

of Sumerwell (5) as described by Tewari and co-workers (8). Each fraction was analyzed for iodine values (I.V.) and saponification equivalent (S.E.). The composition of fractions was calculated by the method of Hilditch (7) and the result recorded in Table I.

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Keyphrases

Cucurbita maxima seed oil
Fatty acid fractionation
Countercurrent distribution with urea
Iodine values
Neutralization value
Saponification equivalents

Isolation of Candicine Chloride, Laurifoline Chloride, and Xanthoxyletin from the Bark of *Zanthoxylum elephantiasis*

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A study of the quaternary alkaloids of the bark of *Zanthoxylum elephantiasis* was made. Utilizing a silicic acid and diatomaceous earth (4:1) chromatographic column, the quaternary alkaloids laurifoline chloride and candicine chloride were separated and identified. In addition, xanthoxyletin was isolated from a petroleum ether extract of the bark. Its identification was made by physical and chemical data.

IN A PREVIOUS publication (1) the presence of quaternary bases in the bark of *Zanthoxylum elephantiasis* Macf. was reported. From the mixture of quaternary bases, only laurifoline chloride was isolated in small quantity by a rather unsatisfactory procedure. An improved procedure for the separation of laurifoline chloride from the mixture and, in addition, the isolation and identification of candicine chloride from the same mixture are reported here.

The petroleum ether extract of the powdered bark when chromatographed on acid alumina with benzene as eluting agent yielded xanthoxyletin (I) previously reported in a species of *Zanthoxylum* (2). Its identity was established by comparison of its physical properties with those reported in the literature and the preparation of the dihydroxanthoxyletin (II).

The NMR spectra of xanthoxyletin is consistent with its structure showing a singlet at $\delta = 1.47$ (6H) for the two methoxy groups at C-2', a singlet at 3.88 (3H) for the methoxy group, and one at 6.52 (1H) for the aromatic proton at C-8. The protons at C-3 and C-4 appeared as a pair of doublets at $\delta = 6.20$ and 7.87 ($J = 9.5$ c.p.s.), respectively, and those at C-3' and C-4' also as a pair of doublets were found at $\delta = 5.73$ and 6.61 ($J = 10$ c.p.s.), respectively.

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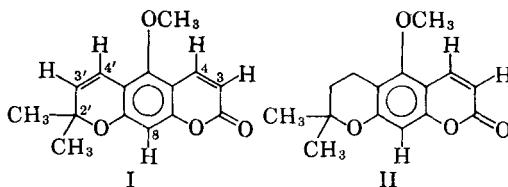
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In dihydroxanthoxyletin the singlet for the two methyl groups at C-2' was shifted upfield to $\delta = 1.38$ and the pair of doublets for C-3' and C-4' were lost and now appeared as a pair of triplets centered at $\delta = 1.82$ (2H) and 2.79 (2H), respectively. The other peaks of the spectrum remained essentially unchanged.

EXPERIMENTAL¹

Separation of Candicine Chloride and Laurifoline Chloride—A quantity of 550 mg. of the mixture of quaternary alkaloid chloride salts obtained by chromatography on alumina as described in the previous publication (1) was dissolved in methanol and adsorbed on 5 Gm. of silicic acid and diatoma-

¹ The melting points are uncorrected and were determined using a Thomas-Hoover Unit-Melt capillary melting point apparatus. The infrared spectra were taken in KBr pellets using a Perkin-Elmer model 237 infrared spectrophotometer. The ultraviolet spectra were determined using a Cary model 15 spectrophotometer. Optical rotations were measured on a Zeiss-Winkel polarimeter in a 2-decimeter polarographic tube. The NMR spectra were determined on a Varian A-60 spectrometer in deuteriochloroform with tetramethylsilane as internal standard. Chemical shifts are reported in δ (p.p.m.) values. Thin-layer chromatography utilized Silica Gel G (Merck) as the adsorbent and the following solvent systems: 1, methanol-water-ammonium hydroxide solution (27%) (2:1:1), and 2, methanol-water-ammonium hydroxide solution (27%) (1:2:2). Dragendorff's test solution was used for the detection of the alkaloids. Silicic acid was obtained from Mallinckrodt Corp., St. Louis, Mo.

TABLE I—SILICIC ACID AND DIATOMACEOUS EARTH CHROMATOGRAPHY OF QUATERNARY ALKALOIDS

Fraction No.	Eluant	Quantity, ml.	Alkaloid, mg.
1	Chloroform	500	...
2	Chloroform-methanol (10%)	1000	...
3	Chloroform-methanol (20%)	500	...
4	Chloroform-methanol (30%)	400	...
5	Chloroform-methanol (30%)	100	Trace, laurifoline chloride
6	Chloroform-methanol (40%)	100	86, laurifoline chloride
7	Chloroform-methanol (40%)	100	62, laurifoline chloride
8	Chloroform-methanol (40%)	100	84, mixture of laurifoline chloride and candicine chloride
9	Chloroform-methanol (40%)	100	77, mixture of laurifoline chloride and candicine chloride
10	Chloroform-methanol (50%)	300	220, candicine chloride
11	Chloroform-methanol (70%)	100	Trace, candicine chloride

ceous earth² (4:1). This was placed on top of a column (2.6 cm. diameter) of 100 Gm. of the same adsorbent which was prepared in the manner described in a previous publication (3). The results are given in Table I.

Fractions 6 and 7 were dissolved in methanol, filtered, and acetone added to point of cloudiness to obtain 90 mg. of laurifoline chloride, m.p. 249–251° dec., $[\alpha]_D^{29} + 40^\circ$ (c 0.985 in methanol). The compound was identical in IR, UV, and R_f (0.16 for solvent system 1 and 0.68 for 2) with that of an authentic sample.

The material in fraction 10 was crystallized from methanol-ether to give 180 mg. of candicine chloride, m.p. 278–279° dec. The IR, UV, and R_f (0.23 for solvent system 1 and 0.75 for 2) were the same with those values of an authentic sample.

Isolation of Xanthoxyletin—The residue from the petroleum ether extract was placed on a column of acid alumina (Woelm, activity grade I, 100 Gm.) and elution started with benzene. Elution with benzene yielded xanthoxyletin (2 Gm.) which crystallized from a mixture of chloroform and diethyl ether, m.p. 133–134° [lit. (2) m.p. 133°].

Anal.—Calcd. for $C_{15}H_{14}O_4$: C, 69.75; H, 5.46. Found: C, 70.28; H, 5.58.

UV spectrum: λ_{max}^{MeOH} 221 m μ (log $\epsilon = 4.37$); 269 (4.42), 346 (4.10), unaffected by dilute acid or alkali.

Hydrogenation of 165 mg. of xanthoxyletin with 50 mg. of PtO₂ in 12 ml. of ethanol showed an uptake of 1 M equivalent of hydrogen and the crystalline dihydroxanthoxyletin (120 mg.) was obtained from ethanol-diethyl ether, m.p. 145–146°; [lit. (2) m.p. 144.5–145.5°]; UV spectrum showed λ_{max}^{MeOH} 332 m μ (log $\epsilon = 4.15$).

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Keyphrases

Alkaloids, quaternary—*Zanthoxylum elephantiasis*
 Candicine Cl, Laurifoline Cl, and Xanthoxyletin—isolation
 Column chromatography—separation
 NMR spectrometry
 UV spectrophotometry—structure
 Optical rotation—identification

² Celite 545. Johns-Manville Corp., New York, N. Y.