained first by simple crystallization; the mother liquid was then evaporated to dryness and the residue crystallized from absolute alcohol to give the α-isomer. Jowett and Pyman considered this to be the first record of the isolation from a plant source of a substance containing an asymmetric nitrogen atom. As they expected, each of the stereochemical configurations had a relatively different physiological behavior, as is the case with compounds with asymmetric carbon atoms such as hyoscyamine, adrenaline, and many others.
D) Benzophenanthidine alkaloids

The benzophenanthidine, also known as \( \alpha \)-naphthaphenanthidine, alkaloids, are derived from the tetracyclic system (XXXI), in which the terminal nuclei are fully aromatic and each carries at least two alkoxyl or one methylenedioxy group. The two central rings are either aromatic or fully reduced, but the nitrogen always has a methyl group.

\[
XXXI
\]

According to Manske (93), the occurrence of benzophenanthidine alkaloids such as chelidonine, sanguinarine (XXXIV) and chelerythrine (XXXV) were restricted to the family Papaveraceae, until 1956, when dihydrotoddaline was isolated from the root bark of *Toddalia aculeata* Pers (94) of the family Rutaceae, and found to be identical with dihydrochelerythrine. However, the first record of a benzophenanthidine alkaloid isolated from a family other than Papaveraceae should be accredited to Ritchie et al. (67) in Australia who in 1953 reported the isolation of chelerythrine from the bark of both *Z. brachyanthum* and *Z. veneficum*. Since then, six other species of *Z.* have been reported to contain benzophenanthidine alkaloids.

In 1959 Arthur et al. (95), in Hong Kong, reported the isolation and characterization of the new benzophenanthidine alkaloids, nitidine (XXXIII) and oxynitidine (XXXIX), from the root bark of *Z.*
Figure 5

Benzophenanthridine Alkaloids in the Genera
Zanthoxylum and Fagara

XXXII Avicine \( R_1 + R_2 = \text{CH}_2 \)

XXXIII Nitidine \( R_1 = R_2 = \text{CH}_3 \)

XXXIV Sanguinarine \( R_1 + R_2 = \text{CH}_2 \)

XXXV Chelerythrine \( R_1 = R_2 = \text{CH}_3 \)

XXXVI Dihydroavicine
\[ X = \text{H}_2 \quad R_1 + R_2 = \text{CH}_2 \]

XXXVII Oxyavicine
\[ X = 0 \quad R_1 + R_2 = \text{CH}_2 \]

XXXVIII Dihydronitidine
\[ X = \text{H}_2 \quad R_1 = R_2 = \text{CH}_3 \]

XXXIX Oxynitidine
\[ X = 0 \quad R_1 = R_2 = \text{CH}_3 \]
nitidum, and avicine (XXXII) from the root bark of Z. avicennae (96). They also reported the second isolation of nitidine from the bark of Z. cuspidatum in 1961 (97).

From the bark of Z. rhetsa, Chatterjee et al. (71), in 1959, reported the isolation of chelerythrine (XXXV); and, in 1962, chelerythrine and dihydrochelerythrine were reported to be the alkaloidal constituents of the bark of F. semiarticulata growing in Hawaii (98). In 1963 the third occurrence of nitidine in the genus Z. was reported by a group of Indian investigators (99) working with the bark of Z. hamiltonianum. Recently, chelerythrine and nitidine were found in several species of Fagara growing in Argentine (12).

It is worth mentioning that most of the benzophenanthridine alkaloids were isolated in the form of quaternary salts, since as free bases they are very unstable. They undergo a rapid disproportionation reaction in alkaline medium, giving a mixture of tertiary bases, the dihydro- and the oxybenzophenanthridine, respectively.

Furthermore, the dihydro- bases are easily oxidized in the air to the yellow oxybases; the latter do not form salts since oxygen is part of a substituted amide function. The oxybases, however, can be chemically reduced in an inert atmosphere (argon) to the dihydro-bases which react to give salts.

5. Simple Amine and Amide Alkaloids

Alkaloids of this type are mostly substituted $\beta$-phenethylamines (100), evidently related to and probably derived from the
naturally occurring aromatic amino acids. It will, therefore, be quite possible to find bases of the same type in plants of widely separated botanical families or even in products of animal metabolism. Reti and others (100), however, seem to indicate that the faculty of producing and storing simple amine alkaloids such as hordenine, candidine, caryneine and mescaline should be considered one of the characteristics of the Cactaceae family.

In the Rutaceae family, there have been several instances of isolation of simple amine alkaloids, such as nor-adrenaline, tyramine and tryptamine in *Citrus aurantium* (101), candidine in *Phellodendron amurense* (101), and aegeline in *Aegle marmelos* (102).

In the general Z. and F., in addition to the occurrence of aliphatic amides, such as herculin, neo-herculin and sanshoil I and II, the following two alkaloid amides, herclavin (0-methyltyramine-N-methylcinnamide) (XL) and fagaramide (XLI), have been reported. In this laboratory, the quaternary alkaloid, candidine chloride (XLII), was isolated from the bark of *Z. martinicense* DC., denoting the first occurrence of a simple alkaloid amine in the genus *Zanthoxylum*. 
Figure 6

Simple Amine and Amide Alkaloids in the Genera *Zanthoxylum* and *Fagara*

![Chemical structures](image)

**XL** Herclavin

**XLI** Fagaramide

**XLII** $R = H$ Candicine chloride

**XLIII** $R = OH$ Coryneine chloride
### TABLE 3

**ZANTHOXYLUM AND FAGARA SPECIES STUDIED AND THEIR CONTAINED ALKALOIDS**

<table>
<thead>
<tr>
<th>No.</th>
<th>Plant Name and Synonyms</th>
<th>Plant Parts</th>
<th>Alkaloids</th>
<th>Category</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>A.</td>
<td>Species Listed Under the Genus Zanthoxylum</td>
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<td>wd</td>
<td>dictamine</td>
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<td>79</td>
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<tr>
<td></td>
<td></td>
<td>wd</td>
<td>skimmianine</td>
<td>F</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wd</td>
<td>magnoflorine</td>
<td>B</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b</td>
<td>laurifoline</td>
<td>B</td>
<td>79</td>
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<td>herclavin</td>
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<td>canadine</td>
<td>B</td>
<td>88</td>
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<td>l,s</td>
<td>+ve test</td>
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### B. Species Listed under the Genus Fagara

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aPlant parts: b, bark; l, leaves; r, root; rb, root bark; s, seed; wd, wood.

bAlkaloid category: A, simple amine or amide; B, benzylisoquinoline; C, canthinone; F, furoquinoline; Q, quinazolinocarboline; Un, uncharacterized.
**TABLE 4**

**SOME PHYSICAL PROPERTIES OF THE ALKALOIDS ISOLATED FROM THE GENUS ZANTHOXYLUM AND THE GENUS FAGARA**

| Alkaloids and Empirical Formula | Melting Point (°C) and/or Optical Rotation | Salts, Derivatives and Melting Point (°C) | Species¹
|--------------------------------|------------------------------------------|------------------------------------------|--------
<p>| Furoquinoline Alkaloids        |                                          |                                          |        |
| Dictamnine ( \text{C}_{12}\text{H}_9\text{O}<em>2\text{N} ) | 132-3 | Picro. 164-5; Hydr. 195; Picrol. 178; Aurich. 152; Chlorpl. sinters at 210 | 1, 2, 24 |
| Skimmianine ( \text{C}</em>{13}\text{H}_1\text{O}<em>4\text{N} ) | 176-7 | Picro. 197 dec.; Perchl. 210 | 1, 7, 24, 31, 36, 47, 48 |
| ( Y) -Fagarine = Aegenine = Haplophine ( \text{C}</em>{13}\text{H}_1\text{O}<em>3\text{N} ) | 142-3 | Picro. 177; chlorpl. 200 dec. | 24, 33 |
| Canthinone Alkaloids           |                                          |                                          |        |
| 6 -Canthinone ( \text{C}</em>{14}\text{H}_8\text{O}_2\text{N}<em>2 ) | 162-3 | Hydr. 244-6; picro. 262-4; Methiod. 261-3 dec. | 11, 28 |
| 5-methoxy-6-canthinone ( \text{C}</em>{15}\text{H}_10\text{O}_2\text{N}_2 ) | 241-2 | Hydr. 201-7 dec.; Picro. 242-4; methiod. 308-9 dec. | 11 |</p>
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<th>Salts, Derivatives, and Melting Point (°C)</th>
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<td>C₁₉H₁₇O₂N₃</td>
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<td><strong>Benzylisoquinoline Alkaloids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tembetarine chloride</td>
<td>236-7</td>
<td></td>
<td>32, 34, 37, 38, 39, 43</td>
</tr>
<tr>
<td>= N-methyl(+)reticuline chloride</td>
<td>29 + 123.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.9 gm. ethanol</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aporphine Alkaloids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laurifoline</td>
<td>24 + 26.3</td>
<td>Chlor. 253 dec.; Picr. 222 dec.;</td>
<td>1, 11, 32, 34, 42</td>
</tr>
<tr>
<td>C₂₀H₂₄O₄N₄</td>
<td>D</td>
<td>Iod. 248-9°</td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaloids and Empirical Formula</td>
<td>Melting Point (°C) and/or Optical Rotation</td>
<td>Salts, Derivatives, and Melting Point (°C)</td>
<td>Species</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>------------------------------------------</td>
<td>--------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Magnoflorine C_{20}H_{24}O_4N^+</td>
<td>15 + 220.1 D</td>
<td>Iod. 248-9; Pier. 206; Styph. 230 dec.</td>
<td>1,23,24,32, 33,34,38,42, 43,44</td>
</tr>
<tr>
<td>N-methyl corydine C_{21}H_{26}N O_4N^+</td>
<td>28 + 154.2 D</td>
<td>Pier. 186-7; Iod. dec. 190-200; Styph. 206-7</td>
<td>39,24,34</td>
</tr>
<tr>
<td>Xanthoplanine C_{21}H_{26}O_4N^+</td>
<td>21 + 71 D</td>
<td>Iod. hemihydrate 148-149 dec.; O-methyl iod. 225-227 dec.</td>
<td></td>
</tr>
<tr>
<td>N-methyl isocorydine = menispernine C_{21}H_{26}O_4N^+</td>
<td>20 + 168 D</td>
<td>Chlor. 217-221; Pier. 203; Styph. 203; Iod. 219; Methiod. 203</td>
<td>6,30,33,39, 42,43,44</td>
</tr>
<tr>
<td>\textit{Z. tinguiosoiba} quatern. alkaloid C_{21}H_{26}O_4N^+</td>
<td>25 + 30.2 D</td>
<td>Chlor. 215-219 dec.; Iod. 226-229 (dec.)</td>
<td>46</td>
</tr>
<tr>
<td>Alkaloids and Derivatives</td>
<td>Melting Point (°C) and/or Optical Rotation</td>
<td>Salts, Derivatives, and Melting point (°C)</td>
<td>Species</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------------------------------</td>
<td>-------------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Protopine Alkaloids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α and β-Alloerytopine</td>
<td></td>
<td>Picr. 208; Aurich. 192; Hydr. 190;</td>
<td>6, 30, 33, 48</td>
</tr>
<tr>
<td>= α-Fagarine</td>
<td>160</td>
<td>Iod. 205</td>
<td></td>
</tr>
<tr>
<td></td>
<td>170</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fagarine II</td>
<td>198-9</td>
<td>Picr. 214; Iod. 234; Aurich. 219;</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methiod. 234 dec.; Hydr. 200-2 dec.;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydrobromide 208-10 dec.</td>
<td></td>
</tr>
<tr>
<td>Protoberberine Alkaloids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Berberine</td>
<td>144</td>
<td>Hydr. 205; Sulfate 274; Picr. 234</td>
<td>2, 3, 4, 9, 14, 15, 19, 22, 25, 33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canadine</td>
<td>133</td>
<td>- Methiodide 220</td>
<td>6, 30</td>
</tr>
<tr>
<td></td>
<td>D ± 298</td>
<td>- Methiodide 264</td>
<td></td>
</tr>
<tr>
<td>α-L-Canadine methochloride</td>
<td>262 = 137</td>
<td>-1-canadine ethiod.;</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>262</td>
<td>-1-canadine ethichlor.</td>
<td></td>
</tr>
<tr>
<td>Palmatine</td>
<td></td>
<td>Iodide 241; Perchlor. 262;</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chlor. 205</td>
<td></td>
</tr>
<tr>
<td>Alkaloids and Empirical Formula</td>
<td>Melting Point (°C) and/or Optical Rotation</td>
<td>Salts, Derivatives, and Melting Point (°C)</td>
<td>Species</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>------------------------------------------</td>
<td>-------------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>Benzophananthridine Alkaloids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitidine ( \left( C_{21} H_{18} O_4 N \right) OH )</td>
<td></td>
<td>Chlor. 285-6 dec.; Acetate 255-60; Pseudocyanide 216; Period. 292-4</td>
<td>10, 13, 17, 32, 33, 42</td>
</tr>
<tr>
<td>Oxynitidine ( C_{21} H_{17} O_5 N )</td>
<td>284-5</td>
<td>Does not produce salts</td>
<td>13, 17, 39</td>
</tr>
<tr>
<td>Dihydronitidine ( C_{21} H_{19} O_4 N )</td>
<td>221-3</td>
<td>Iod. 283-5 and 290-2 dec.; Hydr. 280-3; Methiod. 268</td>
<td></td>
</tr>
<tr>
<td>Avicine ( \left( C_{20} H_{14} O_4 N \right) OH )</td>
<td>Acetate 160 dec.; Chlor. 255-8; Pseudocyanide 340; oxyavicine 259; dihydroavicine 211-2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Chelerythrine = Toddaline ( \left( C_{21} H_{18} O_4 N \right) OH )</td>
<td>207-8</td>
<td>Aurich. 233 dec.; Pseudocyanide 261 dec.; Chlor. 210-4 dec.; Nitrate 240</td>
<td>6, 7, 30, 32, 33, 34, 37, 38, 42, 45</td>
</tr>
<tr>
<td>Dihydrochelerythrine ( C_{21} H_{19} O_4 N )</td>
<td>166-7</td>
<td>Picr. 238; oxychelerythrine 199-201</td>
<td>45</td>
</tr>
<tr>
<td><strong>Simple Amine and Amide Alkaloids</strong></td>
<td></td>
<td></td>
<td>93</td>
</tr>
<tr>
<td>Fagaramide ( C_{14} H_{17} O_5 N )</td>
<td>118-9</td>
<td>Hydr. 177-8 (easily dissociated); Dibromo fagaramide 154-5</td>
<td>35, 48</td>
</tr>
</tbody>
</table>
### TABLE 4—Continued

<table>
<thead>
<tr>
<th>Alkaloids and Emperical Formula</th>
<th>Melting Point (°C) and/or Optical Rotation</th>
<th>Salts, Derivatives, and Melting Point (°C)</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herclavin ( \text{C}<em>{19} \text{H}</em>{21} \text{O}_{2} \text{N} )</td>
<td>76</td>
<td>Chlor. 285 dec.; Iod. 234; Chlorpl. 16, 32, 33, 34; Aurich. 127-9; Picr. 162-3; Picrol. 218-9</td>
<td>3</td>
</tr>
<tr>
<td>Candicine ( \text{C}<em>{11} \text{H}</em>{18} \text{O} \text{N}^{+} )</td>
<td></td>
<td>Chlor. 201; Iod. 205</td>
<td></td>
</tr>
<tr>
<td>Coryneine ( \text{C}<em>{11} \text{H}</em>{18} \text{O}_{1} \text{N}^{+} )</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aHydr., hydrochloride; picr., picrate; picrol., picrolonate; aurich., aurichloride; chlorpl., chloroplatinate; iod., iodide; chlor., chloride; period., periodide; perchlor., perchloride; methiod., methiodide; styph., stiphnate.*

*bThe numbers refer to species mentioned in Table 3.*
EXPERIMENTAL

Source of Plant Material

The plant material used in this investigation was the stem-bark of *Z. elephantiasis* Macf. and *Z. martinicense* DC. (Rutaceae), collected during the summer of 1962 from plants growing at the foot of Long Mountain in Jamaica. The air-dried bark was ground to a moderately coarse particle size by means of a Wiley Mill.

Methodology

It is customary before undertaking a problem of this nature to conduct -- at one's own choice -- certain preliminary investigations of representative samples of the material, with the following main objectives and primary concerns:

1. The determination of the approximate quantitative amount of the total alkaloid content, in order to decide whether or not the problem at hand is worthy of consideration.

2. Ascertaining the probable number of alkaloids present.

3. The planning of an appropriate approach or best methodology for the isolation of at least the major alkaloids present.

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1 The material was collected under the supervision of Dr. Michael P. Cava, present address: Department of Chemistry, Wayne State University, Detroit, Michigan. The material was verified botanically by Dr. Dennis Adams, Department of Botany, University of the West Indies.
Part I of this manuscript deals with the investigation of the alkaloid in the stem-bark of *Z. elephantiasis* Macf. and Part II covers the work done on *Z. martinicense* DC.

**Part I**

**Investigation of the Alkaloids of the Bark of *Z. elephantiasis* Macf.**

**A. Preliminary Investigation**

1. **Quantitative Estimation of Total Alkaloid Content**

   A twenty gram sample of the powdered plant material was placed in a 250 ml. round bottom flask and a volume of 150 ml. of U.S.P. alcohol 95 per cent was added and the mixture refluxed for two hours. The contents of the flask were allowed to cool at room temperature and filtered under suction. The clear filtrate was then evaporated to dryness in vacuo at below 50°C. To the resulting residue in the flask, 50 ml. of 5 per cent aqueous ammonium hydroxide solution was added, followed by an equal volume of ether. The mixture was stirred to dissolve as much of the residue as possible in the two solvents. At this stage a thick emulsion occurred which was difficult to break. The contents of the flask were then transferred to a separatory funnel and another 50 ml. of ether was added, and allowed to stand undisturbed. After about two hours, the upper ether layer was removed, and the aqueous layer was extracted twice more with 50 ml. portions of ether. The ether extractives were combined, dried over anhydrous
sodium sulfate and filtered. The filtrate was concentrated to a small volume (25 ml.) and then extracted with 5 ml. of 5 per cent hydrochloric acid. This aqueous acid extractive was designated as "the total tertiary alkaloid fraction." The alkaline mother liquor, after extraction with ether, containing the quarternary alkaloid base was acidified with hydrochloric acid and filtered. The filtrate was adjusted to 50 ml. with aqueous 2 per cent hydrochloric acid and designated as "the quarternary alkaloid fraction."

As reference standards, for comparison, solutions of brucine (4.0 mg./ml., 1.3 mg./ml. and 0.4 mg./ml.) and solutions of berberine (0.5 mg./ml., 0.17 mg./ml. and 0.05 mg./ml.) were prepared in 2 per cent hydrochloric acid. The concentrations of the standard brucine and berberine were so chosen because upon appropriate treatment, the precipitates obtained would indicate an approximate concentration of 0.1 per cent; 0.033 per cent, and 0.01 per cent, respectively, of total alkaloids in the crude plant material.

Two ml. of each of the above standard reference alkaloid concentrations and of each of the two fractions representing "the total tertiary" and "quaternary alkaloids" in the sample, were placed in separate 12 ml. centrifuge tubes and the volumes made up to exactly 5 ml. with distilled water. Three drops of Valser's reagent were added to each of the eight tubes and the precipitates centrifuged. The heights of the resulting precipitates in the centrifuge tubes were then compared to the height of reference alkaloid standards for the quantitative estimation.
The results obtained indicated an alkaloid content greater than 0.1 per cent for both the tertiary and the quaternary fractions.

The remaining hydrochloric acid extract (3 ml.) of the quantitative screening procedure designated as the total tertiary alkaloid fraction, when left undisturbed in the test tube, for 3 days, was found to give a mass of fine yellowish brown crystalline needles (10 mg.). This crystalline material melted at 150-152°C, gave a positive alkaloid test, and upon exposure to air became efflorescent and turned darker in color.

2. Investigation of the Probable Number of Alkaloids in Z. elephantiasis bark

a) Successive solvent extraction

A sample of 50 gm. of the powder, packed in a thimble, was successively extracted in a small Soxhlet apparatus with Skellysolve\(^2\) F (30-60°), ether, chloroform, and lastly U.S.P. ethanol. Extraction with each of the solvents was allowed to proceed continuously for a 24 hour period. At the end of each extraction, the marc was completely air-dried. Each of the extracts thus obtained was concentrated to a small volume and transferred into a small conical flask of 25 ml. capacity. Upon testing for alkaloids by spotting a small amount on filter paper and spraying with Dragendorff's Reagent (128) all of these extracts gave positive tests.

\(^2\)Skellysolve F. commercial grade.
b) Horizontal paper chromatography of the extracts

The solvent system used consists of the upper phase of a mixture of tertiary amyl alcohol, 5 parts; isoamyl alcohol, 5 parts; formic acid, 4 parts; and water, 10 parts — designated (AAFH) system. After repeated shaking for 10 minutes, the mixture was left to stand for 30 minutes and then the phases were carefully separated.

Horizontal (circular) chromatography was adopted, using sheets of Whatman paper 3 MM (19" x 12.5") as a support. The different extracts were spotted on a circle drawn at 1" radius from the geometric center of the paper sheet. The chromatogram was then allowed to equilibrate for two to three hours, the lower phase of the solvent system being placed in the bottom of the jar. For the development of the chromatogram, the upper phase of the solvent system was introduced, through a hole on one side of the jar by means of a bent funnel, to a petri dish placed in the center. A cotton string hanging from the center of the sheet served to convey, by capillary forces, the solvent needed for effecting separation. Development was allowed to proceed for a sufficient time (10-12 hours) such that the solvent front reached but did not surpass an outer circle drawn at a radius of 5.5 inches.

After drying the developed chromatogram, the fluorescent spots were traced under the ultraviolet light and the sheet was then sprayed with Dragendorff's reagent and allowed to dry in the hood. The appearance of orange stained banana-shaped spots revealed the probable number of alkaloids in the extracts.
Surprisingly, the chromatogram of the Skellysolve F, ether and chloroform extracts showed the same three alkaloidal spots, two of which fluoresced blue and one had a golden yellow fluorescence, of \( R_f \) values 0.86, 0.78, and 0.65, respectively. Paper chromatography of the ethanolic extract, however, showed the same above mentioned three spots, plus three additional alkaloidal spots. Two of the three additional spots exhibited blue fluorescence, while the third was non-fluorescent. Their \( R_f \) values were 0.50, 0.42, and 0.31, respectively. Moreover, a spot near the origin stained red wine or reddish violet with Dragendorff, which indicated the possible presence of choline in this plant.

c) Fractionation of the alcohol extract into tertiary and quaternary alkaloids

The alcohol extract, representing all the alkaloids in the sample, was evaporated to complete dryness in vacuo. The residue was dissolved in 25 ml. of 2 per cent hydrochloric acid, filtered, then the filtrate made distinctly alkaline with NH₄OH (pH paper) and then extracted with chloroform to exhaustion. The chloroform was concentrated to a small volume (10 ml.) and was labelled the tertiary alkaloids. The alkaline mother solution was made acidic with 2 per cent hydrochloric acid and evaporated to complete dryness. The methanol soluble residue was labelled the quaternary alkaloids.

When these fractions were separately spotted and chromatographed, in the manner previously mentioned, it was shown that the
spots of Rf values 0.86, 0.76, and 0.62 represented the three tertiary alkaloids in the bark whereas those of Rf values 0.50, 0.42, and 0.31 represented the quaternary alkaloids. Moreover, from the comparative size and intensities of the Dragendorff's stained spots, it was evident that at least three of the total six alkaloids could be considered major in this plant. The crystalline alkaloid previously isolated (10 mg.) gave the spot with Rf 0.86.

d) Further purification and fractionation of the "tertiary alkaloid fraction"

The concentrated chloroform solution (10 ml.) containing the three tertiary alkaloids was extracted twice with 10 ml. portions of aqueous 2 per cent sodium hydroxide, to separate, if any, the phenolic from the non-phenolic alkaloids. The combined alkaline extract, which was yellowish brown in color, gave a negative alkaloid test indicating the absence of phenolic alkaloids.

When the purified chloroform containing the three tertiary (non-phenolic) alkaloids was shaken with 2 per cent aqueous tartaric acid, it was noticed that, at least partial fractionation occurred. The tartaric acid extract showed the presence of the two blue fluorescent alkaloids of Rf values 0.86 and 0.76, but the golden yellow alkaloidal spot was not evident, indicating that the yellow fluorescent alkaloid did not form a tartarate salt and therefore might be considered as a weak base.
Conclusions

From the foregoing preliminary investigations the following conclusions could be made.

1. The bark of *Z. elephantiasis* contains at least six alkaloids, three of which are tertiary alkaloids, the remaining ones are quaternary. Each group of alkaloids was estimated to occur in greater than 0.1 per cent.

2. Three of the six alkaloids revealed by chromatography could be considered major alkaloids, two of which are tertiary and one quaternary.

3. Extraction of the bark for the purpose of isolating the alkaloids could best be done with ethyl alcohol without the need for previous defatting.

4. Separation of the two major tertiary alkaloids *R* *f* 0.86 and 0.76 from the third alkaloid could be done with aqueous tartaric acid, 2 per cent.

B. General Extraction and Fractionation of the Alkaloids in the Bark of *Z. elephantiasis*

1. Extraction of Total Alkaloids

Coarsely ground bark (1.3 kg.) was extracted continuously in a Soxhlet-type extractor with U.S.P. alcohol (10 l.) for eight days, with a fresh charge of ethanol at the end of every second day. The extraction was discontinued when alkaloids no longer were being extracted, as indicated by a negative test with both Valser's and
Mayer's reagents. The combined ethanolic extract was concentrated in vacuo at 40° to a thick syrupy consistency weighing 485 gm.

2. Fractionation Using 2 Per Cent Aqueous Tartaric Acid

The thick syrupy extract was transferred to a separatory funnel by mixing with sufficient ethanol to produce a viscous fluid consistency. The extract was allowed to drip slowly overnight into a large beaker containing one liter of 2 per cent aqueous tartaric acid solution (pH 4). Mixing of the dripping extract with the acidic solution was done by mechanically stirring at a slow rate. The content of the beaker was then left to digest on a steam bath for one hour, and then it was placed in the refrigerator for 24 hours. Insoluble material separated, leaving a clear dark yellowish brown supernatent solution which was carefully decanted and then filtered. The insoluble material was extracted twice with 750 ml. portions of aqueous 2 per cent tartaric acid, leaving a tartaric acid insoluble residue of 35 gm. which was saved for further work.

3. Treatment of the Tartaric Acid Soluble Extractive

In order to drive off the ethanol the combined decantates of the tartaric acid soluble extractive (2300 ml.) were concentrated under reduced pressure in a rotary-type evaporator to approximately one liter. The solution became turbid and cloudy but was easily clarified from the non-alkaloidal turbidity by filtration through a
celite filter bed, the latter being washed well with fresh tartaric acid solution to free it from adhering alkaloidal material. The clear filtrate was made alkaline to pH 9 with ammonium hydroxide (28%) and then was extracted with chloroform in a large continuous liquid-liquid type extractor for three days, with fresh charge of solvent at the end of each 24 hours. The aqueous mother solution was saved as a source of the quaternary alkaloids. At the end of the extraction, the combined chloroform extracts were concentrated to one liter.

4. Isolation of Two Alkaloids in the Chloroform Extract

Paper chromatography of the concentrated chloroform extract employing the (AAFH) solvent system on page 59 demonstrated the presence of two blue fluorescent alkaloids of Rf values 0.86 and 0.76. The chloroform extract was further purified by shaking in a separatory funnel with five 50 ml. portions of 2 per cent aqueous sodium hydroxide, the combined alkaline extracts were deep yellowish brown in color but free from alkaloids and hence rejected. The chloroform extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure to give 4.8 gm. of crude dark brown alkaloidal residue. The residue was digested with 150 ml. aqueous 5 per cent hydrochloric acid and filtered. The filtrate was made alkaline to pH 9 with concentrated ammonium hydroxide (28%) and extracted with ether at not more than
35°C. in a small continuous liquid-liquid extractor. At the end of three days, slight whitish amorphous encrustations on the walls of the receiver flask started to form. Extraction was then allowed to proceed for two more days to allow for maximum encrustation to occur. The ether extract was decanted, and the alkaloidal amorphous material encrusted on the sides of the flask was washed with acetone in which it was insoluble, and then collected. Recrystallization from methanol yielded 280 mg. of long slightly yellowish needles designated "Base I."

The decanted ether was concentrated to give a dark resinous brown syrup which was dissolved in boiling acetone. On cooling, at room temperature, another crop of 120 mg. of the same base was collected.

The remaining acetone solution was reduced to dryness, the residue dissolved in chloroform (50 ml.) and the latter extracted by shaking in a separatory funnel with 0.1N hydrochloric acid. The acid solution was concentrated to a small volume and when left at room temperature for one day deposited 150 mg. of the crude crystalline hydrochloride of the second base designated "Base II."

5. Purification of Base II

The crude crystalline hydrochloride of Base II (150 mg.) was converted to the free base in the usual way, and the liberated base extracted with ether which was then concentrated to give an amorphous residue 105 mg. Purification of this crude base was done by column chromatography on 5 gm. of Woelm neutral alumina activity grade III. Elution with benzene gave an oily substance in the first fraction
(5 ml.) and the next few fractions, 50 ml., gave an amorphous material. This material crystallized from methanol as fine slightly yellowish needles, 90 mg., which had a melting point of 152-154°. The crystalline material was found not to be stable in air as evident by turning more yellowish on keeping.

Rechromatography, as above, yielded a residue which crystallized from methanol as almost colorless needles, 70 mg., melting at 159-160°.

6. **Treatment of the Tartaric Acid Insoluble Residue**

The viscous resinous residue (35 gm.), left after the extraction of the alcohol extract with the aqueous 2 per cent tartaric acid, was dissolved in the minimum amount of ethanol and extracted with 5 per cent hydrochloric acid (700 ml.). The acid solution, which was shown to contain in addition to the two blue fluorescent alkaloids the third yellow fluorescent alkaloid, was then treated in exactly the same way as the tartaric acid (pp. 63-66) to yield an additional amount of Base I of 80 mg. and 250 mg. of Base II hydrochloride. The latter upon purification gave 220 mg. of pure Base II. The yellow fluorescent alkaloid was not isolated because of its low concentration in the extract.

7. **Preparation of Reineckate of the Quaternary Alkaloids**

The alkaline mother solution, after the extraction of the tertiary alkaloids with chloroform, was acidified to a pH 3 with
dilute hydrochloric acid and the quaternary alkaloids were precipitated with ammonium reineckate\(^3\) following the procedure of Hogg et al. (129).

A saturated aqueous solution of reineckate salt (2 g./100 ml.) was poured slowly with constant stirring into the aqueous alkaloid extract previously adjusted to pH 3, until precipitation ceased. The solution was allowed to stand in the refrigerator for 24 hours, then the pink colored precipitate was collected on a Buchner funnel and dried under suction. The semi-dried precipitate was washed with ether to remove any ether soluble extraneous matter and to facilitate drying. The precipitate was then dried in a desiccator over sulfuric acid for two days, and the resulting lumps were powdered in a glass mortar. The yield was 35 gm. of crude quaternary reineckate.

8. Conversion of the Quaternary Alkaloidal Reineckates to Chlorides by an Ion-Exchange Resin Method

The crude quaternary reineckates (35 gm.) were divided into two portions as follows:

- 20 gm. to be exchanged on ion-exchange resin
- 15 gm. to be treated according to Hogg's et al. method (129).

The 20 gm. portion of the crude quaternary reineckates was dissolved in a mixture of 600 ml. of acetone:water (1:1), by mechanical stirring for 24 hours. The resulting deep reddish violet

solution was filtered under suction and the residue was again treated with 200 ml. of the same solvent mixture. The combined filtrates were allowed to drip overnight into a suspension of 400 gm. of Amberlite\(^4\) IRA 410 resin (chloride form) in acetone:water (1:1), with mechanical stirring at low rate. After 24 hours, exchange was complete as indicated by the light straw yellow color of the solution and the deep reddish violet color of the resin.

After filtration under suction, the resin was washed with methanol to free it from adsorbed alkaloids. Evaporation of the filtrate under reduced pressure, at not more than 40°C, yielded 7.5 gm. of a crude product. Of this material only 3.7 gm. was found to be soluble in boiling methanol. The remaining insoluble material did not give a positive alkaloid test. It dissolved easily in water, from which it recrystallized as large cubic crystals which consisted of inorganic material.

9. Thin-layer Chromatography of the Crude Quaternary Alkaloidal Chlorides

The methanolic solution containing the exchanged quaternary alkaloid chloride when chromatographed on paper chromatography, using the (AAFH) system described on page 59, revealed the presence of alkaloids with \(R_f\) values of 0.50, 0.42, and 0.31, in addition to a spot behaving as choline near the start line. Confirmation of this result was demonstrated by thin-layer chromatography. The adsorbent

\(^4\)Mallinckrodt Chemical Works, St. Louis, New York, Montreal.
used was Silica Gel G, the plates were prepared by using the Stahl-
Desaga apparatus in the conventional manner. The plates were activated
by drying in an oven at 105° for 30 minutes and then stored in a
desiccator over anhydrous calcium sulfate prior to use.

A system of equal volumes of methanol, ammonium hydroxide
solution (28%) and acetonitrile referred to as (MAA) was found to
give a good separation revealing the presence of the same three
quaternary alkaloidal spots, Rf 0.66, 0.42, and 0.22. The first two
spots exhibited a blue fluorescence under ultraviolet light.

10. Column Chromatography of Crude
Quaternary Chlorides and Isol-
ation of One Quaternary
Alkaloid Chloride

A quantity of 3.7 gm. of crude quaternary chloride, dissolved
in a small volume of methanol, was adsorbed on 10 gm. of Woelm acid
alumina, grade I. After drying, the lumpy mixture was triturated in
a glass mortar and then sifted through a 100 mesh wire screen. The
resulting fine powder was added to the top of a previously packed
column.

The column (1" x15") was wet-packed with 150 gm. of Woelm
acid, alumina, grade I, in benzene.

Elution with benzene, benzene:chloroform (1:1), and chloroform
500 ml., respectively, eluted only a blue fluorescent non-alkaloidal
material. When the polarity of chloroform introduced in the column

5Silica Gel G, Merck, supplied by Brikmann Instrument In-
was increased by the gradual addition of methanol in the proportions shown in Table 5, a blue fluorescent zone started to move slowly down the column. The separation achieved was checked by Silica Gel G thin-layer chromatography, adopting the (MAA) solvent system. The results obtained indicated that fractions eight to fourteen contained all the alkaloidal components of the crude quaternary alkaloid chloride mixture. The combined effluents of fractions eight to fourteen were then concentrated under reduced pressure to a syrupy consistency. After a period of one week, at room temperature, microrosette crystals were found to be embedded in the dark thick matrix. The flask containing the extract was then inverted over a suction funnel and the contents allowed to drip. The crystalline masses adhering to the sides and bottom of the flask were scraped and washed with chloroform containing 1 per cent methanol. A quantity of 130 mg. of colorless prismatic crystals was recovered. The crystals gave positive alkaloidal tests with Dragendorff's, Valser's, and Mayer's reagents. Recrystallization was carried out in absolute ethanol, melting point 253°C with decomposition. The filtrate from which the crystals were obtained was boiled with absolute ethanol and upon cooling and leaving undisturbed an additional amount of 60 mg. of the same alkaloid was obtained. The mother solution was then saved for future work.
TABLE 5
COLUMN CHROMATOGRAPHY OF THE CRUDE QUATERNARY CHLORIDES OF *Z. ELEPHANTIASIS*

<table>
<thead>
<tr>
<th>Composition of Effluent</th>
<th>No. of Fractions</th>
<th>Volume</th>
<th>Alkaloid Test</th>
<th>Rf Valuesa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>1</td>
<td>500</td>
<td>-ve</td>
<td></td>
</tr>
<tr>
<td>Benzene:chloroform</td>
<td>2</td>
<td>500</td>
<td>-ve</td>
<td></td>
</tr>
<tr>
<td>(1:1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroform</td>
<td>3</td>
<td>500</td>
<td>-ve</td>
<td></td>
</tr>
<tr>
<td>Chloroform:methanol</td>
<td>4</td>
<td>300</td>
<td>-ve</td>
<td></td>
</tr>
<tr>
<td>(99:1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(98:2)</td>
<td>5</td>
<td>300</td>
<td>-ve</td>
<td></td>
</tr>
<tr>
<td>(97:3)</td>
<td>6</td>
<td>300</td>
<td>-ve</td>
<td></td>
</tr>
<tr>
<td>(96:4)</td>
<td>7</td>
<td>300</td>
<td>-ve</td>
<td></td>
</tr>
<tr>
<td>(95:5)</td>
<td>8</td>
<td>300</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td>(94:6)</td>
<td>9</td>
<td>300</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td>(93:7)</td>
<td>10</td>
<td>300</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td>(92:8)</td>
<td>11</td>
<td>300</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td>(91:9)</td>
<td>12</td>
<td>300</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td>(90:10)</td>
<td>13</td>
<td>300</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td>(85:15)</td>
<td>14</td>
<td>300</td>
<td>+ve</td>
<td></td>
</tr>
</tbody>
</table>

aSolvent system (MAA), Silica Gel G plates.

11. Conversion of the Remainder of the Quaternary Reineckates to Chlorides and Isolation of One Quaternary Alkaloid Chloride by Column Chromatography

The remaining 15 gm. sample of the crude quaternary alkaloid reineckates was worked up according to the method of Hogg et al. (129) to give 6.6 gm. of crude quaternary alkaloid chlorides. Extraction of this crude residue with methanol left 3.9 gm. of methanol insoluble, non-alkaloidal residue. The methanol containing the alkaloid
chlorides was chromatographed on Woelm acid alumina activity, grade I, in the same manner as described on page 69. However, the packing of the column was done in chloroform and elution was started with chloroform (500 ml.) and then chloroform containing 2 per cent methanol (500 ml.). The effluent showed non-alkaloidal fluorescent material. However, when chloroform containing 5 per cent, 10 per cent, and 15 per cent of methanol (each 500 ml.) was introduced in the column, the effluents showed in each case the three alkaloidal components of the mixture.

From the combined effluents, after concentration to dryness and crystallization from absolute ethanol, 90 mg. of the crystalline quaternary alkaloid previously isolated (page 70) was obtained. The mother solution after the crystallization of the quaternary alkaloid chloride was combined with the previous mother solution (page 70) and saved for future work.

C. The Identification of the Three Crystalline Alkaloids Isolated from the Bark of *Z. elephantiasis* Macf.

In this investigation, all melting points were taken on a Thomas Hoover Uni-Melt Capillary melting point apparatus. The infrared spectra were taken in potassium bromide pellets using a Perkin-Elmer model 237, spectrophotometer. The ultraviolet spectra were determined using a Perkin-Elmer, model 4000, spectrocord. The N.M.R. spectra were obtained using a Varian A-60 nuclear magnetic resonance spectrometer. The optical rotation was determined using a Carl Zeiss
Circle Polarimeter $0.01^\circ$. The micro-analysis of the three alkaloids was performed by Alfred Bernhardt Mikroanalytisches Laboratorium in Max-Planck-Institut für Kohlen-Forschung, Hohenweg 17, 433 Mulheim (Ruhr), Germany.

1. Identification of Base I (5-methoxy-6-canthinone)

Base I was sparingly soluble in cold absolute ethanol or acetone but dissolved on boiling. On cooling, it crystallized from these solvents into slightly pale yellow thin needle-shape crystals. Recrystallization from chloroform-methanol (1:1) mixture gave an almost colorless material, m.p. 237-8°C with decomposition.

Analysis -- calculated for $\text{C}_{15}\text{H}_{10}\text{O}_2\text{N}_2$: C, 71.99; H, 4.03; N, 11.20; O-CH$_3$, 12.38. Found: C, 72.00; H, 4.12; N, 11.33; O-CH$_3$, 12.77. The infrared spectra of the alkaloid (Fig. 9) was obtained in a potassium bromide pellet; the spectra showed absorption at 5.99 $\mu$ and 6.13 $\mu$ indicating the presence of a conjugated amide carbonyl in the molecule. The ultraviolet spectra of base I taken in both neutral and alkaline solution in 95 per cent ethanol showed the following absorption data (Table 6).

There was no shift in the spectra in alkaline medium. The ultraviolet spectrum obtained is characteristic of extended carbazoles or $\beta$-carboline chromophores (130).
From the physical data thus obtained, for Base I, and from a search in the literature of the alkaloids in the family Rutaceae, and in particular of the genus Zanthoxylum, it was possible to suggest that the alkaloid in question was a canthinone derivative, namely, 5-methoxy-6-canthinone. Reported melting point 240° dec. Ultraviolet absorption data in dioxane are shown in Table 6.

The identity of Base I with 5-methoxy-6-canthinone was confirmed by comparison with an authentic sample graciously supplied by Professor J. R. Price. The infrared spectrum of Base I was found to be exactly superimposable with that of the authentic sample. Moreover, the mixed melting point did not show any depression.

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6 Dr. J. R. Price, Division of Industrial Chemistry, C.S.I.R.O., Melbourne, Australia.
a) Preparation of 5-methoxy-6-
canthinone hydrochloride

A filtered hot solution of Base I (15 mg.) in about 3 ml. of
1N hydrochloric acid was allowed to stand at room temperature over­
night. The hydrochloride formed was deposited in fine yellow needles,
m.p. 206-7°C with decomposition, as reported in the literature.

b) Preparation of 5-methoxy-6-
canthinone picrate

The picrate was precipitated from an alcoholic solution of the
base, with a saturated alcoholic solution of picric acid. The col­
lected precipitate was crystallized from methanol as fine yellow
needles melting at 242-244°C (reported m.p. 242°C).

c) Preparation of 5-methoxy-6-
canthinone methiodide

A solution of 20 mg. of Base I in chloroform (20 ml.), 3 ml.
of methyl iodide was added and the whole refluxed for 12 hours. At
the end of the period, the deep orange red solution was dried in
vacuo. The residue was crystallized from water to give orange red
plates melting with decomposition at 308-9°C (reported m.p. 308-9°C).

2. Identification of Base II (6-
canthinone)

The needle-shape crystals of Base II, purified by chromato­
graphy on Woelm alumina, Grade III, turned slightly yellow upon
exposure to air. Recrystallization from methanol gave a product
melting at 159-196°C.
The infrared spectra of Base II (Fig. 10) taken in potassium bromide pellet, showed absorption at 6.08 μ and 6.15 μ indicating the presence of a conjugated amide carbonyl.

The ultraviolet spectrum of Base II taken in both neutral and alkaline solution in 95 per cent ethanol showed an absorption pattern similar to that of Base I. This indicated that Base II might be also a canthinone compound. The data obtained are shown in Table 7.

TABLE 7

ULTRAVIOLET ABSORPTION DATA OF 6-CANTHINONE

<table>
<thead>
<tr>
<th></th>
<th>( \lambda_{\text{Max}} ) (mu)</th>
<th>( \log \varepsilon ) max</th>
<th>( \lambda_{\text{Max}} ) (mu)</th>
<th>( \log \varepsilon ) max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base II in neutral ethanol</td>
<td>252</td>
<td>4.08</td>
<td>347</td>
<td>3.84</td>
</tr>
<tr>
<td></td>
<td>259</td>
<td>4.03</td>
<td>362.5</td>
<td>4.09</td>
</tr>
<tr>
<td></td>
<td>269</td>
<td>4.01</td>
<td>381</td>
<td>4.06</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>3.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Canthinone in dioxane (64)</td>
<td>251</td>
<td>4.09</td>
<td>299</td>
<td>3.91</td>
</tr>
<tr>
<td></td>
<td>259</td>
<td>4.05</td>
<td>347</td>
<td>3.94</td>
</tr>
<tr>
<td></td>
<td>269</td>
<td>4.03</td>
<td>362</td>
<td>4.17</td>
</tr>
<tr>
<td></td>
<td>293 (shoulder)</td>
<td>3.90</td>
<td>381</td>
<td>4.14</td>
</tr>
</tbody>
</table>

There was no shift in the spectra in alkaline medium.

From the data obtained, it was possible to suggest that Base II was canthinone, an alkaloid previously isolated from \( Z. \ suberosum \) by Ritchie et al. (67). Reported melting point 162.5-163.0°C. Ultraviolet absorption data in dioxane (64) are shown in Table 7.
For confirmation of the identity of Base II with 6-canthinone comparison with an authentic sample generously provided by Dr. E. Ritchie\(^7\) was made. The infrared absorption spectrum of Base II and that of authentic 6-canthinone was completely superimposable. In addition, the mixed melting point of the hydrochloride, picrate, and methiodide salts of Base II, prepared in the same manner as in the case of 5-methoxy-6-canthinone, corresponded with those reported in the literature for 6-canthinone (64).

The hydrochloride melts with decomposition at 242°C; the picrate melts at 262-4°C and the methiodide at 271-273°C.

3. **Identification of the Quaternary Alkaloid (Laurifoline Chloride)**

The quaternary chloride crystallized in the form of colorless prisms from ethanol and melted with decomposition at 253°C. Other data was \( \left[ \alpha \right]_D^{20} + 14^\circ \) (C, 6.22 in methanol), analysis calculated for \( \text{C}_{20}\text{H}_{10}\text{O}_{2}\text{N}_2 \): C, 63.56; H, 6.46; N, 3.71; Cl, 9.40; O-\text{CH}_3, 16.52; N-\text{CH}_3, 8.10. Found: C, 63.56; H, 6.46; N, 3.66; Cl, 9.26; O-\text{CH}_3, 16.06; N-\text{CH}_3, 8.12.

The infrared spectrum (Fig. 11) taken in potassium bromide pellet showed absorption at 3.07 \( \mu \) (\(-\text{OH}\)); 3.37 \( \mu \) (\(-\text{CH}_3\)) and 6.30 \( \mu \) (aromatic rings). The ultraviolet spectrum in neutral methanol solution showed the following absorption data: \( \lambda_{\text{max.}} \) methanol 228 \( \mu \) (log \( \varepsilon \) 4.61), 282 \( \mu \) (log \( \varepsilon \) 4.17), and 306 \( \mu \) (log \( \varepsilon \) 4.26).

\(^7\)Dr. E. Ritchie, Department of Organic Chemistry, The University of Sydney, Sydney, N.S.W., Australia.
The above data suggested that the alkaloid in question belonged to the aporphine type in which position 11 is free (131). In alkaline solution, the ultraviolet spectrum indicated a strong bathochromic shift of the maximum at 308 μμ (log ε 4.26) to a higher maximum at 335 μμ (log ε 4.28). This shift in alkaline solution confirmed the presence of the phenolic -OH indicated by the absorption at 3.07 μμ in the infrared spectrum.

The physical data possessed by the quaternary alkaloid isolated from Z. elephantiasis was typical of that reported for laurifoline chloride, one of the phenolic 1,2,9,10-tetrasubstituted aporphine alkaloids, found in the genus Zanthoxylum (78). Reported melting point 253°C dec.; ultraviolet absorption data (132) 227 μμ (log ε 4.48); 281 μμ (log ε 4.04), and 307 μμ (log ε 4.14).

The compound was identified to be laurifoline chloride on the basis of infrared and ultraviolet spectral comparison with an authentic sample. A mixed melting point determination did not show any depression. In addition, the picrate was prepared and found to have a melting point of 220°C dec.; reported 222°C dec. (78).

Preparation of Laurifoline Picrate

Laurifoline chloride 20 mg. was dissolved in 2 ml. of water, saturated aqueous sodium picrate solution was added dropwise until

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8 A sample was provided by Dr. M. Tomita, Pharmaceutical Institute, Medical Faculty, University of Kyoto, Japan, and by Dr. V. Deulofeu, Department of Organic Chemistry, Faculty of Sciences, Buenos Aires, Argentina.
Figure 7
Structure of the Alkaloids Isolated from the bark of *Z. elephantiasis* Macf.

5-Methoxy-6-canthinone

6-Canthinone

Laurifoline chloride
no more precipitate was formed. The filtered crude picrate was easily crystallized from hot water to give yellow needles, m.p. 220° dec.

4. Nuclear Magnetic Resonance Spectra of the Three Alkaloids

The determination and interpretation of the n.m.r. spectra of 5-methoxy-canthinone, canthinone, and laurifoline chloride were made by Dr. Sunil Talapatra, Post Doctoral Fellow, Department of Chemistry, The Ohio State University.

a) N.M.R. spectrum of 5-methoxy-6-canthinone

The n.m.r. spectra of 5-methoxy-6-canthinone were taken in trifluoroacetic acid as the solvent (-1.237) and tetramethylsilane (TMS) as the internal standard (7 = 10).

The methoxyl protons, attached to a carbon adjacent to a carbonyl appeared at 5.747, appreciably downfield. All other signals appeared only in the aromatic region from 2.437 to 1.077 in a complex pattern.

b) N.M.R. spectra of 6-canthinone

The nuclear magnetic resonance spectra of 6-canthinone were taken in deuterochloroform with tetramethylsilane (TMS) as the internal standard (7 = 10).

The spectra showed the signals appearing only in the aromatic region from 3.287 to 0.1327 in a complex pattern.

9Present address: Department of Chemistry, University College of Science, Calcutta, India.
c) N.M.R. of laurifoline chloride

The n.m.r. spectra of laurifoline chloride was determined in trifluoroacetic acid as the solvent (-1.23\(\tau\)) and TMS as the internal standard (\(\tau = 10\)).

The resonance spectra gave the following signals: 6, singlet, at 5.95\(\tau\) \(\text{N}^+\) (CH\(_3\))\(_2\); 3, singlet, at 6.53\(\tau\) (C\(_2\) or C\(_{10}\)-O-CH\(_3\)); 3, singlet, at 6.85\(\tau\) (C\(_{10}\) or C\(_2\)-O-CH\(_3\)); 1, singlet, at 3.17\(\tau\), (C\(_3\)-H); 1, singlet, at 2.97\(\tau\) (C\(_8\)-H); 1, singlet, at 1.85\(\tau\), (C\(_{11}\)-H). Benzylic hydrogens alpha to nitrogen gave broad and buried signals at 6 - 6.4\(\tau\).

Part II

Investigation of the Alkaloids of the Bark of

*Z. Martinicense* DC.

The objectives of this portion of the study were to screen the bark of *Z. martinicense* for its alkaloid contents, to outline the best methodology to be adopted for the isolation of the alkaloids and to isolate and identify some of the constituents.

A. Preliminary Investigations

1. Quantitative Estimation of Total Alkaloids in the Bark of *

   *Z. martinicense*

   The approximate quantitative estimation of the total alkaloids in the bark of *Z. martinicense* was done following the same procedure described on page 56, with a slight modification of using chloroform instead of ether for the extraction of the tertiary alkaloids. The
results indicated that both the tertiary and the quaternary alkaloid fraction contained more than 0.1 per cent of total alkaloids.

2. Determining the Probable Number of Alkaloids

a) Extraction with Skellysolve F

A 300 gm. sample of the powdered bark was packed in a modified Soxhlet extractor and extracted continuously for two days with Skellysolve F (boiling point 30-60°). The extractive was deep greenish-yellow in color and gave a positive test for alkaloids with Dragendorff's, Mayer's and Valser's test solutions. To insure complete defatting and extraction of all the alkaloids soluble in Skellysolve F, re-extraction for 24 hours of the air-dried marc with a fresh charge of Skellysolve F was done. The combined extracts were evaporated to dryness, and the residue dissolved in 50 ml. of U.S.P. alcohol. When this alcoholic solution was left overnight in the refrigerator, a mass of dark brown semi-crystalline material was deposited. The material was collected by filtration and washed with cold acetone to give about 250 mg. of a yellowish-white semi-crystalline residue. Preliminary qualitative tests indicated that this material was not an alkaloid, it did not reduce Fehling solution before and after attempted hydrolysis with hydrochloric acid, and it gave a pink-violet color with Liebermann-Burchard (133) test revealing its possible triterpenoid nature. Because of the characteristic color the material was suspected of being pentacyclic to the
triterpene lupeol, the latter being reported to occur in various species of *Zanthoxylum* (67,71,111). The identification of the triterpene is described on page 88.

b) Extraction with U.S.P. alcohol

The defatted marc was extracted continuously for two days with U.S.P. alcohol. The alcohol extract was concentrated to a small volume *in vacuo* at not more than 45°C. Upon standing the concentrated extract deposited in the bottom of the flask and on the sides, prismatic crystals. The crystalline material was filtered off and washed with cold ethanol to give a residue of 0.5 gm. This material did not give a positive alkaloid test and was thought to be glycosidal in nature by virtue of the fact that it did not give a positive Fehling's test except after hydrolysis. When the crystals were heated in a crucible, they charred with an odor characteristic of sugars. This substance was then suspected of being sucrose, a disaccharide commonly encountered in alcoholic extracts of plant material. The identity of the isolated crystals is described on page 93.

c) Chromatographic behavior of the Skellysolve F and the alcohol extracts

Horizontal paper chromatography, using the system (AAFH) described on page 59, revealed that the Skellysolve F extract of the bark of *Z. martinicense* contained, besides other non-alkaloidal
fluorescent spots, three alkaloidal spots of \( R_f = 0.88, 0.70, \) and 0.60. The first two spots fluoresced blue, the third spot, \( R_f = 0.60, \) exhibited a strong golden yellow fluorescence on paper.

The separation of the alkaloids in the alcohol extract of *Z. martinicense*, using the same system as above, showed the presence of five distinctly separated alkaloidal spots with \( R_f = 0.80, 0.70, 0.60, 0.46, \) and 0.38. Moreover, a tailing spot appearing near the starting line faded after it was sprayed with Dragendorff's reagent.

In order to tell whether the alkaloids with \( R_f = 0.88 \) and 0.70 in the Skellysolve F extract were the same as those in the alcohol extract, thin-layer chromatography on alumina plates was utilized, using a solvent system of benzene:chloroform (2:1). In this system, the Skellysolve F extract showed three alkaloidal spots of \( R_f = 0.83 \) (blue fluorescent), 0.38 (reddish violet fluorescence) and 0.2 (blue fluorescent). In this system, the alkaloids in the alcohol extract did not show any separation and remained stationary at the starting line.

d) Fractionation of the alcohol extract into tertiary and quaternary alkaloid fractions

The alcohol extract of the defatted bark was evaporated to complete dryness in vacuo and the residue (18 gm.) was dissolved in 200 ml. of 5 per cent dilute hydrochloric acid and filtered. The clear filtrate was made distinctly alkaline by the addition of ammonium hydroxide solution (28%) and then extracted by shaking with
chloroform in a separatory funnel. The chloroform extract was con-
centrated and labeled the "tertiary fraction." The remaining alkaline
solution was adjusted to pH 3 with dilute hydrochloric acid and
divided into two equal portions. One portion was treated with an
aqueous saturated solution of picric acid. The precipitate of
alkaloid picrate was collected by filtration, redissolved in methanol
and converted to the chlorides by using the ion exchange resin Amber-
lite IRA 410. The other portion was treated with 2 per cent ammonium
reineckate solution. The precipitate of alkaloid reineckate was
collected by filtration, redissolved in acetone:water (1:1) and
converted to the chloride in the same manner as above.

When the tertiary alkaloid fraction and the two quaternary
alkaloid chloride solutions previously mentioned were spotted on
Whatman 3MM paper and chromatographed using the (AAFI) system, the
chromatogram of the tertiary alkaloid fraction indicated the presence
of two alkaloidal non-fluorescent spots of Rf values 0.50 and 0.58.
The chromatogram of the two quaternary fractions showed the presence
of five well separated alkaloidal spots of Rf 0.30, 0.70, 0.60, 0.46
and 0.38, in addition to the tailing alkaloidal spot at the starting
line.
3. Conclusions

From the foregoing preliminary investigations the following conclusions could be made:

1. The bark of *Z. martinicense* DC. was shown to contain several alkaloids which occur in more than 0.2 per cent based on dry weight of the crude bark.

2. Three of the alkaloids in the bark could be completely extracted in the process of defatting with Skellysolve F. Defatting of the bark of *Z. martinicense*, in contrast to *Z. elephantiasis* should be considered an essential procedure not only for the sake of the extraction of the three alkaloids and the triterpene, but also for facilitating subsequent extraction of the rest of the alkaloidal constituents using U.S.P. alcohol.

3. At least two tertiary and five quaternary alkaloids were revealed on the chromatogram, when the fractionated alcohol extract of the defatted powdered bark of *Z. martinicense* was analyzed by paper chromatography.

B. General Extraction and Fractionation of the Alkaloids in the Bark of *Z. martinicense*

1. Extraction of the Crude Powder with Skellysolve F (b.p. 30-60°)

Defatting of the coarsely ground bark (7.15 Kg.) with Skellysolve F (total 30 liters) was done in a continuous Soxhlet-type extractor capable of holding a maximum of 2.5 kg. at a time. Extraction in each case was allowed to proceed for one week, with a
fresh charge of solvent at the end of the third day. The Skellysolve extract, after each extraction, was concentrated in vacuo at not more than 40° to give a deep greenish-yellow gummy residue. The combined extracts weighed 230 gm.

a) Isolation of the triterpene lupeol

The gummy Skellysolve extract (230 gm.) was dissolved in about 800 ml. of U.S.P. alcohol by the aid of steam bath and was filtered under suction while it was still hot. The solution upon standing for a few days in a cool place deposited a dark semi-crystalline material which, when collected on a filter and washed with cold acetone, gave 8 gm. of white semi-crystalline material. A positive pink-violet Libermann-Burchard's test confirmed its nature. For the purification of this triterpene, the residue was dissolved in hot ethanol, mixed with two grams of activated charcoal and refluxed for 30 minutes. The content of the flask was then rapidly filtered, under suction, while the solution was still hot. Upon cooling, dense white feathery shaped crystals appeared in the solution. After collecting the first crop of the crystalline triterpene, the filtrate was concentrated on a steam-bath and when cooled gave a second crop of crystals having a m.p. of 210-211°C. Several recrystallizations from ethanol were needed before the melting point reached a consistent m.p. of 214°C, which is the same as reported for lupeol (135).
b) The identification of lupeol

The pure crystalline triterpene was identified as lupeol by virtue of comparison of the infrared spectra of lupeol, lupeol benzoate and lupeol acetate with those of authentic samples. The samples used for comparison were kindly supplied by Dr. T. J. Fitzgerald who, in this laboratory, isolated lupeol from the bark of *Sweetia panamensis* (134).

The melting point of lupeol 214°C; of lupeol acetate, 217°C; and of lupeol benzoate 262-4°C were the same as reported in the literature (135). Moreover, the mixed melting point of the isolated lupeol, its benzoate, and its acetate did not show any depression with those of the authentic samples.

c) Isolation of a crude mixture of the alkaloids in the Skellysolve F extract

The alcoholic solution of the gummy Skellysolve F extract, freed from the deposited lupeol, was allowed to drip into a beaker containing one liter of 5 per cent aqueous hydrochloric acid. Mixing of the extract with the acidic solution was done by mechanically stirring at a slow rate. The content of the beaker was then filtered under suction, concentrated under reduced pressure to a volume of one liter, refiltered through a celite bed, and left in the refrigerator overnight. It was noticed that when ether was added to a few ml. of the filtrate, a yellowish-orange, light flocculent alkaloidal precipitate was formed. The whole filtrate was then treated with
ether, about one liter, until apparently no more precipitation was produced. The precipitate was collected on a filter, washed with ether and dried in a desiccator to give 1.1 gm. of an orange-yellow, very light powder. The ether in the filtrate was driven off by allowing a stream of air to pass through the filtrate while the flask was placed under the hood.

The filtrate was then made alkaline to pH 10 (hydrion paper) and continuously extracted with chloroform in a liquid-liquid extractor. When the chloroform was dried over anhydrous sodium sulfate and evaporated to dryness, it left a thick oily residue which showed traces of alkaloids as judged by the visual size of the spots and intensity of the Dragendorff's stain. The amorphous, orange-yellow, light powder, however, when chromatographed showed the presence of three alkaloids, two of which were major in terms of quantity.

d) Separation of two alkaloids from the amorphous orange-yellow mixture of alkaloids

Preliminary attempts to separate the components of the crude amorphous alkaloid mixture (1.1 gm.) indicated that cellulose column chromatography might be useful for this purpose. Cellulose was chosen because when a methanolic solution of the alkaloid mixture was spotted on Whatman 3MM paper and developed in a system of benzene:chloroform (2:1), the two alkaloids possessing the blue fluorescence were found to travel near the solvent front whereas the yellow fluorescent alkaloid remained at the starting line. Other
fluorescent non-alkaloidal impurities were seen distributed in between the origin and solvent front.

For the resolution of the alkaloid components of the mixture, a column (0.5" x 8") was packed with 50 gm. of cellulose powder made into a slurry in a mixture of benzene:chloroform (2:1). Elution was started with the same solvent mixture and the column was checked with ultraviolet light to observe the distribution of the zones and, in particular, the movement of the yellow fluorescent zone down the column. The polarity of the solvent was increased by increasing the percentage of chloroform in the elution solvent mixture. The effluent fractions were checked by thin-layer chromatography using benzene: chloroform (2:1) as a solvent system. The results obtained are shown in Table 8. Fractions 1 to 4 containing the two blue fluorescent alkaloids were combined and concentrated to dryness to give a thick, dark brown, oily liquid weighing 0.42 gm. and was designated (X). Fractions 5 to 12 containing mainly the yellow fluorescent alkaloid were combined and concentrated to give an amorphous yellow residue of 0.45 gm. and was designated (Y).

Attempts to crystallize each of (X) and (Y) fractions failed to give any crystalline material; however, when (Y) was dissolved by boiling in dilute hydrochloric acid and was allowed to cool, slender yellow needle-like crystals were easily formed. The crystals, representing the hydrochloride salt of the yellow fluorescent alkaloid, were very thin and light in weight, 20 mg., m.p. 178°C. Recrystallization from ethanol:methanol mixture gave 12 mg., m.p. 195°C.
<table>
<thead>
<tr>
<th>Solvent</th>
<th>Fraction</th>
<th>Volume</th>
<th>Fluorescence</th>
<th>Alkaloida,b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Benzene:chloroform (2:1)</td>
<td>1</td>
<td>200 ml.</td>
<td>Blue</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>200 ml.</td>
<td>Blue</td>
<td>++</td>
</tr>
<tr>
<td>Benzene:chloroform (1:1)</td>
<td>3</td>
<td>200 ml.</td>
<td>Blue</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>200 ml.</td>
<td>Blue</td>
<td>+</td>
</tr>
<tr>
<td>Benzene:chloroform (1:2)</td>
<td>5</td>
<td>200 ml.</td>
<td>Blue</td>
<td>+</td>
</tr>
<tr>
<td>Chloroform</td>
<td>7</td>
<td>200 ml.</td>
<td>Yellow</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>200 ml.</td>
<td>Yellow</td>
<td>+</td>
</tr>
<tr>
<td>Chloroform: methanol (9:1)</td>
<td>9</td>
<td>200 ml.</td>
<td>Yellow</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>200 ml.</td>
<td>Yellow</td>
<td>+</td>
</tr>
<tr>
<td>Chloroform: methanol (1:1)</td>
<td>11</td>
<td>200 ml.</td>
<td>Yellow</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>200 ml.</td>
<td>Yellow</td>
<td>+</td>
</tr>
</tbody>
</table>

*a* 1, alkaloid, Rf 0.83 blue fluorescence  
2, alkaloid, Rf 0.38 reddish violet fluorescence  
3, alkaloid, Rf 0.2 blue fluorescence.  
As chromatographed on thin-layer alumina plates using a solvent system of benzene:chloroform (2:1).  

*b* Comparative amount of alkaloids indicated by size of spots ++++, ++, +.
The ultraviolet spectrum of the isolated yellow alkaloid hydrochloride gave \( \lambda_{\text{max}} \) methanol 228 mp \( (E^1 \text{ per cent } 433.3) \), 274 mp shoulder \( (E^1 \text{ per cent } 583.3) \), 283 mp \( (E^1 \text{ per cent } 616.6) \) and 320 mp \( (E^1 \text{ per cent } 308.3) \). No shift in alkaline solution was observed; however, the peak of 228 mp and the shoulder at 274 mp disappeared.

The infrared absorption spectra taken in potassium bromide pellet gave the following absorptions: at 3.0 \( \mu \), at 3.4 \( \mu \), at 6.7 \( \mu \), at 7.8 \( \mu \), at 9.0 \( \mu \), and at 9.6 \( \mu \).

The alkaloid gave positive Labat (136) and Hansen (137) tests for the methylenedioxy group.

For the resolution of the alkaloid components of fraction (X), a column (0.25" x 10") was packed with 30 gm. of Woelm neutral alumina grade I. Elution was started with benzene, 3 fractions each of 150 ml. and then with benzene 5, 10 and 20 per cent methanol, each 200 ml.

When the effluents were concentrated and chromatographed on thin-layer alumina plates, in the same manner as described previously, the first benzene effluent fraction did not show any alkaloid; however, the second and third benzene fractions contained one blue fluorescent alkaloid of \( R_f 0.83 \). From the two combined fractions, only 5 mg. of a colorless crystalline alkaloid was obtained. The remainder of the fractions of the effluents were shown to contain negligible amounts of the mixture of the two blue fluorescent alkaloids.
2. Extraction of the Defatted Powder with U.S.P. Alcohol

The defatted powdered bark of *Z. martinicense* (7.15 Kg) was placed in a large glass tank and macerated with 10 liters of U.S.P. alcohol for a period of three days. At the end of the maceration period, the supernatant menstrum (3 liters) was decanted, filtered under suction, and saved. The wet powder was then packed in an all glass cylindrical-type percolator (6" x 32"). A fresh charge of 10 liters of U.S.P. alcohol containing 50 ml. of concentrated hydrochloric acid was introduced into the percolator, and percolation was allowed to proceed at a rate of 30 drops per minute. Another 10 liters of U.S.P. alcohol, free from hydrochloric acid, were needed to completely extract the alkaloids from the bark.

a) Isolation and identification of sucrose

The alcohol extract (3 liters), resulting from the maceration of the defatted crude powder, was concentrated to about 500 ml. in vacuo at 40°C and allowed to stand undisturbed overnight. About 8 gm. of the crystalline material was removed by filtration, purified by washing with cold ethanol and recrystallized several times from methanol, m.p. 185-186°C.

The identity of the isolated crystals with sucrose was based on:

1. Similarity in melting point; authentic sucrose melts at 185°C (138).

2. Similarity of *R*ₚ value of the compound and its hydrolysates with that of sucrose and its hydrolysis products. Glucose and
fructose were used as controls. The solvent system consisted of the upper phase of a mixture of ethyl acetate, glacial acetic acid and water (3:1:3). Whatman No. 1 chromatographic paper was used as a support.

Final confirmation of the identity of the crystalline compound as sucrose was provided by comparison of its infrared spectrum with that of an authentic sucrose sample. Both spectra, taken in potassium bromide pellet were superimposable and showed a broad hydroxyl band at 3.08 μ.

b) Treatment of the alcohol extract

The combined alcohol percolate was concentrated in vacuo at not more than 45°C, to a thick syrupy consistency weighing about 896 gm. This syrupy extract was well mixed with a total of four liters of 5 per cent aqueous hydrochloric acid in the manner described on page 63. The resulting acid extract was concentrated under reduced pressure to about two liters, was filtered and adjusted to pH 2 followed by extraction with chloroform using a liquid-liquid extractor. The chloroform extract, thus obtained, was dried over anhydrous sulfate, and then evaporated to complete dryness in vacuo to give an amorphous light brown granular residue, 48 gm., designated "Residue A." The mother acid solution, after extraction with chloroform, was made alkaline with ammonium hydroxide solution (28%) and exhaustively extracted with chloroform in the same manner as above. The resulting dark brown dried residue (12 gm.) was designated "Residue B."
The alkaline mother solution was made acid to pH 3 and the quaternary alkaloids were precipitated as reineckates in the usual manner (page 67). The dried reineckate weighted about 120 gm.

c) Treatment of "Residue A" and isolation of alkaloids from it

"Residue A," 48 gm., was dissolved in 600 ml. of a 2 per cent aqueous hydrochloric acid by the aid of boiling on a steam bath (only 35 gm. went into solution). The solution obtained was filtered hot and the filtrate was allowed to cool overnight. A slight orange precipitate appeared in the solution, which was filtered and saved. The filtrate was adjusted to pH 2 and extracted thoroughly with ethyl ether in a liquid-liquid extractor. The resulting ether extractive was concentrated to dryness (2 gm.) and labelled Ea (ether acid). The mother acidic solution was then adjusted to pH 9-10 by the addition of ammonium hydroxide solution (28%) and again extracted thoroughly with ether in the same liquid-liquid extractor. The ether concentrated extract (4 gm.) was labelled Eb (ether base). Both ether extracts, Ea and Eb, when chromatographed on thin-layer silica Gel G plates using the (EAA) system, showed several non-alkaloid blue fluorescent spots, in addition to traces of the same two alkaloids previously isolated from the Skellysolve extract. The presence of these two alkaloids in the alcohol extract was probably due to incomplete defatting of the starting material.

The mother alkaline solution, after extraction with ether, was then thoroughly extracted with chloroform in a liquid-liquid
extractor. When the chloroform extract was dried over anhydrous sodium sulfate and brought to complete dryness in vacuo about 10 gm. of a granular light-colored tertiary alkaloid residue was obtained. The alkaloidal residue (10 gm.) was dissolved in 300 ml. of benzene: methanol (100:5) mixture to give a light brownish solution. When this solution was allowed to concentrate by evaporation on a steam-bath, a dense floculent precipitate of white flake-like crystals was formed. The solution was then cooled at room temperature and filtered under suction to give the first crop of tertiary alkaloid residue. The mother filtrate was repeatedly treated in the same manner as described previously, to give three more crops of alkaloidal residue. The total weight of the four crops amounted to about 8.9 gm.

Horizontal paper chromatography of a sample of the tertiary alkaloidal residue, using the (AAARH) system revealed the presence of two alkaloidal spots of close Rf values, 0.50 and 0.58.

In searching for an effective and rapid chromatographic technique for the resolution of the tertiary alkaloid mixture, thin-layer chromatography on Silica Gel G plates was found to be comparatively more successful than either Alumina Gel G plates or microcellulose, Avicel10 plates. Of the several solvent systems tried, only those which were very polar affected separation. On Silica Gel G plates, a system of absolute ethanol, ammonium hydroxide solution (28%) (2:15) designated as (EA) system was found to resolve the components

10Technical grade for thin-layer chromatography, FMC Corporation, Newark, Delaware.
of the tertiary mixture into three alkaloidal spots; however, the three spots were very close to one another, \( R_f \) values 0.88, 0.76, and 0.60.

As a result of trials done to improve the resolution of the (EA) system, it was noticed that the addition of acetone to the (EA) system caused a drastic change in the chromatographic pattern, the mobility of the components of the tertiary alkaloid mixture was variously affected. When a mixture of 10 parts of (EA) system was added to 15 parts of acetone and the resulting solvent system, designated as (EAA), was freshly used, the chromatographic pattern obtained on Silica Gel G plates revealed that the tertiary alkaloid mixture consisted of at least four different alkaloidal spots, two of which appeared as major ones.

The mother solutions, from which the tertiary alkaloid residue was obtained, were combined, evaporated to dryness, and saved for future work.

d) Purification of "Residue B" and isolation of alkaloids from it

In an attempt to separate the components of "Residue B," a sample (200 mg.) was chromatographed on a 10 gm. column of Woelm acid alumina grade III. It was observed that elution of the column with ethyl acetate and ethyl acetate containing up to 5 per cent methanol removed undesirable non-alkaloidal material. Ethyl acetate containing 10 per cent methanol increasing to 30 per cent eluted the
alkaloids as a mixture. The evaporation of the effluents left a
colorless amorphous alkaloidal residue. Although this small scale
experiment failed to give separation of individual components, it
was considered useful for the purification of "Residue B." Thus,
"Residue B" (12 gm.) was placed on a column (1.75" x 15") containing
500 gm. of Woelm acid alumina, grade III, and first eluted with ethyl
acetate, 1000 ml.; then with ethyl acetate containing 5 per cent
methanol, 500 ml.; followed with ethyl acetate containing 10, 20
and 30 per cent methanol, 1000 ml. each, respectively. Evaporation
of the effluents in vacuo produced a total of 4.9 gm. of clean
tertiary alkaloidal mixture.

The isolation of 4.9 gm. of tertiary alkaloids from "Residue B"
and 8.9 gm. for Residue A" (page 94) brought to a total of 13.8 gm.
of tertiary alkaloid mixture which were originally extracted from
7.15 kg. of the crude bark. The approximate percentage of the
tertiary alkaloid mixture in the crude bark of Z. martinicense would
therefore amount to about 0.18 based on dry weight.

e) Properties of the isolated
tertiary alkaloid mixture

All attempts made to separate the components of the tertiary
alkaloid mixture, by such techniques as gradient pH extraction,
adsorption, partition, and gradient elution chromatographic methods,
were not successful.

Except for a mixture of benzene:methanol (100:5), the crystal-
ization of this alkaloid mixture would have remained a problem.
The tertiary alkaloid mixture, however, was easily crystallized from benzene:methanol (100:5) into colorless crystalline form, the crystals melted with decomposition at a range from 220° to 228°C.

The infrared spectra of the dried crystalline mixture in potassium bromide pellet showed absorption at 3 μ indicating the presence of a hydroxyl group; however, when the spectrum was taken in chloroform no such absorption was seen. The presence of a hydroxyl group was ruled out as a result of the following tests.

1. The alkaloid mixture failed to give any color with an aqueous, alcoholic or chloroformic (5%) solution of ferric chloride.

2. When a methanolic solution of the mixture was spotted on paper and the dried spot sprayed with 2% phosphomolybodic acid solution in acetone:water (1:1) followed by exposure to vapors of concentrated ammonium hydroxide solution, no blue-black color was formed indicating the absence of hindered phenolic group.

3. The mixture failed to give an acetate and was recovered unchanged at the end of the reaction. This was confirmed by checking the melting point which was found unchanged as well as the infrared spectra.

4. The mixture remained unchanged when its methanolic solution was treated with an excess of an ethereal solution of diazomethane and left in contact, for three days, at room temperature.

5. The ultraviolet spectrum of the mixture taken in neutral methanol did not show any shift when rendered alkaline. The mixture
showed absorption at 212 μm (E 1 per cm. 120.3), 231-233 μm shoulder (E 1 per cm. 69.5), and at 285-287 μm (E 1 per cm. 43.5).

The presence of methylenedioxy group in the mixture was indicated by positive Labat and Hansen tests (136-137).

f) Conversion of the quaternary alkaloid reineckate to chlorides

Seventy gm. of a total of 120 gm. of crude quaternary alkaloid reineckate were converted to alkaloid chlorides using Amberlite IRA 410 ion exchange resin, chloride form.

The crude reineckate was extracted with acetone, by stirring with three 400 ml. portions, leaving behind about 15 gm. of acetone insoluble non-alkaloidal residue. The acetone solution was concentrated to about 500 ml. in vacuo followed by the addition of an equal volume of water. This solution was then added, dropwise, to about 1200 gm. of Amberlite IRA 410 ion exchange resin in the form of its chloride, suspended in 2000 ml. of acetone:water (1:1). Mixing of the dripping extract with the resin was done by mechanical stirring at a slow rate for 24 hours. At the end of this period the mixture was filtered under suction to give a yellowish brown filtrate. The resin was then washed by macerating and stirring for two hours in 1000 ml. of methanol:water (1:1). The filtered washing was combined with the original filtrate and concentrated in vacuo at not more than 40° to a volume of 800 ml. Lyophilization of the concentrated extract gave a tan-colored powder which, as soon as it came in contact
with air, was found to liquify and turn dark brown. The residue weighed about 18 gm. and dissolved completely in methanol.

Horizontal paper chromatography of the methanolic extract using 3MM Whatman paper and the solvent system (AAFH) revealed the presence of five quaternary alkaloid chlorides. In a search for a more rapid, but not less effective chromatographic system than the (AAFH) system (10-12 hours development), thin-layer chromatography on Silica Gel G was tried. Of the many solvent systems tried, propanol:ammonium hydroxide solution (28%) (1:1) designated (PE) system was found to be best. Resolution of the quaternary chlorides with this system showed five alkaloidal spots of $R_f$, 0.72, 0.64, 0.53 (blue fluorescent), 0.44, and 0.32. This was in addition to the presence of a spot behaving like choline $R_f$ 0.06.

g) Purification of the crude quaternary chloride and isolation of its alkaloids

The methanolic extract containing about 18 gm. of crude quaternary alkaloid chloride was chromatographed on a column (2" x 30") packed with one kg. of deactivated Woelm acid alumina. Deactivation of the alumina was done by shaking thoroughly one kg. of acid alumina, grade I, in a two liter conical flask, with successive portions of 100 ml. of benzene:methanol (100:5) until no more heat was generated as a result of the deactivation. The alumina was then treated with a sufficient quantity of the same solvent mixture to produce a slurry which was then used for the packing of the column.
The packed column was allowed to equilibrate for two days and was then topped with 80 gm. of the deactivated alumina on which the crude quaternary chloride was adsorbed.

The column was first eluted with about 5 liters of a mixture of benzene:methanol (100:5), the effluents being collected in ten fractions, each 500 ml. These effluents were found to be devoid of any alkaloid. However, when the column was eluted with another five liters of a mixture of benzene:methanol (100:15) and the effluents evaporated to dryness, they gave a total of 7.47 gm. of an amorphous, slightly yellowish powder of quaternary chloride mixture (five alkaloids).

Further elution of the column with two liters of a mixture of benzene:methanol (50:50) gave only an additional 0.3 gm. of the same mixture, the last portions of effluent being devoid of alkaloids.

h) Isolation of one quaternary chloride from the quaternary alkaloid mixture

Although fractional crystallization of the quaternary alkaloid mixture was not possible from a number of solvents, yet when the mixture was dissolved in absolute ethanol:ethyl acetate (1:1) and concentrated by evaporation on a steam bath, a small amount of crystalline material separated out. Repeated fractional crystallization led to the isolation of about 80 mg. of this alkaloidal crystalline material which was shown on Silica Gel G thin-layer chromatography using the (P.E.) system to give one spot, Rp 0.32. From the
comparative size of the spot, the isolated alkaloid chloride could be considered a minor constituent.

i) Identification of the quaternary chloride (candicine chloride)

The quaternary chloride crystallized from absolute ethanol: ethyl acetate (1:1) in the form of colorless microcrystals. The crystals melted with decomposition at 276-278°C. Analysis calculated for: C_{11}H_{17}NOCl: C, 61.24; H, 8.36; Cl, 16.43; N, 6.49; O, 7.30. Found: C, 61.05; H, 8.46; Cl, 16.53; N, 6.68; O, 7.26. The infra-red spectrum taken in potassium bromide pellet showed weak absorption at 2.9 μ (-OH), and strong absorptions at 3.15 μ, 6.2 μ (aromatic ring), 6.6 μ, 8.05 μ, 8.2 μ, 11.1 μ and 12.1 μ (Figure 12).

The ultraviolet absorption spectrum in neutral methanolic solution gave \( \lambda_{\text{max}} \) 277 μ (log E 3.13), 225 μ (log E 3.96). In alkaline solution the absorption bands were bathochromically shifted 21 μ and increased in intensities. The ultraviolet spectrum gave \( \lambda_{\text{max, methanol}} \) 297 μ (log E 3.43) and 246 μ (log E 4.1).

The shift in the alkaline solution confirmed the presence of a phenolic hydroxy group as indicated in the infrared absorption spectrum.

The ultraviolet absorption spectrum of the quaternary chloride was indicative of a simple \( \beta \)-phenethyl amine (139) alkaloid.

To the author's knowledge no quaternary simple \( \beta \)-phenethyl amine alkaloid was reported from the genus Zanthoxylum; however,
these types of alkaloids were mainly reported to be in the Cactaceae family (140).

One of the cactus alkaloids, candicine \( \text{C}_{14}\text{H}_{18}\text{ON}^+ \) (\( \alpha \)-p-hydroxyphenethyl trimethyl ammonium hydroxide) was found to fit the analytical data obtained for the isolated quaternary alkaloid. However, there exists also an isomer of candicine which has the hydroxyl group in the meta- instead of in the para position. This isomer is the alkaloid leptodactyline isolated in 1953 from the skin of two South American amphibians, \textit{Leptodactylus pentadactylus} and \textit{L. ocellatus} (141). Candicine, like other p-hydroxy compounds, such as tyrosine and tyramine, was reported to give a positive red color with Millon's\(^{11}\) reagent (100). This test was also positive with the isolated alkaloid, which indicated that the alkaloid in question might be candicine chloride, reported m.p. 285\(^0\)C with decomposition.

The identity of the isolated alkaloid was established as candicine chloride by virtue of comparison of its melting point, its mixed melting point and its infrared spectrum with candicine chloride (m.p. 278\(^0\)C with decomposition), prepared from hordenine sulfate following the procedure reported by Nakano (142).

\( j \) Synthesis of candicine chloride from hordenine sulfate

1) Extraction of hordenine base from the sulfate. -- An amount of 2 gm. of hordenine sulfate\(^{12}\) dissolved in 10 ml. of distilled

\(^{11}\) Millon's reagent gives a red color with protein, the phenolic group in tyrosine is functionally responsible for the formation of this red color with protein.

\(^{12}\) Merck and Co., Incorporation, Rahway, New Jersey.
water was transferred to a separatory funnel containing 50 ml. of ether. The aqueous layer was made distinctly alkaline by the addition of dilute 10 per cent ammonium hydroxide solution. The precipitate which formed was found to dissolve rapidly in the ether by gentle shaking. The ether layer was separated and the aqueous phase was repeatedly extracted with three or more portions of 50 ml. ether. The combined ether extracts were washed with distilled water, dried over anhydrous sodium sulfate, and concentrated on a steam-bath until hordenine crystallized out. Recrystallization from ethanol gave 1.7 gm. of a crystalline base, m.p. 118°C, reported m.p. for hordenine 117-118°C (145).

2) Preparation of hordenine methiodide (candicine iodide). — An amount of 1.2 gm. of hordenine base dissolved in 25 ml. of methanol was refluxed with 4 ml. of methyl iodide for 30 minutes. The reaction mixture was evaporated until a slightly yellowish crude candicine iodide crystallized. Recrystallization from methanol gave 1.4 gm. of colorless prismatic crystals of candicine iodide, m.p. 231-232°C., reported 230-231-234°C (142).

3) Conversion of candicine iodide to candicine chloride. — To a solution of 0.5 gm. of candicine iodide in 15 ml. of methanol a suspension of freshly prepared silver chloride (0.25 gm. in 10 ml. of distilled water) was added and the mixture was stirred by means of a magnetic stirrer for 40 minutes. At the end of this period, the solution containing the precipitated silver iodide and the excess
of silver chloride was filtered under suction. The filtrate, which contained candicine chloride, was evaporated in vacuo to complete dryness. The residue was crystallized from absolute ethanol to give 0.32 gm. of candicine chloride, m.p. 278°C with decomposition, reported m.p. 285 dec. (100).

Figure 8
Structure of the Quaternary Chloride Isolated from the Bark of Z. martinicense

Candicine chloride
DISCUSSION

The occurrence of alkaloids of the canthinone type in plants was first reported in 1952 when Haynes, Nelson, and Price described the isolation of 6-canthinone (65), 5-methoxy-6-canthinone (65) and 4-methylthio-6-canthinone (66) from Pentaceras australis Hook (Rutaceae). In a subsequent study of three Australian Zanthoxylum species, Cannon, Hughes, Ritchie, and Taylor reported the isolation of 6-canthinone from Z. suberosum C. T. White (67). The isolation of canthinones from the bark of Z. elephantiasis marks the second report of the occurrence of this type of alkaloid in a species of Zanthoxylum.

There have been recent reports of the isolation of canthinone alkaloids from plants belonging to a family other than the Rutaceae. In 1961, 4,5-dimethoxy-6-canthinone (68) was isolated from Picrasma ailanthoides (Simarubaceae); in 1965, 4-methoxy-6-canthinone (69) was isolated from Charpentiera obovata Gand. (Amaranthaceae).

The quaternary aporphine alkaloid, laurifoline, was isolated for the first time from Cocculus laurifolius (Menispermaceae) by Tomita and Kusuda (78). The presence of aporphine alkaloids in the genus Zanthoxylum is not uncommon, but the isolation of laurifoline from Z. elephantiasis represents only the third report of the occurrence of laurifoline in a Zanthoxylum species. Tomita and Ishii (79)
reported, in 1958, the isolation of laurifoline from the bark of Z. ailanthoides. The only other isolation of laurifoline from any plant is from the bark of Fagara pterotæ L. (Zanthoxylum pterotæ H.B.K.) (76). Recently, in a private communication with Deulofeu,¹ he stated that laurifoline has been isolated from two other species of the Fagara growing in Argentina; however, the work is still unpublished (127).

The first recorded isolation of the quaternary β-phenethyl amine alkaloid, candidine, was reported by Reti (140) in 1933 to occur in the Argentine cactus Trichocereus candicans (Cactaceae). Three other members of the genus Trichocereus were next reported to contain candidine. The isolation of candidine from plants belonging to a family other than the Cactaceae was first reported in 1954 by Nakano (142) in his studies of the alkaloids of Magnolia grandiflora L. (Magnoliaceae). By 1958, Rabitzsch (143) reported that among other hydroxyphenyl alkylamines, candidine was detected in germinating barley seedling roots, Hordeum vulgare. Unlike the other alkaloids detected, candidine appeared only on the third day of germination and completely disappeared after 25 days. Price (101), in his discussion of the alkaloids of the Rutaceae, mentioned the occurrence of candidine in the bark of Phellodendron amurense. The isolation of candidine in this laboratory from the bark of Z. martinicense marks, to the author's knowledge, its first occurrence in a member

¹ V. Deulofeu, Department of Organic Chemistry, Faculty of Sciences, Buenos Aires, Argentina.
of the genus Z. or the genus F. (Rutaceae). However, it was not until the author’s private communication with Deulofeu that he was informed of the work, still unpublished, about the isolation of candicine from seven Fagara species in Argentina. Deulofeu also stated that coryneine (3-hydroxy-candicine) was isolated along with candicine from one of the studied species.
SUMMARY

A survey regarding the taxonomy and the geographical distribution of the genus Zanthoxylum L. and its closely related genus Fagara L. has been presented. Particular emphasis has been made on the nomenclatural confusion existing between the members of the two genera. Reference has been made to two excellent articles which recently clarified the relationship of Z. and F. in such a way that today less confusion exists.

A complete and up-to-date literature survey of the alkaloids in both genera Z. and F. has also been presented. A general discussion of the five main classes of alkaloids isolated and fully characterized has been given. Recently proposed structural revisions of some aporphine alkaloids and differences in opinions about the structure of one quinazolino-carboline alkaloid have been included. The survey was supplemented with two tables; in one, a compilation of all the studied species and their contained alkaloids was presented, and in the other, some of the physical data of the alkaloids and their derivatives were described.

Detailed preliminary investigation of the alkaloidal contents of the bark of two Z. species: Z. elephantiasis Macf. and Z. martinicense DC. has been described and the results discussed.
Horizontal paper chromatography demonstrated the occurrence of six alkaloids in the bark of the first species and at least ten alkaloids in the bark of the second species. Both species were shown to contain each, tertiary and quaternary alkaloid content, of more than 0.1 per cent.

The extraction, fractionation, isolation, and purification of single and mixtures of alkaloids have been reported.

The use of ion exchange resin, Amberlite IRA 410, for the conversion of the quaternary alkaloid reineckate to chloride was successfully used and described. The method can be regarded as simple, rapid and dependable for the quaternary alkaloids of the two Z. species.

The three crystalline alkaloids, 6-canthinone, 5-methoxy-6-canthinone, and laurifoline chloride, isolated from the bark of Z. elephantiasis were identified on the basis of comparison of their melting points, ultraviolet and infrared spectra with authentic samples. The n.m.r. spectra of the alkaloids have been also reported.

The minor quaternary alkaloid, candicine chloride, isolated from the bark of Z. martinicense, was identified by virtue of comparison of its melting point, its mixed melting point, and its infrared spectra with candicine chloride prepared from hordenine.

The defatting of the crude bark of Z. martinicense, the fractionation of the extract and the isolation, by the use of cellulose and alumina column, of two crystalline alkaloids has been described. A non-alkaloidal Skellysolve F soluble triterpene, giving
a pink violet color with Liebermann-Burchard's test was isolated and identified as lupeol. Melting points, mixed melting point, infrared spectrum of lupeol, its prepared benzoate and acetate, were identical with those of authentic samples of lupeol, its benzoate and acetate.

The isolation and identification of sucrose from the alcohol macerate of the defatted powder of Z. martinicense has been reported.

The isolation of a pure crystalline mixture of tertiary alkaloids from the bark of Z. martinicense, in a yield of about 0.18 per cent, has been described. Likewise, the isolation, in a yield of about 0.15 per cent, of an amorphous mixture of quaternary alkaloids has been effected.
Infrared Spectra of Base I, 5-methoxy-6-canthinone, (A), and an Authentic Sample of 5-methoxy-6-canthinone, (B).
Infrared Spectra of Base II, 6-canthinone, (A), and an Authentic Sample of 6-canthinone, (B).
Figure 11

(A)

(B)

Infrared Spectra of the Isolated Laurifoline Chloride, (A), and an Authentic Sample of Laurifoline Chloride, (B).
Infrared Spectra of the Isolated Candicine Chloride, (A), and that Synthesized from Hordenine, (B).
Chart II

Flow Sheet for the Extraction and Fractionation of the Alkaloids in *Z. elephantiasis* Bark

1.3 Kg. powdered bark
- extracted with 95% U.S.P. alcohol
- extract concentrated to syrup
- syrup extracted with 2% tartaric acid

Residue 35 gm.

Acid Solution
- NH₄OH (28%)  
- CHCl₃

Acid Solution
- CHCl₃ extract
  - 2% NaOH

Alkaline Solution
- made acid to pH 3
- ppt'd alkaloids as reineckates

Crude Alkaloid Reinekate

Crude Alkaloid Chloride

Laurifoline Chloride

Acid solution was treated in the same manner as Acid solution to give additional amounts of Base I and Base II.
CHART III

Flow Sheet for the Extraction and Fractionation of the Alkaloids in Z. marticense Bark

7.15 Kg. powdered bark

defatted with Skellysolve F

Dried marc.
- macerated with 10 liters of U.S.P. alcohol, macerate
- percolated with 10 liters of U.S.P. alcohol containing 50 ml. conc. HCl
- percolated with 10 liters of U.S.P. alcohol

Combined macerate and percolates
- conc. to syrupy consistency
- extracted with 5% HCl
- acid extracts conc. to 2 liters
- pH adjusted to 2
- extracted with CHCl₃ continuously

Skellysolve F extract conc'd

gummy extract (230 gm)

Alcohol Solution

Acid Solution

The addition of ether to the concentrated acid solution gave rise to an orange-yellow alkaloidal precipitate (1.1 gm).
Two alkaloids were obtained in crystalline form by fractionation first on a cellulose and then on an alumina column.
Ea and Eb contained traces of the minor alkaloids of the Skellysolve extracts.
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