The action of sulfuric acid on the *cis*-isomer (XXXIIIb. $R^1 = H$ and $R^2 = COOH$) proceeded similarly giving the comparable yield (86%) of the identical xylenol (XXXV), m.p. 160–162°. Recrystallization from ethyl acetate–hexane formed white prisms, m.p. and mixed m.p. 164–166°. Any other traceable materials could not be obtained from the mother liquors.

Acknowledgment. We are indebted to Mr. K. Kotera, the Tanabe Seiyaku Co., Ltd., Osaka, Japan, for determination and interpretation of the infrared spectra in this work.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF HAWAII]

Hawaiian Plant Studies. VI. The Structure of Holeinine[1]

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A new colorless alkaloid, *holeinine*, was isolated from *Ochrosia sandwicensis* A. Gray. It was shown to be N_b-methylisoreserpilinium chloride.

In the course of our research on the constituents of Hawaiian plants it seemed attractive to investigate the alkaloids of *Ochrosia sandwicensis* A. Gray. *Ochrosia* is one of some 300 genera in the plant family APOCYNACEAE and its *ca.* thirty-six species[3] are distributed from Madagascar through Malaya and tropical Australia into Polynesia.[4] *O. sandwicensis* is the only endemic Hawaiian representative of the genus. It is reported[4] to be botanically related to *O. elliptica* and *O. oppositifolia*. The Hawaiian name of the tree, which is rather scarce now, is *holei*. A yellow *tapa* dye used to be extracted from the wood and the roots[4]; the bark and leaves were extracted with boiling water and the steaming extract was used in sweat bath treatments.[5]

When the present study was initiated, the chemical literature of the genus *Ochrosia* consisted of one paper by Greshoff[6] in which he reported the presence of alkaloids in four *Ochrosia* species. He surmised the presence of three separate bases by virtue of color and solubility. While this work was in progress Buzas *et al.*[7] isolated and characterized a yellow base from *O. oppositifolia;* Goodwin and co-workers[8] isolated four alkaloids from *O. elliptica*, of which they identified the known isoreserpiline and characterized three new compounds, ellipticine, methoxyellipticine and elliptinine; from a small collection of *O. sandwicensis*[9] Goodwin[8] isolated ellipticine, methoxyellipticine, and a new colorless alkaloid; the synthesis of ellipticine, which represents the first example of a novel ring system was reported by Woodward, Hochstein, and Iacobucci[10]; and, most recently, Moore[11] reported the isolation of five new alkaloids in addition to isoreserpiline from *O. poweri*.

During the early stages of this work we, too, isolated ellipticine and methoxyellipticine, but we discontinued our efforts in that direction when Goodwin informed us of her work prior to publication. This paper deals with the structure determination of a new colorless alkaloid from *O. sandwicensis*, which we have called holeinine after the Hawaiian name of the *Ochrosia* tree.

The plant material was collected on the island of Maui in the Auahi lava fields, about eight miles from Ulupalakua at an elevation of *ca.* 3000 feet.[12] The dried and milled root and trunk bark was extracted by conventional procedures (see experimental). The crude alkaloids were obtained as a brown solid, which was repeatedly treated with benzene. This treatment removed a mixture of yellow bases, ellipticine, methoxyellipticine and, after chromatography on acid-washed alumina a third yellow base, m.p. 289–296° dec., whose spectral characteristics were similar to, but not identical with, those of ellipticine. It was obtained in 0.04% yield and has not been investigated further. The benzene-insoluble residues were extracted with water. Removal of the water, treatment of the solid residue with methanol and chro-

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[1] Part V of this series: C. E. Swanholm, H. St. John, and P. J. Scheuer, *Pacific Sci.*, **14**, 68 (1960).

[2] In part from the M.S. thesis of J. T. H. Metzger, University of Hawaii, 1961.

[3] M. Pichon, *Bull. muesum nat. hist. nat.* (*Paris*) [ii], **19**, 205 (1947).

[4] J. F. Rock, *The Indigenous Trees of the Hawaiian Islands*, Published under patronage, Honolulu, 1913, p. 413.

[5] D. M. Kaaiakamanu and J. K. Akina, *Hawaiian Herbs of Medicinal Value*, Board of Health of the Territory of Hawaii, Honolulu, 1922, p. 44.

[6] M. Greshoff, *Ber.*, **23**, 3537 (1890).

[7] A. Buzas, M. Oswiecki, and O. Schindler, *Compt. rend.*, **247**, 1390 (1958).

[8] S. Goodwin, A. F. Smith, and E. C. Horning, *J. Am. Chem. Soc.*, **81**, 1903 (1959).

[9] Goodwin *et al.* name their plant material *O. sandwicensis* A. DC. According to Rock[4] this name is synonymous with *Rauwolfia sandwicensis* A. DC. and the authority for *O. sandwicensis* is A. Gray.

[10] R. B. Woodward, G. A. Iacobucci, and F. A. Hochstein, *J. Am. Chem. Soc.*, **81**, 4334 (1959).

[11] B. P. Moore, Abstracts of Papers, *International Symposium on the Chemistry of Natural Products*, Australia, 1960, p. 39.

[12] The assistance of Mr. Henry C. Inciong is gratefully acknowledged.

matography on acid-washed alumina furnished as the major crystalline levorotatory alkaloid (0.2% of the dry bark) holeinine, m.p. 283–285° dec., after several recrystallizations from methanol-ethyl acetate. Its purity was established by paper chromatography. Holeinine was very soluble in water, methanol, and ethanol and slightly soluble in chloroform; its composition was $C_{24}H_{31}N_2O_5Cl$ and the substance was shown to contain three $O\text{-}CH_3$, one $N\text{-}CH_3$, one $C\text{-}CH_3$ groups, and one active hydrogen. Holeinine formed a crystalline perchlorate, m.p. 228–231°, in good yield. The ultraviolet spectrum (Fig. 1) with maxima at 221.5, 296.2, 302, and 307 mμ was reminiscent of the spectrum of reserpiline where the 2,3-dimethyl-5,6-dimethoxyindole chromophore is added to that of a β-alkoxyacrylic ester.[13,14] Two sharp bands of nearly equal intensity at 1695 and 1634 cm.$^{-1}$ constituted the most characteristic feature of the infrared spectrum of holeinine. These bands had been assigned previously[13,14,15] to the β-alkoxyacrylic ester system.

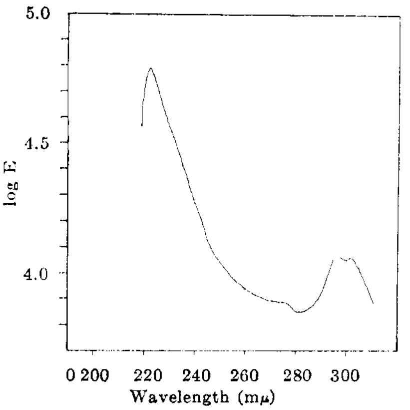
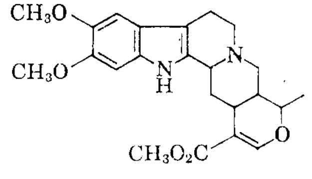


Fig. 1. Ultraviolet spectrum of holeinine

The analytical and spectral data pointed to a close relationship of holeinine with the known alkaloids reserpiline and isoreserpiline which possess structure I.[14,15] The N-methyl group known to be present in holeinine could *a priori* be at either the indole nitrogen or at the basic nitrogen atom. If the former hypothesis were correct, holeinine would be a hydrochloride salt and a corresponding free base should be obtainable. Yet no free base could be produced by alkaline treatment in aqueous or nonaqueous media or by ion exchange. It seemed therefore likely (and the result of the Zerewitinoff determination would tend to support this) that the N-methyl group was attached to N_b and that holeinine was a quaternary N-methochloride of isoreserpiline. While such a structure would be a departure from previously reported alkaloids of the Apocynaceae, one of the alkaloids isolated by Schlittler and Hohl[16] from the South American *Strychnos melinoniana* possesses structure II.



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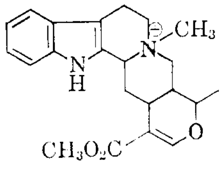
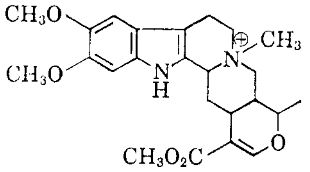


II

In order to prove this relationship holeinine was pyrolyzed in high vacuum as described by Karrer and Schmid.[17] The pyrolysis product could be purified only with difficulty (see Experimental), but finally yielded small crops of white crystals from petroleum ether, m.p. 210–211°, from which a crystalline perchlorate, m.p. 215–219°, could be prepared. In order to demonstrate that pyrolysis had caused no changes in the molecule other than the removal of the quaternary methyl group the N-methochloride was reformed *via* the iodide and proved to be identical with holeinine.

An authentic sample of isoreserpiline methanesulfonate[18] was transformed into its free base, m.p. 210–212°. This was shown to be identical with our pyrolysis product by melting point, mixture melting point, and infrared spectra. Holeinine therefore has structure III and constitutes the first example of a quaternary N-methochloride of the ajmalicine type to be isolated from a species of the Apocynaceae. It thus forms an interesting bridge between melinonine A of the Loganiaceae and isoreserpiline of the Apocynaceae. In this connection it would be interesting to study the constituents of the Hawaiian genus *Labordia*, which is a member of



III

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(13) F. E. Bader, *Helv. Chim. Acta,* **36,** 215 (1953).

(14) M. W. Klohs, M. D. Draper, F. Keller, and W. Malesh, *Chem. & Ind.* (*London*), **1954,** 1264.

(15) A. Stoll, A. Hofmann, and R. Brunner, *Helv. Chim. Acta,* **38,** 270 (1955).

(16) E. Schlittler and J. Hohl, *Helv. Chim. Acta,* **35,** 29 (1952).

(17) P. Karrer and H. Schmid, *Helv. Chim. Acta,* **29,** 1853 (1946).

(18) We are grateful to Dr. A. Hofmann of Sandoz A. G. for this sample.

Loganiaceae but which according to some botanists[19] should be placed in Apocynaceae.

EXPERIMENTAL[20]

Isolation procedure. The stripped bark was dried in a forced draft oven at 55° for 48 hr. The dried bark was ground in a Wiley mill to pass a 1-mm. screen. Three batches of bark totalling 1.3 kg. were stirred with 3 l. of ethanol containing 1% tartaric acid at 55° for 3 days. Three such extractions were necessary for each batch in order to exhaust the plant material of alkaloids as determined by a very weak test with Mayer's reagent. The combined ethanolic extracts were stripped *in vacuo* and concentrated to 1 l. The concentrate was mixed with 600 ml. of 2*N* hydrochloric acid and filtered through a bed of filter aid. The filter cake was washed with further quantities of acid and then with water. The combined acidic filtrates (*ca.* 2 l.) were extracted with 5 × 800 ml. of chloroform. The chloroform extract was washed with 10% sodium carbonate solution, dried over magnesium sulfate, and distilled *in vacuo* to dryness. Extraction of the chloroform residue with benzene, first at room temperature and then in a Soxhlet, furnished a yellow solid. This was a mixture of ellipticine and methoxyellipticine as determined by paper chromatograms and by comparison of authentic samples obtained from Dr. Goodwin.[21]

The benzene solution after removal of ellipticine and methoxyellipticine was chromatographed on acid-washed alumina (Woelm). In addition to a number of gummy and oily fractions a crystalline bright yellow solid was eluted with chloroform-methanol. Recrystallization from methanol-benzene furnished crystalline material, m.p. 289–296° dec. in 0.04% yield (based on dry bark). Single spots were obtained in paper chromatograms developed with *n*-butyl alcohol–hydrochloric acid–water (100-20-sat.) and with *n*-butyl alcohol–acetic acid–water (4-1-5, organic phase).

The thimble residue after exhaustive extraction with benzene was extracted with water. The red to yellow aqueous extracts were distilled to dryness *in vacuo* yielding a tarry residue. Trituration of this residue with methanol furnished a glassy brown residue in *ca.* 0.75% yield. This residue was dissolved in chloroform and chromatographed on a wet-packed column of acid-washed alumina (Woelm). Elution with chloroform containing increasing amounts of methanol produced from fractions 2 to 18 2.5 g. (0.6% of dry bark) of a crystalline white to pale yellow solid. Recrystallization of this material from methanol–ethyl acetate furnished 2.1 g. of white needles, m.p. 283–285° dec. Ascending paper chromatography on Whatman No. 1 paper in *n*-butyl alcohol–hydrochloric acid–water (100-20-sat.) produced a single yellow spot (R_f 0.70) which was pink under ultraviolet light.

Holeinine (III). A sample was crushed and dried for 4 hr. at 56°/4 mm. When the crushing of the sample was omitted, methanol was retained and lead to low C and high H values.

Anal. Calcd. for $C_{21}H_{31}O_5N_2Cl$: C, 62.26; H, 6.75; N, 6.05; Cl, 7.66; 3 OCH_3, 20.11; 1 NCH_3, 3.24; 1 CCH_3, 3.24; 1 active H, 0.22. Found: C, 62.03, 61.90; H, 6.78, 6.79; N, 6.03; Cl, 7.51; OCH_3, 20.05; NCH_3, 4.51; CCH_3, 2.64, 2.84; active H, 0.26, 0.25.

Rotation $[\alpha]_D^{23}$ −134.5° ± 1° (*c*, 2.97, methanol).

Spectra. Ultraviolet spectrum is reproduced in Fig. 1. Infrared bands (potassium bromide disk): 3570 (w), 3395 (w), 2900 (m), 1695 (s), 1634 (s), 1480 (s), 1460 (m), 1440 (m), 1390 (w), 1310 (s), 1210 (s), 1178 (w), 1158 (w), 1125 (s), 1100 (s), 1025 (m), 855 (m), 773 (m) $cm.^{-1}$

Holeinine perchlorate. Prepared from 50 mg. holeinine in 2.5 ml. water and 4 drops of 20% perchloric acid at the boiling point. After recrystallization from water the salt formed long white needles, m.p. 228–231°. Ascending paper chromatogram in *n*-butyl alcohol–hydrochloric acid–water (100-20-sat.) showed one pink spot in ultraviolet light. An analytical sample was dried at 78°/0.01 mm.

Anal. Calcd. for $C_{24}H_{31}O_9N_2Cl$: C, 54.63; H, 5.93; N, 5.32. Found: C, 54.93; H, 6.15; N, 5.58, 5.68.

Rotation $[\alpha]_D^{23}$ −90° (*c*, 3, pyridine).

Pyrolysis of holeinine. One hundred-milligram batches were dissolved in the minimum amount of hot methanol and introduced into the well of the sublimer. The solvent was removed *in vacuo* so as to produce a thin and even coating of holeinine. Pressure was reduced to 5×10^{-4} mm. and the sublimer was submerged in a Wood's metal bath preheated to 255°. The temperature was then increased as rapidly as possible and sublimation of a white solid occurred within 5 min. at 260–275°. Pyrolysis was discontinued as soon as a yellow oil began to be condensed on the cold finger which was charged with a Dry Ice–acetone mixture The yellowish product (46–60 mg.) was scraped off the cold finger. It turned dark rapidly and could not be purified by crystallization and Norit treatment or chromatography over basic or acid-washed alumina. Purification was eventually achieved, albeit in small yield, by extraction with anhydrous ether in which the yellow oily material remained insoluble. The ethereal extracts were combined and distilled to dryness furnishing a tan amorphous residue. Crystallization from benzene–petroleum ether (b.p. 60–80°) yielded tan powders with melting ranges from 111–126°. This material was crystallized from petroleum ether yielding small white crystals growing in clusters, m.p. 210–211°. A perchlorate was prepared, m.p. 215–219°.

Holeinine from pyrolysis product. The pyrolysis product was refluxed in benzene with an excess of methyl iodide for 30 min. The resulting yellowish precipitate was filtered off and recrystallized once from ethanol-ether. A benzene-ether slurry of this *N*-methyl iodide was added to freshly prepared silver chloride and refluxed for 1 hr. The benzene-ether solution was decanted and the silver halide residues were extracted with methanol. The combined organic solution was evaporated to dryness and the residue recrystallized from methanol–ethyl acetate, yielding small white rods, m.p. 285–288° dec. Mixture melting point with holeinine was undepressed.

Comparison of pyrolysis product with isoreserpiline. An authentic sample of isoreserpiline was prepared from 22 mg. of isoreserpiline methanesulfaonate[18] by liberating the free base with ammonia and extracting with ether. The resulting off-white solid was obtained crystalline after crystallization from benzene–petroleum ether, chromatography on acid-washed alumina and recrystallizations from petroleum ether, yielding white prisms, m.p. 211–212°. Mixture melting point with the pyrolysis product of holeinine was undepressed and the infrared spectra (potassium bromide) were identical.

Acknowledgment. The initial phases of this research were supported by a Frederick Gardner Cottrell grant of Research Corporation and carried out with the assistance of Mr. H. C. Botelho. The major portion of the research was aided by a grant (RG-5095) from the National Institutes of Health, Division of General Medical Sciences. The authors express their gratitude for this generous financial support.

HONOLULU 14, HAWAII

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[19] J. F. Rock, *loc. cit.*, p. 403.

[20] Melting points were determined on a Kofler block and are uncorrected. Analyses by Dr. Alfred Bernhardt, Mülheim, Germany. Ultraviolet spectra were measured on a Beckman DK-2 and infrared spectra on a Beckman IR-2A instrument.

[21] Dr. Goodwin's assistance with these samples and by informing us of her work is gratefully acknowledged.