TABLE I.—SEDIMENTATION COEFFICIENTS (S) AND PEAK AREAS (cm.^{2}) FOR AMPHOTERICIN B MICELLES WHEN AMPHOTERICIN CONCENTRATION IS KEPT CONSTANT (100 mcg./ml.) AND CETYL DIMETHYL BENZYL AMMONIUM CHLORIDE AND DMSO LEVELS ARE VARIED

Cetyl Dimethyl Benzyl Ammonium Chloride, mcg./ml.	DMSO, %[a]					
	0.25	0.50	1	2	4	8
12.5	88[b] (0.008)[c]	38 (0.012)	23 (0.017)	31 (0.016)	60 (0.009)	662 (0.079)
25	43 (0.011)	21 (0.007)	13 (0.007)	9 (0.007)	28 (0.011)	437 (0.149)
50	23 (0.016)	12 (0.003)	23 (0.008)	20 (0.005)	24 (0.057)	58 (0.011)
100	34 (0.011)	31 (0.012)	24 (0.017)	46 (0.024)	76 (0.036)	42 (0.014)
200	47 (0.012)	37 (0.010)	41 (0.008)	67 (0.038)	139 (0.060)	140 (0.145)

[a] DMSO, dimethylsulfoxide. [b] S, sedimentation coefficient. [c] Peak area.

TABLE II.—SEDIMENTATION COEFFICIENTS (S) AND PEAK AREAS (cm.^{2}) FOR AMPHOTERICIN B MICELLES WHEN DMSO[a] CONCENTRATION IS KEPT CONSTANT (1%) AND CETYL DIMETHYL BENZYL AMMONIUM CHLORIDE AND AMPHOTERICIN B LEVELS ARE VARIED

Cetyl Dimethyl Benzyl Ammonium Chloride, mcg./ml.	Amphotericin B, mcg./ml.					
	12.5	25	50	100	200	400
12.5	160[b] (0.04)[c]					207 (0.020)
25		269 (0.007)			54 (0.014)	
50	91 (0.08)		58 (0.006)	24 (0.004)		49 (0.048)
100			53 (0.006)	25 (0.004)		
200		196 (0.011)			77 (0.008)	
400	485 (0.026)					331 (0.070)

[a] DMSO, dimethylsulfoxide. [b] S, sedimentation coefficient. [c] Peak area.

These results show that in formulating an aqueous system for a water-insoluble substance the concentrations of the substance and other components of the system are critical in determining the physical characteristics of a molecular species. The results suggest that physical chemical analysis, with an analytical ultracentrifuge, as used in these studies, can help select the more stable aqueous system for a relatively water-insoluble substance. This method proved practical when a stable water-miscible amphotericin B formulation was prepared as medication in the drinking water of poultry (5). Using this procedure, other water-insoluble substances, such as the polyene antifungal antibiotic nystatin, can be formulated into water-miscible systems.

REFERENCES

(1) Donovick, R., Steinberg, B. A., Dutcher, J. D., and Vandeputte, J., *Del Giornale Microbiologia*, **2**, 147(1956).
(2) Zimm, B. H., and Crothers, D. M., *Proc. Natl. Acad. Sci.*, **48**, 905(1962).
(3) Archibald, W. J., *J. Phys. Colloid Chem.*, **51**, 1204 (1947).
(4) Engelberg, J., *Anal. Biochem.*, **6**, 530(1963).
(5) Kahn, S. G., and Slocum, A., *Poultry Sci.*, **45**, 761 (1966).

Isolation and Identification of Three Alkaloids from the Bark of *Zanthoxylum elephantiasis*

By ALBERT T. AWAD, JACK L. BEAL, SUNIL K. TALAPATRA*, and MICHAEL P. CAVA†

The bark of *Zanthoxylum elephantiasis* (*Rutaceae*) was found to contain three major alkaloids which were identified as 6-canthinone, 5-methoxy-6-canthinone, and laurifoline.

THE GENUS *Zanthoxylum* (*Rutaceae*) contains some 200 species dispersed over the world, many of which have been studied because of their alkaloid content. Price (1) in an excellent review of the distribution of alkaloids in the *Rutaceae* alludes to the nomenclature confusion between the two genera *Zanthoxylum* and *Fagara* when he points out that a number of *Fagara* species are alternatively named as *Zanthoxylum* and vice versa. Indeed, the particular *Zanthoxylum* species which forms the subject of the present investigation was at one time placed in the genus *Fagara* (2). Fosberg (3) recently clarified the situation regarding the relationship of *Fagara* and *Zanthoxylum* in such a way that today less confusion exists.

Zanthoxylum elephantiasis Macf. (*Z. aromaticum* DC., *Fagara elephantiasis* Kr. and Urb.) is indigenous to the Caribbean region having been found

Received April 26, 1966, from the College of Pharmacy, Ohio State University, Columbus 43210.
Accepted for publication September 27, 1966.
This investigation was supported by research grant GM-05640 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.
Presented to the Pharmacognosy and Natural Products Section, A.PH.A. Academy of Pharmaceutical Sciences, Dallas meeting, April 1966.
* Present address: Department of Chemistry, University College of Science, Calcutta, India.
† Present address: Department of Chemistry, Wayne State University, Detroit, Mich.

in Costa Rica, Mexico, Cuba, Hispaniola, and in Jamaica, where it is known popularly as "yellow sanders." It is a tree of moderate size, 15–40 ft. in height, which is conspicuous in having corky conical spiny knobs on the bark around the base of the trunk (2). This report concerns the isolation and identification of three alkaloids from the bark of *Z. elephantiasis*.

EXPERIMENTAL[1]

The bark used in this investigation was collected during the summer of 1962 at the foot of Long Mountain in Jamaica.[2] The coarsely ground bark (1.3 Kg.) was extracted in a modified Soxhlet with ethanol until a negative test was obtained with Valser's test solution. The ethanolic extract was concentrated *in vacuo* to a thick syrupy consistency and extracted 3 times with 750-ml. portions of 2% tartaric acid solution, leaving 35 Gm. of insoluble residue. The combined acid extracts were concentrated *in vacuo* to about 1 L. and then filtered. The filtrate was made alkaline (about pH 9.0) with ammonium hydroxide solution and then extracted with chloroform in a liquid–liquid continuous extractor. The alkaline aqueous solution containing quaternary salts was treated with ammonium reineckate solution according to the method described by Hogg *et al.* (4) to give 35 Gm. of crude alkaloid reineckate.

Isolation of Alkaloids from Tertiary Bases.—The chloroform extract containing the tertiary alkaloids was extracted with 50-ml. portions of 2% sodium hydroxide solution. The combined alkaline extracts were a deep yellowish-brown color and gave a negative test for alkaloids. The chloroform solution was washed with distilled water, dried over anhydrous sodium sulfate, and reduced to dryness *in vacuo* to give 4.8 Gm. of residue. The residue was digested in 150 ml. of 5% hydrochloric acid solution. The resulting solution was filtered and then made alkaline with ammonium hydroxide solution (pH 9.0) and extracted with ether in a small continuous liquid–liquid extractor. Partial evaporation of the ether at room temperature caused the separation of a white amorphous encrustation on the sides of the flask. The ether solution was decanted and the encrusted material was washed with acetone and then crystallized from methanol to give 280 mg. of long needles (5-methoxy-6-canthinone). The decanted ether solution was evaporated to give a viscous dark brown liquid, which after dissolving in boiling acetone, afforded an additional 120 mg. of 5-methoxy-6-canthinone.

The remaining acetone solution was evaporated to dryness and the residue was dissolved in chloroform which was then extracted with 0.1 *N* hydrochloric acid. The aqueous acid extract was concentrated and left at room temperature for 1 day when 150 mg. of an alkaloid hydrochloride separated. The hydrochloride was dissolved in distilled water and the solution was then made alkaline with ammonium hydroxide and extracted with ether. The ether extract was evaporated to dryness to give 105 mg. of amorphous residue. This residue was chromatographed on 5 Gm. of Woelm neutral alumina (grade III) using benzene as the eluant to obtain 90 mg. of crystals, m.p. 150–152°. Rechromatography as above followed by crystallization from methanol gave colorless needles, m.p. 159–160° (6-canthinone).

Treatment of Tartaric Acid Insoluble Residue.—The 35 Gm. of tartaric acid insoluble residue was digested with 5% hydrochloric acid solution. Work up of this acid solution in the same manner as the tartaric acid soluble fraction yielded an additional 80 mg. of 5-methoxy-6-canthinone and 220 mg. of 6-canthinone. A third alkaloid was demonstrated to be present in a small amount by paper and thin-layer chromatography. However, its isolation has been postponed until more plant material can be collected and processed.

Identification of 5-Methoxy-6-canthinone (I).—5-Methoxy-6-canthinone was crystallized from methanol as pale yellow needles, m.p. 237–238° dec. Positive identification of the alkaloid was made by comparison with an authentic sample[3] (mixed melting point determination, infrared, and ultraviolet spectra). In addition, the hydrochloride, the picrate, and the methiodide were prepared as derivatives and were found to have melting points corresponding to those reported in the literature (5).

Identification of 6-Canthinone (II).—6-Canthinone was crystallized from methanol as colorless needles which quickly became yellow on exposure to air (probably loss of solvent of crystallization), m.p. 159–160°. Positive identification of the alkaloid was made by comparison with an authentic sample[4] (mixed melting point determination, infrared, and ultraviolet spectra). In addition, the hydrochloride, the picrate, and the methiodide were prepared as derivatives and were found to have melting points corresponding to those in the literature (6).

Isolation of Laurifoline Chloride.—The 35-Gm. sample of alkaloid reineckate was divided into two portions. A 15-Gm. sample was converted to chloride by the method described by Hogg *et al.* (4), while a 20-Gm. sample was converted to chloride by utilizing an ion-exchange resin described as follows. The 20-Gm. sample was dissolved in a mixture of 600 ml. of acetone and water (1:1). The solution was filtered and then added dropwise to a suspension of 400 Gm. of ion-exchange resin[5] IRA 400 (chloride form) in 800 ml. of acetone and water (1:1) and the mixture was then mechanically stirred for 24 hr. At this time the mixture was filtered, giving a light, straw-colored filtrate which when evaporated to dryness *in vacuo*, gave 7.5 Gm. of residue. Extraction of the residue with methanol left 3.8 Gm. of methanol insoluble, nonalkaloidal material. Thin-layer chromatography of the methanolic solution of crude quaternary chlorides using Silica Gel G as the adsorbent and acetonitrile–methanol–ammonium hydroxide solution (equal parts) as the

[1] All melting points were determined using a Thomas-Hoover Uni-Melt capillary melting point apparatus. The infrared spectra were taken in KBr using a Perkin-Elmer model 237 infrared spectrophotometer. The ultraviolet spectra were determined using a Perkin-Elmer model 4000 Spectracord. Alkaloid tests employed were Valser's and Dragendorff's test solutions.

[2] The material was collected under the supervision of Michael P. Cava and was verified botanically by Dr. Dennis Adams, Department of Botany, University of the West Indies. A fresh bark sample has a local anesthetic effect on the lips and tongue when chewed; this effect is almost entirely lost in the dried bark (Cava, M. P., personal observations).

[3] The authors are grateful to Dr. J. R. Price, Division of Industrial Chemistry, C.S.I.R.O., Melbourne, Australia, for the authentic sample of 5-methoxy-6-canthinone.

[4] The authors are grateful to Dr. E. Ritchie, Department of Organic Chemistry, The University of Sydney, Sydney, New South Wales, Australia, for the authentic sample of 6-canthinone.

[5] Marketed as Amberlite by Rohm & Haas, Philadelphia, Pa.

solvent revealed three Dragendorff positive spots, R_f 0.22, 0.42, and 0.66.

In an attempt to separate the quaternary chlorides the methanolic solution was adsorbed on Woelm acid alumina, grade I, and chromatographed on a column (2.5 cm. × 38 cm.) containing 100 Gm. of the same type of alumina. The column was eluted successfully with benzene, benzene–chloroform (1:1), chloroform, chloroform containing 5% methanol, chloroform containing 10% methanol, and chloroform containing 15% methanol. Only nonalkaloidal material was removed until the chloroform–methanol solvent was introduced. The chloroform–methanol mixtures (2100 ml.) eluted the alkaloid components as a mixture.

The combined eluates containing alkaloid material when concentrated to a syrupy consistency and left for 3 days at room temperature deposited crystals which were recovered by filtration and washed with chloroform containing 1% methanol, yielding 130 mg. of crystalline quaternary chloride. The filtrate was evaporated to dryness and then dissolved in absolute ethanol, to yield an additional 60 mg. of the same crystalline material.

The 15-Gm. sample of alkaloid reineckate that was worked up according to the method of Hogg *et al.* (4) gave 6.6 Gm. of crude quaternary chloride.

Identification of Laurifoline Chloride.—The above isolated quaternary chloride crystallized from ethanol as colorless prisms, m.p. 253° dec. $[\alpha]_D^{20} + 14°$ (c, 6.22 in methanol); $\lambda_{max.}^{methanol}$ 228 mμ (log ε 4.61), 282 (4.17), 308 (4.26). A strong bathochromic shift at the higher wavelength maxima was observed in an alkaline solution: $\lambda_{max.}^{methanol}$ 335 mμ (log ε 4.28).

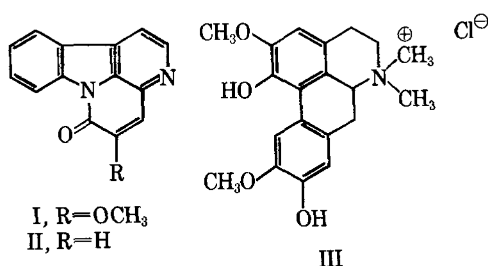
Anal.—Calcd. for $C_{20}H_{24}NO_4Cl$: C, 63.56; H, 6.46; Cl, 9.40; N, 3.71; N—CH_3, 8.10; OCH_3, 16.52. Found: C, 63.56; H, 6.46; Cl, 9.26; N, 3.66; N—CH_3, 8.12; OCH_3, 16.06.

The compound was identified as laurifoline chloride (III) on the basis of infrared and ultraviolet spectral comparison with an authentic sample.[6] In addition, the picrate was prepared and found to have the same melting point (220°) as that reported in the literature (7).

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 (6), 5-methoxy-6-canthinone (5), and 4-methylthio-6-canthinone (8) from *Pentaceras australis* Hook (*Rutaceae*). In a subsequent study of three Australian *Zanthoxylum* species, Cannon,

[6] The authors thank Dr. M. Tomita, Pharmaceutical Institute, Medical Faculty, University of Kyoto, Japan, and Dr. V. Deulofeu, Department of Organic Chemistry, Faculty of Sciences, Buenos Aires, Argentina, for authentic samples of laurifoline chloride.



Hughes, Ritchie, and Taylor reported the isolation of 6-canthinone from *Z. suberosum* C. T. White (9). 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 (10) was isolated from *Picrasma ailanthoides* (*Simaroubaceae*); in 1965, 4-methoxy-6-canthinone (11) 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 (7). 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 (12) reported, in 1958, the isolation of laurifoline from the bark of *Z. ailanthoides*. The only other reported (13) isolation of laurifoline from any plant is from the bark of *Fagara pterota* L. (*Z. pterota* H.B.K.).

REFERENCES

(1) Price, J. R., in "Chemical Plant Taxonomy," Swain, T., ed., Academic Press Inc., New York, N. Y., 1963, pp. 429–452.
(2) Fawcett, W., and Rendle, A. B., "Flora of Jamaica," vol. IV, British Museum, London, England, 1920, pp. 171–172.
(3) Fosberg, F. R., *Taxon*, **8**, 103(1959).
(4) Hogg, R., Beal, J. L., and Cava, M. P., *Lloydia*, **24**, 45(1961).
(5) Haynes, H. F., Nelson, E. R., and Price, J. R., *Australian J. Sci. Res.*, **A5**, 563(1952).
(6) *Ibid.*, **A5**, 387(1952).
(7) Tomita, M., and Kusuda, F., *Chem. Pharm. Bull. (Tokyo)*, **1**, 1(1953).
(8) Nelson, E. R., and Price, J. R., *Australian J. Sci. Res.*, **A5**, 768(1952).
(9) Cannon, J. R., Hughes, G. K., Ritchie, E., and Taylor, W. C., *Australian J. Chem.*, **6**, 86(1953).
(10) Inamoto, N., Masuda, S., Shimamura, O., and Tsuyuki, T., *Bull. Chem. Soc. Japan*, **34**, 888(1961).
(11) Scheuer, P. J., and Pattabhiraman, T. R., *Lloydia*, **28**, 95(1965).
(12) Tomita, M., and Ishii, H., *Yakugaku Zasshi*, **78**, 1441 (1958).
(13) Albonico, S. M., Kuck, A. M., and Deulofeu, V., *Chem. Ind. London*, **1964**, 1580.