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Alkaloids of *Ochrosia elliptica* Labill.[1]

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Four alkaloids have been isolated from leaves of *Ochrosia elliptica* Labill. One has been shown to be identical with isoreserpiline. The other three, ellipticine, methoxyellipticine and elliptinine, have been characterized and certain features of their structures have been suggested.

The plant *Ochrosia elliptica* Labill. (family Apocynaceae) is a small tropical evergreen tree. The genus is a member of the tribe Plumiereae along with the alkaloid producing genera Rauwolfia, Aspidosperma and Holarrhena. Fifteen species of *Ochrosia* have been described, occurring in Australia, the Andaman Islands, Madagascar, Hawaii and many other Pacific Islands. The species *elliptica* was first described in New Caledonia.[3,4]

The only recorded previous chemical examination of *Ochrosia* in which alkaloids were isolated was carried out by Greshoff[5] at the Botanical Garden, Buitenzorg, Java. He found the bark of the Javanese species *acuminata, ackeringae* and *coccinea* to be rich in alkaloids and distinguished three bases by virtue of their color and solubility; it is not possible to be certain that they correspond to any of the alkaloids found in the present study. The observation that *O. elliptica* Labill. (*Bleekaria calocarpa* Hassk.) was alkaloid-containing was also made by Greshoff.

Isolation.—In the present study four crystalline alkaloids were isolated and assigned the names elliptine (now isoreserpiline), ellipticine, methoxyellipticine and elliptinine; ellipticine and methoxyellipticine were distinguished by their bright yellow color and sparing solubility. The isolation studies were carried out on a total of 67 lb. of leaves (wet weight); in all, twelve isolation runs were performed. There appeared to be some variation in the alkaloidal content of the plant material. No single procedure was found to be suitable for all of the alkaloids. Although isoreserpiline was found to belong to the class of alkaloids whose salts are easily extractable from acid solutions with chloroform, this property was not utilized; procedure A, provided the best yield of isoreserpiline (0.28%). The isolation of a mixture of ellipticine and methoxyellipticine presented no difficulty but their separation proved to be extremely tedious. Alumina chromatography was used in this work; other adsorbents were not investigated and attempts to devise a countercurrent distribution system were not successful. Ellipticine and methoxyellipticine, when crystalline, are sparingly soluble in most solvents used in chromatography, and it was found best to apply a concentrate of the alkaloid extract directly to the chromatographic column (procedure A). After the elution of isoreserpiline, the column was developed with increasing amounts of methanol and the separation was followed by paper chromatography. The yields of the yellow alkaloids were variable; the following are representative: ellipticine, 0.004%; methoxyellipticine, 0.007%; and a mixture of the two, 0.002%.

Elliptinine, the most elusive of the four alkaloids, was isolated in only two instances; the second time in only trace amounts. The isolation providing the better yield (0.02%, procedure B) was characterized by the fact that most of the other alkaloids had been removed by crystallization prior to chromatography. Elliptinine was eluted immediately following methoxyellipticine. A thorough investigation of the mother liquors of the chromatographic fractions which yielded crystalline methoxyellipticine and of the subsequent fractions from other isolations failed to give any crystalline elliptinine, although its presence in small amount was observed by its characteristic ultraviolet spectrum and by paper chromatographic examination. The alkaloids are well resolved in paper chromatography; R_f values of 0.65 for methoxyellipticine, 0.70 for ellipticine and 0.76 for elliptinine were observed in a butanol–acetic acid system. Elliptinine may be differentiated from the yellow alkaloids by virtue of its easy solubility in methanol.

Isoreserpiline (Elliptine).—The analytical results indicated that the alkaloid, m.p. 210–211.5°, $C_{23}H_{28}O_5N_2$, contained three methoxyl groups, no methylimino group, one active hydrogen at room temperature and a second one at 100°. Variable Kuhn–Roth values were obtained. The infrared and ultraviolet spectra were characteristic of the β-alkoxyacrylic ester system, ROC=CCOOCH$_3$.[6] The presence of an ester function was confirmed by alkaline hydrolysis. The alkaloid was found to contain no readily acetylated hydroxyl group and no easily hydrogenated double bond. Structural studies were not carried out, since it was noted (in 1955) that the m.p., rotation, and spectra reported for isoreserpiline,[7] an alkaloid from *Rauwolfia canescens* L., corresponded with those of the *Ochrosia* alkaloid. The structure proposed for

(1) Presented in part before the 14th International Congress of Pure and Applied Chemistry, July 1955, Zurich.

(2) Ciba Pharmaceutical Company, Summit, N. J.

(3) J. J. H. de la Billardiere, Sertum Austro-Caledonicum, 25 (1824).

(4) The genus is not known in the New World except through importation. It is believed that the first introduction of *O. elliptica* was at the Royal Botanic Garden, Port-of-Spain, Trinidad, where it is called *O. moorei*. The USDA Plant Introduction Station in Florida has a specimen of *O. elliptica* which was presumably started from seeds from the Trinidad tree. The plant material used in this study was obtained from clippings of the Florida tree and consisted of relatively new growth of leaves and stems. The fruit was found to be alkaloid-free; neither the trunk bark nor the root bark was examined.

(5) M. Greshoff, *Ber.*, **23**, 3537 (1890).

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

(7) A. Stoll, A. Hofmann and R. Brunner, *ibid.*, **38**, 270 (1955).

isoreserpiline by the Swiss group, based on analytical and spectral data, is suported by these observations. The name to be retained in the literature should be isoreserpiline. The active hydrogen data could be interpreted as reaction with the indole N–H at room temperature and an ether cleavage reaction[8] of one of the aromatic methoxyl groups at 100°.

Ellipticine and Methoxyellipticine.—The preparation of homogeneous solvent-free samples of these alkaloids for analysis was a matter of considerable difficulty but it was eventually accomplished with the results that ellipticine was assigned the formula $C_{17}H_{14}N_2$ and methoxyellipticine $C_{18}H_{16}ON_2$ with one methoxyl group.[9] Both alkaloids were analyzed for terminal methyl groups (in two laboratories) with negative results. They are assumed to have the same basic structure since they differ empirically only by a methoxyl group and they have similar ultraviolet, visible and infrared spectra.

Previously encountered yellow alkaloids from plants of the Apocynaceae are β-carboline anhydronium bases. This system is characterized by the observations that the ultraviolet spectrum in alcohol is unaffected by the addition of acid whereas the addition of alkali produces a bathochromic shift. The unusually complex ultraviolet spectra of ellipticine in alcohol (Fig. 1) and in

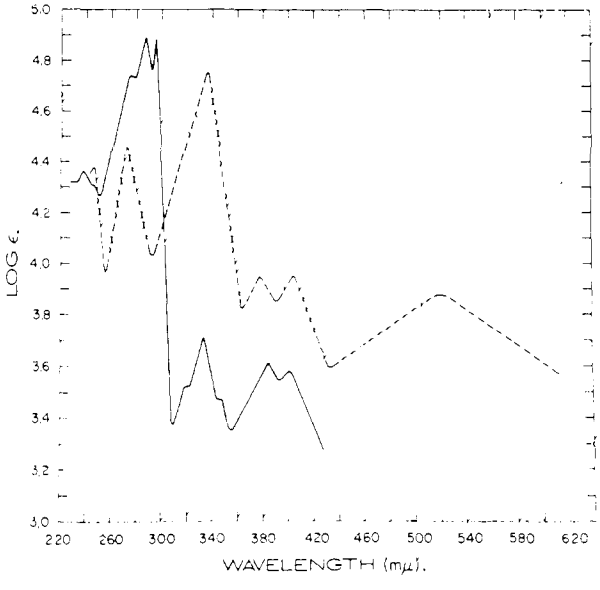
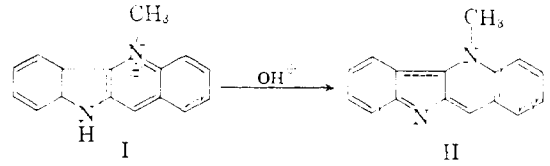


Fig. 1.—Ultraviolet spectra of: ———, ellipticine in ethanol; ---------, ellipticine methiodide in 0.05 *N* potassium hydroxide solution in ethanol.

0.01 *N*-alcoholic potassium hydroxide were identical in the wave lengths of the maxima and minima; however, the spectrum in acid solution was shifted toward the red. The same results were found for methoxyellipticine. Thus it was evident that these yellow bases represented a new structural departure for alkaloids of the Apocynaceae.

Ellipticine and methoxyellipticine were converted to methiodides whose analyses confirmed the assigned empirical formulas for both alkaloids. When alcoholic solutions of the methiodides which were deep yellow and orange, respectively, were made alkaline, reddish-purple solutions were formed, and if sufficient water were present, amorphous, reddish-purple solids precipitated. This behavior was interpreted as being due to the formation of anhydronium bases of the type found for the purple alkaloid cryptolepine (II).[10] The European workers have shown that cryptolepine (II) is the anhydronium base of quindoline methiodide (I). Although the absorption maxima in the



I II

visible are nearly the same (530 mμ) for both ellipticine methiodide and quindoline methiodide (in 0.01 *N* alcoholic potassium hydroxide), the maxima in the ultraviolet are not identical. Similarly the ultraviolet spectra of the parent bases, ellipticine and quindoline, are similar but not identical.[11] It is evident that ellipticine and methoxyellipticine are not quindoline derivatives; however, the color changes described for the methiodides must be due to anhydronium base formation and it is therefore suggested that the nitrogen functions in these yellow alkaloids are of the same type as in quindoline; in an abbreviated form the nitrogen functions may be described as —NH-(C=C)C=N—.

If it may be assumed that the ring system of these alkaloids is isomeric with quindoline, then all of the atoms would be accounted for except for C_2H_4 which could be satisfied either by an ethyl substituent or by two methyl groups. The nuclear magnetic resonance spectra[12] of ellipticine and methoxyellipticine showed conclusively that an ethyl substituent was not present and that two chemically shifted methyl groups were present. These conclusions are apparent from examination of the n.m.r. spectrum of methoxyellipticine (Fig. 2); the signal at 67 c.p.s. relative to benzene has been assigned to the hydrogen nuclei of the methoxyl group and those at 90 and 109 c.p.s. to the hydrogen nuclei of methyl groups which are attached to carbon atoms having no attached hydrogen atoms. The n.m.r. spectrum of ellipticine was identical with the exception of the signal for the methoxyl group. The fact that Kuhn-Roth oxidation did not afford acetic acid indicated that the methyl groups were situated on an aro-

(8) M. S. Kharasch and O. Reinmuth, "Grignard Reactions of Nonmetallic Substances," Prentice-Hall, Inc., New York, N. Y., 1954, p. 1013.

(9) The empirical formulas mentioned earlier[1] for these alkaloids were based on samples which may have been inadequately purified or were solvated.

(10) E. Gellert, Raymond-Hamet and E. Schlittler, *Helv. Chim. Acta*, **34**, 642 (1951).

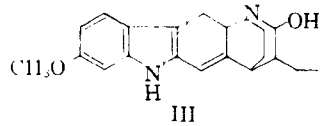
(11) The quinindoline system was also eliminated for ellipticine on the basis of its ultraviolet spectrum. The spectra of quindoline and quinindoline have been published by G. R. Clemo and D. G. I. Felton, *J. Chem. Soc.*, 671 (1951).

(12) We are indebted to Dr. J. N. Shoolery and Mr. L. F. Johnson Varian Associates, Palo Alto, Calif., for these data.

matic nucleus[13]; evidence bearing on this point and the structure of ellipticine has been provided by Dr. F. A. Hochstein of Charles Pfizer and Co.[14]

Ellipticine methiodide was readily reduced by sodium borohydride to form a tetrahydro derivative.[15] Methyl tetrahydroellipticine was found to be identical in melting point and ultraviolet and infrared spectra on direct comparison with an alkaloid from the Peruvian "Quillo Bordon"[14] and with the reported melting point and ultraviolet spectrum of alkaloid B from *Aspidosperma ulei* Mgf.[16]; more detailed comparisons are being carried out in other laboratories.

Ellipticine.—This colorless, optically active alkaloid, m.p. 231–233°, was found to have the formula $C_{20}H_{24}O_2N_2$ and to contain one methoxyl, no methylimino, and one terminal methyl group. The infrared spectrum indicated that no carbonyl groups were present; this included the 6 μ region with respect to amide groups and conjugated unsaturated esters of the type proposed for akuammicine.[17] The ultraviolet absorption spectrum of ellipticine was very interesting in that it consisted of a shoulder at 222 mμ and a broad maximum at 311 mμ; acidification produced a slight hypsochromic shift to 308 mμ. In a small scale hydrogenation experiment from which a crystalline product could not be obtained, one mole of hydrogen was absorbed and the maximum in the ultraviolet spectrum was shifted from 311 to 298 mμ. In the infrared spectrum of elliptininium perchlorate very intense bands at 2.99 (broad) and 3.16 μ were observed and, in addition, a very weak band was present at 5.97 μ which although having the expected wave length for an iminium ion was of much too low intensity. The bands near 3 μ were assigned to indole NH and NH^+, respectively. On the basis of the infrared interpretation, a normal enamine system, $-C=C-N-$, is not present.[18] An interesting speculation based on the Woodward[19] scheme for the derivation of a linear carbazoquinuclidine system from ajmaline or a biogenetic precursor is structure III which is compatible with—but of course not required by—the analytical and spectral evidence so far available for ellipticine.



III

Ochrosia sandwicensis **A.DC.**—Ellipticine and methoxyellipticine were isolated in the examination of a small quantity of this Hawaiian species. Isoreserpiline and elliptinine were not detected, but a new colorless alkaloid was found in small amount. This alkaloid has been partially characterized as its hydriodide, m.p. 215° dec., $[\alpha]_{589}$ 27°. The free base has ultraviolet absorption maxima at 238 and 290 mμ and the spectrum is unaffected by acidification.

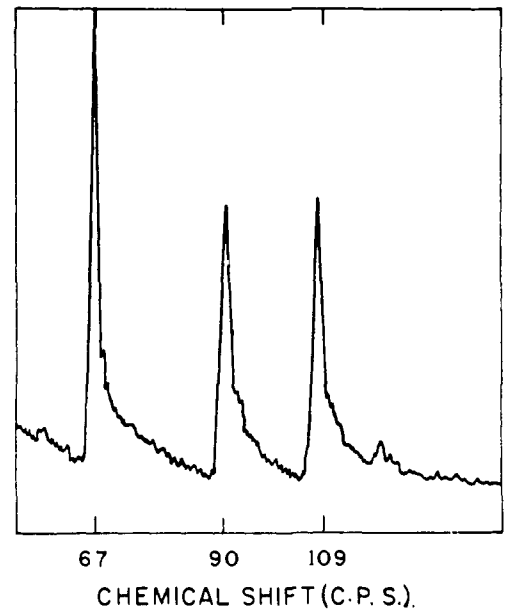


Fig. 2.—Nuclear magnetic resonance spectrum of methoxyellipticine at 40 mc. in pyridine solution referred to benzene.

Acknowledgments.—We are especially grateful to Dr. F. A. Hochstein of Chas. Pfizer & Co. for a stimulating exchange of information and for samples of "Quillo Bordon" alkaloids. We are grateful to Dr. H. A. Lloyd of this Laboratory for samples of quindoline and quinindoline and to Dr. J. Schmutz, Dr. A. Wander, A. G. Bern, for samples of some of his *Aspidosperma* alkaloids. We are indebted to the Section of Plant Introduction, Agricultural Research Service, U. S. Department of Agriculture, for obtaining and identifying the plant material and to Dr. Karl H. Korte, Division of Forestry, Kahului, Maui, T. H., for the *Ochrosia sandwicensis* used in this work.

Experimental[20]

Isolations. Procedure A.—Two kilograms of ground air-dried leaves and stems of *Ochrosia elliptica* were stirred at 55° with 10 l. of 95% ethanol. The alcoholic extract was filtered and the plant material was treated again with ethanol at 55°. The combined alcoholic extracts were concentrated *in vacuo* to about 2 l. of black, sirupy residue. The concentrate was poured slowly into 15 l. of water containing 2 kg.

(13) N. A. Hughes and H. Rapoport, THIS JOURNAL, **80**, 1604 (1958).

(14) F. A. Hochstein, private communication.

(15) The reduction of unsaturated heterocyclic methiodides has been described by B. Witkop, THIS JOURNAL, **75**, 3362 (1953) and by B. Witkop and J. B. Patrick, *ibid.*, **75**, 4474 (1953).

(16) J. Schmutz and F. Hunziker, *Helv. Chim. Acta*, **41**, 288 (1958).

(17) K. Aghoramurthy and R. Robinson, *Tetrahedron*, **1**, 172 (1957).

(18) An enamine system in which the corresponding iminium ion is incapable of existence for steric reasons (as would be the case if the nitrogen were part of a quinuclidine system) may also be discarded on spectral grouns (*cf.* C. A. Grob, A. Kaiser and E. Renk, *Chemistry & Industry*, 598 (1957).

(19) R. B. Woodward, private communication; *cf.* also R. B. Woodward, *Angew. Chem.*, **68**, 13 (1956).

(20) Melting points were taken on a Kofler block. Rotations and ultraviolet spectra were determined in absolute ethanol, and infrared spectra were taken in chloroform, unless otherwise specified. Instrumental measurements were made by Mr. and Mrs. H. F. Byers, Misses P. A. Wagner and C. Monaghan, and Mrs. K. Warren. Infrared spectra were obtained with a Perkin–Elmer spectrophotometer model 21, ultraviolet spectra with a Cary recording spectrophotometer model 11 and rotations were measured in 2-dm. cells using a Rudolph photoelectric polarimeter model 80. The nuclear magnetic resonance spectra of ellipticine and methoxyellipticine were measured in pyridine with a Varian High Resolution spectrometer model V4300B at Varian Associates, Palo Alto, Calif. Extractions were made by Mr. D. L. Rogerson and Mr. J. D. Link. Analyses were made by Mr. W. Manser, Zurich, and the Clark Microanalytical Laboratory, Urbana, Ill.

of Supercel (with vigorous stirring) and the *p*H of the mixture was adjusted from 5 to 4 with 75 cc. of glacial acetic acid. The mixture was filtered through a large Buchner funnel and the pale green filter cake was resuspended in 7 l. of water containing 50 cc. of glacial acetic acid. (After filtration, the filter cake was washed with a minimum of water, dried at 32° *in vacuo*, and extracted in a soxhlet extractor with ether and alcohol. Although a Mayer test was positive, crystalline alkaloids could not be obtained from these extracts after chromatography or countercurrent distribution attempts.) The combined filtrates were mixed well with a large volume of chloroform and made alkaline with solid sodium carbonate. The basic solution was extracted in six portions with 10 × 200 cc. of chloroform for each portion. The final basic solutions still gave weak alkaloid tests. The combined chloroform extracts were dried over magnesium sulfate and concentrated to 2.5 l.

This chloroform extract was added to a column (5 × 62 cm.) of 1 kg. of acid-washed alumina. The summary of the chromatogram has been arranged in the following order: fraction, solvent, volume of eluant and weight of residue: 1, chloroform (2.5 l.), 0.891 g., non-alkaloidal; 2–4, chloroform (3 l.), 10.91 g., from which 5.50 g. of crystalline isoreserpiline was obtained on crystallization from ether; 5–10, chloroform (4 l.) and 1% methanol–chloroform (3 l.), 2.87 g., non-crystalline, combined with the mother liquors of 2–4 and other suitable fractions from other chromatograms for rechromatography; additional amounts of isoreserpiline were obtained but no other crystalline alkaloids were found; 11–14, 2, 3 and 5% methanol–chloroform (6 l.), 1.24 g., non-crystalline, but ellipticine was indicated in 14 by paper chromatogram[21]; 15–16, 5% methanol–chloroform (2 l.) 1.63 g., red-brown tar and yellow solid which on trituration with chloroform yielded 25 mg. of ellipticine (an additional 10 mg. was obtained from the chloroform on concentration); 17–19, 5% methanol–chloroform (1.5 l.), 0.79 g. crude residue which afforded 21 mg. of ellipticine which was contaminated with methoxyellipticine (according to paper chromatogram); 20, 5% methanol–chloroform (0.7 l.), 0.19 g. crude; 8 mg. of crystalline methoxyellipticine contaminated with ellipticine; 21–24 5% methanol–chloroform (6 l.), 1.89 g. of residue from which a yellow solid was obtained in the usual way; after three recrystallization from methanol, 119 mg. of methoxyellipticine was obtained.[22] Elution was continued but no crystalline solids could be obtained.

Procedure B.—One kilogram of dried, ground plant material was extracted three times with 1% ethanolic tartaric acid solution at 55°. A 2.5–l. concentrate was mixed with 1.5 l. of 2 *N* hydrochloric acid and filtered through Supercel. The filter cake was washed with dilute acid and with water. The combined filtrates were washed with chloroform (5x); the last wash was still deeply colored. The combined chloroform extracts were shaken with 10% sodium carbonate solution, dried and the solvent was evaporated. The benzene-soluble portion of the residue afforded no crystalline alkaloids on chromatography. The acidic solution was made alkaline with solid sodium carbonate and extracted with ethyl acetate. Continuous extraction of the aqueous phase with chloroform afforded 1.27 g. of black tar. The ethyl acetate extracts were extracted with 15% acetic acid (it was necessary to add ether—about one-half the volume of the organic phase—to break up emulsions). The acid extracts were made alkaline and extracted with ethyl acetate. After drying and removing the solvent, 6 g. of brown, tarry residue was obtained which was dissolved in 20 ml. of chloroform with warming. On cooling, a yellow solid precipitated (186 mg.) which according to paper chromatography was a mixture consisting mostly of methoxyellipticine. The mother liquor was diluted with 3 volumes of benzene and chromatographed on a column of acid-washed alumina (3 × 30 cm.). The chromatogram was developed with increasing amounts of chloroform. From fractions 6–15, a total of 62 mg. of the yellow alkaloids was obtained in crystalline form and fractions 16–21 yielded 30 mg. of noncrystalline residue. Fractions 22–29, eluted with a total of 550 ml. of benzene–chloroform (1:2), afforded 0.528 g. of residue consisting of colorless solid and reddish oil; trituration with chloroform gave 0.203 g. of elliptinine.

Isoreserpiline.—A sample of this alkaloid obtained as described in procedure A was found to be homogeneous by chromatography on acid-washed alumina (eluting with benzene and increasing amounts of ethyl acetate in benzene); fractions 3 and 35 were identical in m.p. and infrared spectra. Isoreserpiline was found to be very soluble in most solvents although it appeared to decompose rather quickly in chloroform and carbon tetrachloride; the alkaloid may be crystallized from ether or methanol–water (3:1). The analytical sample crystallized from aqueous methanol in colorless needles, m.p. 210–211.5° (lit.[7] 211–212°); $[\alpha]^{24}_{589}$ −90°, $[\alpha]^{24}_{546}$ −110° (*c* 0.73) (lit.[7] $[\alpha]^{20}_{D}$ −88°); and $[\alpha]^{24}_{589}$ −77°, $[\alpha]^{24}_{546}$ −98° (*c* 0.48, pyridine) (lit.[7] $[\alpha]^{20}_{D}$ −82°, $[\alpha]^{20}_{546}$ −100°); λ_{max} 228, 300 and 304 mμ (log ε 4.58, 4.01 and 4.02, respectively); λ_{min} 275 and 302 mμ (log ε 3.79 and 4.01, respectively) (lit.[7] λ_{max} 229 and 304 mμ (log ε 4.56 and 4.02, respectively); λ_{max} (CCl_4)[23] 2.87, 2.94(sh), 2.97, 3.33, 3.38, 3.43, 3.52, 3.56, 3.62, 5.89 and 6.12 μ).

Anal. Calcd. for $C_{23}H_{28}O_5N_2$: C, 66.97; H, 6.84; N, 6.79; 3 OCH_3, 22.57; (C)CH_2, 3.64; active hydrogen, 0.12. Found: C, 67.00, 66.94; H, 6.76, 6.92; N, 6.92; OCH_3, 21.96; (N)CH_3, 1.01; (C)CH_3, none, 3.39; active hydrogen 0.19 at 0° and 0.23 at 100°.

The salts of isoreserpiline (hydrochloride and perchlorate) afforded poor analytical results. The methanesulfonate which was prepared in methanol–ether, m.p. 254–258° dec., $[\alpha]^{25}_{589}$ −30° (*c* 0.52, water) (lit.[7] 282–284° dec., $[\alpha]^{20}_{D}$ −35° (water)) gave analytical results in agreement with a monohydrate but on drying provided poor results. A sample of this salt, m.p. 235–240°, provided by the Swiss group, was similar in infrared and ultraviolet spectra with that prepared in this study. A more direct comparison of the alkaloids was not possible.

Hydrogenation Conditions.—A solution of 0.4 g. of isoreserpiline in 10 ml. of methanol was added to a pre-treated mixture of 70 mg. of Pd-C and 5 ml. of methanol. No hydrogen was absorbed after 2 hours and isoreserpiline was recovered unchanged.

Acetylation Conditions.—Isoreserpiline was recovered unchanged after treatment with acetic anhydride–pyridine at room temperature. Under more drastic conditions (refluxing acetic anhydride in the presence of potassium acetate), a colorless, crystalline compound, m.p. 213–214°, was obtained after chromatography. The infrared and ultraviolet spectra were in agreement with an N-acetylindole: λ_{max} 257 mμ and shoulders 285–300 and 310 mμ; 5.92 (v.s.) and 6.14(s) μ; however, the analysis was not in good agreement with this structure possibly because of a persistent impurity.

Anal. Calcd. for $C_{25}H_{30}O_6N_2$: C, 66.06; H, 6.65; N, 6.16; 3 OCH_3, 20.48; 2(C)CH_3, 6.62. Found: C, 64.21; H, 6.49; N, 6.22; OCH_3, 19.96; (C)CH_3, 7.44.

Hydrolysis.—A solution of 0.4 g. of isoreserpiline in 30 ml. of 1% sodium hydroxide in methanol–water (1:1) was refluxed for 3 hours. The solution was taken to *p*H 6 with hydrochloric acid and evaporated to dryness. Trituration with water afforded a colorless solid having a carboxylate anion (zwitterion) band in the infrared (3.0 and 6.12 μ). The compound was very unstable and was converted to its hydrochloride, m.p. 205–208°, λ_{max} (mull) 3.0, 5.98 and 6.12 μ. This compound was rather hygroscopic and unstable. Although the analysis was not good, the loss of one methoxyl group was clearly indicated.

(21) Paper chromatograms were obtained using Whatman 1 paper and the upper phase of a mixture of 1-butanol–acetic acid–water (4:1:5). The alkaloids were detected by their fluorescence under ultraviolet light and by spraying with Dragendorff reagent. The fluorescent maxima of ellipticine and methoxyellipticine, measured in alcohol solution with activation at 294 mμ, were found to be approximately 440 and 500 mμ, respectively; an experimental model of the Bowman–Aminco Spectrophotofluorometer was used to obtain these data.

(22) In this manner it was possible to obtain ellipticine which was completely homogeneous according to paper chromatography; however the paper chromatograms of methoxyellipticine even after repeated crystallizations showed a persistent trace impurity having the R_f of ellipticine but giving a blue-green fluorescence rather than the brilliant yellow-green fluorescence of ellipticine. Since analytically pure methoxyellipticine was obtained, this phenomenon was not investigated further.

(23) Measured using a Beckman IR-3 spectrophotometer.

Anal. Calcd. for $C_{22}H_{27}O_4N_2Cl$: C, 60.75; H, 6.26; 2 OCH_3, 14.27. Found: C, 61.29; H, 6.17; OCH_3, 14.41.

Ellipticine.—The most convenient solvent for crystallization was methanol, from which ellipticine crystallized as bright, lemon-yellow needles; however, it was apparent from initial analytical data that methanol of crystallization was difficult to remove completely. The analytical sample was prepared from yellow crystalline material (48 mg.) obtained from a chromatographic fraction by crystallization from chloroform; recrystallization from ethyl acetate afforded a mixture of orange rosettes, orange rods and yellow rosettes. The different crystal modifications were indistinguishable by ultraviolet spectra and paper chromatography. The analytical sample melted at 311–315° dec., λ_{max} (satd. chloroform solution): 2.86 and 6.23 μ; pK_a 5.87. The value of the pK_a of quindoline was found to be 3.6.[24] Ultraviolet: λ_{max} 227–234(sh), 238, 245(sh), 276, 287, 295, 318–322(sh), 333, 343–347(sh), 384 and 401 mμ (log ϵ 4.32, 4.36, 4.31, 4.74, 4.90, 4.88, 3.52, 3.71, 3.47, 3.61 and 3.58, resp.); λ_{min} 251, 279, 292, 309, 355 and 393 mμ (log ϵ 4.27, 4.73, 4.76, 3.37, 3.35 and 3.55, resp.). In 0.01 *N* ethanolic potassium hydroxide: no change in wave length of maxima and minima but a slight change in extinction coefficients. In 0.1 *N* hydrochloric acid in ethanol: λ_{max} 241, 249, 271(sh), 307, 335 and 426 mμ (log ϵ 4.41, 4.39, 4.29, 4.90, 3.75 and 3.68, resp.); λ_{min} 246, 260, 330 and 382 mμ (log ϵ 4.38, 4.13, 3.40 and 3.36, resp.).

Anal. Calcd. for $C_{17}H_{14}N_2$: C, 82.90; H, 5.73; N, 11.37; (C)CH_3, 6.10. Found: C, 82.83; H, 5.77; N, 11.16; (C)CH_3, none.

Methiodide.—A solution of 44 mg. of ellipticine in 100 ml. of acetone was allowed to stand with an excess of methyl iodide (10 ml.). The bright yellow, crystalline precipitate (59 mg.) was collected, washed with acetone, and crystallized from methanol. The methiodide decomposed at about 360° without melting. Ultraviolet: λ_{max} 223, 242, 251, 270–285(sh), 311, 337–345(sh), 362 and 432 mμ (log ϵ 4.47, 4.43, 4.40, 4.24–4.40, 4.90, 3.51–3.57, 3.74 and 3.71, resp.); λ_{min} 235, 248, 262, 335 and 390 mμ (log ϵ 4.38, 4.39, 4.13, 3.49 and 3.49, resp.). In 0.05 *N* alcoholic potassium hydroxide: λ_{max} 246, 272, 336, 376, 403 and 520 mμ (log ϵ 4.38, 4.46, 4.75, 3.95, 3.95 and 3.88, resp.); λ_{min} 255, 292, 363, 390 and 432 mμ (log ϵ 3.97, 4.03, 3.82, 3.85 and 3.59, resp.).

Anal. Calcd. for $C_{18}H_{17}N_2I$: C, 55.68; H, 4.41; N, 7.22; I, 32.69. Found: C, 55.55; H, 4.58; N, 7.37; I, 32.70.

Anhydronium Base.—In a very small scale experiment, the anhydronium base was found to be precipitated as an amorphous red-purple solid after the addition of 10% sodium hydroxide solution to a methanol solution of ellipticine methiodide. It formed a violet-purple solution in chloroform which was completely decolorized on shaking with water; the yellow aqueous phase was not changed on the addition of a small amount of alkali but with excess alkali a red-purple precipitate formed. Cryptolepine was not as easily hydrated; its purple chloroform solution was unchanged on shaking with water. This difference in stability of the anhydronium bases and the differences in pK_a values probably result from the same structural features.

Methyltetrahydroellipticine.—A solution of the methiodide, prepared from 113 mg. of ellipticine, in 100 ml. of methanol and 10 ml. of water[25] was treated with excess sodium borohydride. The orange color was instantaneously discharged on the addition of the borohydride. After standing for 1 hour, most of the methanol was evaporated *in vacuo*. The concentrate was diluted with water and extracted with methylene chloride. Crystallization of the methylene chloride residue from ethyl acetate gave 109 mg. of yellowish solid, m.p. 215–220°. The product crystallized from methanol as colorless needles, m.p. 218–22°; m.m.p. with the "Quillo Bordon" alkaloid 216–220°; the infrared spectra of the two were identical; ultraviolet: λ_{max} 227–231(sh), 243, 250(sh), 264, 286–288(sh), 296, 318(sh), 328 and 342 mμ (log ϵ 4.56, 4.74, 4.63, 4.40, 4.08, 4.35, 3.50, 3.64 and 3.62, resp.); λ_{min} 259, 278, 308 and 335 mμ (log ϵ 4.34, 3.86, 3.36 and 3.53, resp.).

(24) We are grateful to Dr. Norbert Neuss and Dr. Harold Boaz, Eli Lilly Co., Indianapolis, Ind., for these data.

(25) R. Mirza, *J. Chem. Soc.*, 4400 (1957).

Anal. Calcd. for $C_{18}H_{20}N_2$: C, 81.78; H, 7.63; N, 10.60. Found: C, 81.88; H, 7.65; N, 10.59.

Methoxyellipticine.—An analytical sample was prepared from the sample described in the isolation section by sublimation at 180° at about 0.001 mm. Methoxyellipticine could also be crystallized from ethyl acetate as bright yellow needles, m.p. 280–285° dec., suitable for analysis; equivalent weight: A solution of 29.06 mg. of methoxyellipticine in glacial acetic acid required 2.341 ml. of 0.0448 *N* perchloric acid in acetic acid; calcd. 276 found 278. Ultraviolet: λ_{max} 246, 277, 294, 307(sh), 337, 353, 403 and 410–420(sh) mμ (log ϵ 4.42, 4.66, 4.73, 4.49, 3.81, 3.54, 3.57 and 3.55–3.52, resp.); λ_{min} 223, 254, 281, 327, 348 and 365 mμ (log ϵ 4.24, 4.33, 4.64, 3.65, 3.50 and 3.22, resp.). In 0.01 *N* potassium hydroxide in ethanol: essentially identical with the spectrum in alcohol alone. In approx. 0.1 *N* hydrochloric acid in ethanol: λ_{max} 225, 245–261(plateau), 278, 314, 360, 379 and 450 mμ (log ϵ 4.26, 4.41–4.40, 4.36, 4.75, 3.79, 3.88 and 3.60, resp.); λ_{min} 218, 231, 270, 288, 344, 368 and 400 mμ (log ϵ 4.22, 4.24, 4.33, 4.28, 3.67, 3.75 and 3.34, resp.).

Anal. Calcd. for $C_{18}H_{16}ON_2$: C, 78.23; H, 5.84; N, 10.14; OCH_3, 11.23; (C)CH_3, 5.44. Found: C, 78.09, 78.19; H, 5.89, 5.81; N, 10.29; OCH_3, 11.06; (C)CH_3, none.

Methiodide.—A solution of 105 mg. of methoxyellipticine in 150 ml. of acetone was allowed to stand with an excess of methyl iodide (10 ml.). The orange, crystalline precipitate (126 mg.) was collected and washed with acetone. The analytical sample crystallized as fine orange needles from methanol. The methiodide decomposed at about 340° without melting. Ultraviolet: λ_{max} 222(sh), 246–254(plateau), 260–265(sh), 281, 316, 364, 384 and 448 mμ (log ϵ 4.56, 4.43, 4.41, 4.38, 4.74, 3.78, 3.85 and 3.60, resp.); λ_{min} 238, 272, 290, 347, 373 and 407 mμ (log ϵ 4.36, 4.36, 4.32, 3.67, 3.74 and 3.43 resp.). In ethanolic potassium hydroxide solution: λ_{max} 245, 273, 338, 370–377(sh), 409 and 540 mμ (log ϵ 4.33, 4.46, 4.69, 394–393, 3.93 and 3.69, resp.); λ_{min} 237, 252, 295, 393 and 445 mμ (log ϵ 4.31, 4.32, 4.21, 3.84 and 3.10, resp.).

Anal. Calcd. for $C_{19}H_{19}ON_2I$: C, 54.56; H, 4.58; N, 6.70; I, 30.34. Found: C, 54.37; H, 4.56; N, 6.80; I, 30.47.

Elliptinine.—Elliptinine was found to be very soluble in methanol and sparingly soluble in ethyl acetate, benzene and chloroform. The tan solid, m.p. 213–219° (isolated as described), on recrystallization from methanol–benzene afforded a colorless crystalline solid, m.p. 229–233°. The analytical sample, m.p. 231–233°, $[\alpha]^{24}_{569}$ −255° and $[\alpha]^{24}_{436}$ −593° (*c* 0.200), was crystallized from methanol–benzene. Equivalent weight: The titration of elliptinine (15.669 mg.) in glacial acetic acid with 0.0448 *N* perchloric acid to the methyl violet end point required 1.100 ml.; calcd. 324, found 318. Ultraviolet: λ_{max} 222(sh) with end absorption and 311 mμ (log ϵ 4.37 and 4.27, resp.); λ_{min} 270 mμ (log ϵ 3.39); in 0.1 *N* hydrochloric acid in ethanol; λ_{max} 217 and 308 mμ (log ϵ 4.45 and 4.24, resp.); λ_{min} 265 mμ (log ϵ 3.50). Infrared: λ_{max} (satd. chloroform solution): 2.80(v.w.), 2.88(m), 6.16(m), and 6.30(m) μ; λ_{max} (Nujol mull): 2.90(v.w.), 3.02(m), 6.16(m) and 6.28(w) μ.

Anal. Calcd. for $C_{20}H_{24}O_2N_2$: C, 74.04; H, 7.46; N, 8.64; OCH_3, 9.57; (C)CH_3, 4.63. Found: C, 73.90, H, 7.05; N, 8.72; OCH_3, 9.83; (C)CH_3, 4.02; (N)CH_3, 0.73.

Perchlorate.—A small sample of elliptinine was converted to the perchlorate in methanol; λ_{max} (mull): 2.99(s), 3.16 (s), 3.35(Nujol), 5.97(v.w.), 6.15(m), and 6.27(m) μ.

Hydrogenation.—A solution of 57 mg. of elliptinine in 6 ml. of methanol was added to a pre-treated mixture of 50 mg. of Pd–C and 5 ml. of methanol and stirred in an atmosphere of hydrogen at atmospheric pressure and room temperature for 2 hours. The change in volume represented the absorption of one equivalent of hydrogen. After removal of the catalyst and solvent, a colorless solid residue, λ_{max} 298 mμ, was obtained. This could not be induced to crystallize.

Ochrosia sandwicensis A. DC.—One kilogram of ground leaves was treated according to procedure B. After chromatography, a total of 13 mg. of yellow, crystalline solid was obtained. This was shown to be a mixture of ellipticine and methoxyellipticine by paper chromatography. Isore-

serpiline and elliptinine were not detected, although a colorless alkaloid (90 mg.) not found in *O. elliptica* was eluted after the yellow alkaloids. Suitable solvents for crystallization could not be found and the alkaloid was converted to its hydriodide in acetone. The salt (which was quite soluble in acetone) was recrystallized from water, m.p. 215° dec., $[\alpha]^{23}_{589}$ +27° and $[\alpha]^{23}_{460}$ +58° (*c* 0.45). The ultraviolet spectrum was examined: λ_{max} 238 and 290 mμ and λ_{min} 258 mμ; infrared spectrum: λ_{max} (mull) 3.01 and 6.24 μ.

BETHESDA 14, MD.

[CONTRIBUTION FROM THE LABORATORY OF CHEMISTRY OF NATURAL PRODUCTS, NATIONAL HEART INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Alkaloids of *Lunasia amara* Blanco. Structure of Lunacrine

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Lunacrine has been assigned the structure I on the basis of the chemical reactions and interpretations of spectra described in this paper.

Lunacrine was first isolated in 1900 by Boorsma[1] from the bark of *Lunasia costulata* Miq. The alkaloid was characterized by its melting point (114°), solubility, precipitation reactions and color reactions. Wirth[2] carried out the first studies related to the structure of lunacrine in 1931, and reported the empirical formula to be $C_{16}H_{20}O_3N$. The presence of one methoxyl and one methylimino group, and the formation on zinc dust distillation of quinoline, skatole and ammonia were also reported.[2] A few years later, Dieterle and Beyl[3] corrected the obviously impossible formula and gave $C_{16}H_{19}O_3N$ which was later confirmed by Steldt and Chen.[4] The alkaloid described by the German workers,[3] although agreeing in melting point (116°) with that reported by others,[2,4] was said to be optically inactive whereas values had been given for $[\alpha]_D$ of −38°[2] and, later, −58°.[4] Lunacrine was stated to have a methylenedioxy group by virtue of the formation of the calculated amount of precipitate on heating with phloroglucinol–sulfuric acid.[3] Lunacrine methiodide on treatment with silver oxide followed by 40% alkali was reported to give a product, m.p. 85–86°, allegedly isomeric with lunacrine.[3] The alkaloid was found to be stable toward permanganate, chromic oxide and other oxidizing agents and was not affected by catalytic hydrogenation conditions.[3]

In the present study, lunacrine, m.p. 117–119°, $[\alpha]_{589}$ −50.4°, was isolated from the leaves of *Lunasia amara* Blanco of Philippine origin. The empirical formula and the presence of one methoxyl and one methylimino group were confirmed by analysis and, in addition, Kuhn–Roth determination indicated one terminal methyl group (or *gem*-dimethyl). The resistance of lunacrine toward catalytic hydrogenation was confirmed. No evidence was obtained for the presence of a methylenedioxy group; Labat and chromotropic acid color tests were negative. An examination of the infrared spectrum in the 3 μ region revealed no band at 3.60 μ, a diagnostic band for the methylenedioxy group; analysis of the 9.5–10.6 μ region was obscured by the presence of other bands.[5,6] The absence of a methylenedioxy group was shown conclusively by the absence of any signal in the appropriate region of the n. m. r. spectrum.[7,8]

The infrared spectrum[9] of lunacrine was devoid of absorption in the 5 μ region while the 6 μ region was very rich in bands; structural assignments were not possible. The ultraviolet spectrum[9] in alcohol was unaffected by the addition of alkali, but a marked change occurred on acidification (Fig. 1); the long wave length bands (313 and 326 mμ) exhibited a hypsochromic shift (300 mμ). Thus the nitrogen atom is a part of the chromophore and involved in a structure which would give this behavior in acid. This immediately eliminated from consideration the most prevalent nitrogen-containing nucleus in the Rutaceae alkaloids, *viz.*, quinoline whose long wave length ultraviolet absorption maxima exhibit pronounced bathochromic shifts in acid solution. This behavior of lunacrine was in fact reminiscent of 1-methyl-2-phenyl-4-quinolone, a derivative prepared from 4-methoxy-2-phenylquinoline, the first alkaloid isolated in the present study.[10] In the spectrum of this 4-quinolone the typical bifurcated maxima (325 and 337 mμ) were shifted to a single maximum at 304 mμ in acid. This is apparently a characteristic phenomenon of the 4-quinolone system as it has been found that the long wave length maxima (324 and 337 mμ) of 1-methyl-4-quinolone are shifted hypsochromically (plateau 303–313 mμ) in *N* hydrochloric acid.[11] After examination of the literature,[12] it would ap-

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(2) E. H. Wirth, *Pharm. Weekblad*, **68**, 1011 (1931); *C. A.*, **26**, 557 (1932).

(3) H. Dieterle and H. Beyl, *Arch. Pharm.*, **275**, 174, 276 (1937).

(4) F. A. Steldt and K. K. Chen, *J. Am. Pharm. Assoc., Sci. Ed.*, **32**, 107 (1943).

(5) L. H. Briggs, L. D. Colebrook, H. M. Fales and W. C. Wildman, *Anal. Chem.*, **29**, 904 (1957).

(6) We are indebted to Dr. H. M. Fales for this information.

(7) S. Goodwin, J. N. Shoolery and L. F. Johnson, THIS JOURNAL, **81**, in press (1959).

(8) The first n.m.r. spectrum of lunacrine was obtained and interpreted by Dr. H. Conroy of Brandeis University, to whom we are indebted.

(9) Details of the infrared and ultraviolet spectra are given in the Experimental section.

(10) S. Goodwin, A. F. Smith and E. C. Horning, *ibid.*, **79**, 2239 (1957).

(11) G. W. Ewing and E. A. Steck, *ibid.*, **68**, 2181 (1946), found no shift in the spectrum of 4-quinolone in 0.01 *N* hydrochloric acid; however, in *N* hydrochloric acid, the strength generally used in this study, a hypsochromic shift to give a broad maximum at 300–307 mμ was observed.

(12) *Cf.* E. A. Steck, G. W. Ewing and F. C. Nachod, *ibid.*, **71**, 238 (1949), for the spectra of 6-, 7- and 8-methoxy-3-methyl-4-quinolones.