amount of water and chromatographed on paper. The first dialysate (16 hours) contained maltose and maltotriose and the second (72 hours), maltotetraose in addition.

Discussion

Liver Glucosyl Oligosaccharides.—The data prove the existence in an aqueous homogenate of fresh liver of maltose, maltotriose, maltotetraose and a number of higher glucosyl homologs. This statement is based on the following observations. The active material ("X") was isolated from liver by a procedure (adsorption on and elution from charcoal) known to be applicable to oligosaccharides. Its properties (solubility in water and in alcohol and its passage through cellophane) excludes high molecular weight polysaccharides such as glycogen. It formed a polyacetate whose elemental analysis, optical rotation and molecular weight suggested a mixture of oligosaccharides. It was unstable in alkali and its reducing power increased greatly following acid hydrolysis. The major components of this mixture were isolated by column and paper chromatography. Minor components were prepared as a mixture (D). It was possible to prepare a pure acetyl derivative of the fastest component and its elemental analysis and rotation corresponded to maltose. Acid hydrolysis of "X" and of its isolated components A, B, C and D yielded only glucose which was identified by valid criteria. The first three members of this series were identified beyond question as maltose, maltotriose and maltotetraose (Table I). When the $\log_{10}(1/Rf - 1)$ of the spots in Fig. 2 was plotted against hexose units per molecule, a straight line resulted (Fig. 3) which indicates a homologous series.[15-17]

The origin of these oligosaccharides, present in liver homogenate, is the subject of current research in this Laboratory. Certain of these experiments to be reported elsewhere indicate that the question of the significance of liver glucosyloligosaccharides deserves more than passing attention.

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The Alkaloids of *Tabernanthe iboga*. Part III.[1] Isolation Studies

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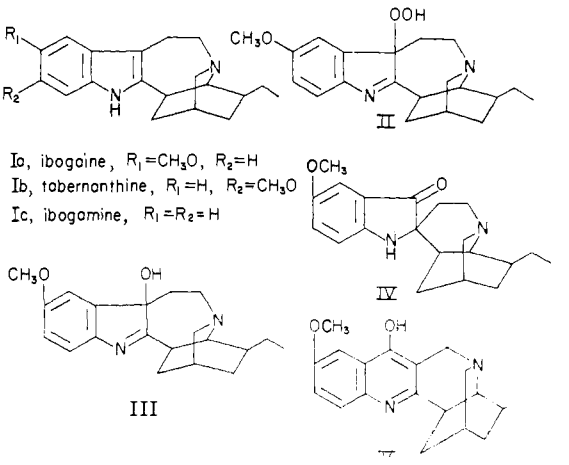
The present investigation of *Tabernanthe iboga* root records the isolation of twelve compounds, which includes not only the four known alkaloids ibogamine, ibogaine, tabernanthine and iboluteine but also iboquine, desmethoxyiboluteine, the hydroxyindolenine derivatives of ibogaine and ibogamine, voacangine, gabonine, kisantine and kimvuline. Of these, the latter three have not been described previously.

The current interest in *Tabernanthe iboga* initiated a more detailed investigation of the alkaloidal content of the root. Emphasis was placed both on the preparation of the major alkaloids in a state of high purity and on the isolation of the greatest possible number of minor alkaloids.

The three indole alkaloids ibogaine (Ia), tabernanthine (Ib) and ibogamine (Ic) known from the earlier investigations of *Tabernanthe iboga* root[2] are presumed to differ only with respect to a methoxyl on ring A.[3] A fourth alkaloid, iboluteine (IV), has been isolated from a plant extract[4] and has been shown to be a 5-methoxypseudoindoxyl related to ibogaine.[5] Catalytic oxidation of ibogaine followed by reduction and alkaline rearrangement led to its formation in good yield. Two intermediates of the reaction sequence, the hydroperoxy (II), and the hydroxyindolenine (III) derivatives of ibogaine were obtained in crystalline form. Desmethoxyiboluteine was prepared by a similar treatment of ibogamine but without isolation of the intermediate compounds. Air oxidation of ibogaine yielded not only iboluteine but also iboquine (V) which was formulated as a 6-methoxyquinolol.[4]



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In the present study, ibogaine readily was obtained on direct crystallization of the total alkaloids. Chromatography of the mother liquor material on alumina gave ibogamine in high purity followed by ibogaine which was eluted together with small amounts of tabernanthine. While the separation of the latter two alkaloids may be achieved by a tedious fractional crystallization, a simple method has been developed based on the relative solubilities of the hydrochlorides in acetone. Addition of hydrochloric acid to an acetone solution of the bases precipitates ibogaine hydrochloride. Tabernanthine is obtained by direct crystallization of the bases recovered from the filtrate. A similar method has been published which takes advantage of the greater solubility of tabernanthine hydrochloride in chloroform.[6] In our hands the separation claimed could not be realized.

Several minor alkaloids were isolated from the amorphous material remaining after the above chromatography. The hydroxyindolenine derivatives of ibogaine and ibogamine which were obtained in larger amounts than the other minor alkaloids had not previously been isolated from a plant extract. While they may occur naturally, their origin might equally well be attributed to the facile autoxidation of the parent alkaloids. The presence of iboluteine, desmethoxyiboluteine and iboquine in the plant extract may well arise as a consequence of this type of aerial oxidation.

The presence of voacangine confirms the relationship of *Tabernanthe* and *Voacanga* alkaloids. The first indication was recorded by Janot and Goutarel who obtained ibogaine by saponification and decarboxylation of voacangine.[7] Its occurrence in *Tabernanthe iboga* had not been noted prior to the present isolation.

Gabonine, kisantine and kimvuline are new *Tabernanthe* alkaloids and deserve additional comment. Gabonine has been isolated from numerous *Tabernanthe* samples and is one of more widely distributed *Tabernanthe* alkaloids. Micro-analysis indicates an empirical formula of $C_{21}H_{28}N_2O_4$ and the presence of two methoxyl groups. The compound is recovered unchanged following attempted reduction with $LiAlH_4$. The ultraviolet absorption with maxima at 253, 287 and 345–349 mμ differs from that of any other *Tabernanthe* alkaloid. Only a very weak band is present in the OH–NH region of the infrared absorption spectrum.

Another alkaloid, kisantine, analyzes for $C_{21}H_{28}N_2O_5$ and has two methoxyl groups. The ultraviolet spectrum has maxima at 213–214 and 274–276 mμ with a shoulder at 296 mμ. Infrared data indicate an –NH group and a band at 1670 cm.$^{-1}$ which possibly represents an unsaturated ester moiety.

Kimvuline which was isolated from the recrystallization mother liquor of crude ibogaine has been assigned the empirical formula $C_{20}H_{26}N_2O_2$. One oxygen is represented in a methoxyl while the second probably occurs as a hydroxy group. This oxygen function is not readily apparent in the infrared spectrum since strong hydrogen bonding is present which does not change on dilution in chloroform solution. The ultraviolet spectrum is typical of indoles and remains unchanged in alkaline medium.

The authors wish to express their thanks to Mr. B. Korzun for the paper chromatography used throughout this investigation, and to Mr. L. Dorfman and his staff for the analytical and spectral data.

Experimental

Ibogaine.[8]—*Tabernanthe iboga* (96.7 kg.) was extracted four times by recycling methanol at 60–65° through a stationary bed of the ground root for two hours. The total extract (1320 l.) was evaporated *in vacuo* to 18 l. to which was added 9.6 l. of water, 28.8 l. of 15% acetic acid and 1.25 kg. of Filter-Cel. The mixture was stirred, filtered, and the filter cake washed with 2.4 l. of a solution prepared by mixing 0.18 l. of acetic acid, 0.8 l. of methanol and 1.6 l. of water. The combined filtrate and washings were extracted twice with 19.2-l. portions of petroleum naphtha (b.p. 60–90°). The petroleum naphtha extracts were back washed with 3.8 l. of 15% acetic acid. To the aqueous phase, 25.2 l. of ethylene dichloride was added followed by 19.1 l. of ammonium hydroxide over a period of 90 min. The layers were separated and the aqueous phase re-extracted five times with 12.8-l. portions of ethylene dichloride. The extracts were combined and washed with three 25-l. portions of water, dried over anhydrous sodium sulfate, filtered, and concentrated to 2.5 l. Ethanol (2 l.) was added, the solution again concentrated to 2.5 l., and a further 6 l. of ethanol added. After chilling for two days 456 g. of crude ibogaine was collected, m.p. 140–143°. The filtrate was evaporated to four liters and chilled overnight yielding a second crop of 474.5 g. melting at 137–140°. The filtrate was evaporated to dryness and the dark amorphous residue of 1.16 kg. was reserved for the isolation of the minor alkaloids.

The crude ibogaine (474 g.) in 2890 ml. of ethanol was treated with 50 g. of Norite, filtered, and allowed to crystallize yielding 379 g. melting at 143.5–146°. A second treatment with Norite and crystallization from ethanol yielded 309 g. melting at 148–149°. This product in 3,090 ml. of benzene was filtered through 620 g. of neutral alumina (Activity II) and the column washed with an equal volume of benzene. Evaporation of the solvent and crystallization of the residue from 1400 ml. of ethanol yielded 259 g. of pure ibogaine melting at 152–153°. Chromatography on paper showed only a trace of impurity. For analysis a sample was dried overnight in high vacuum at room temperature.

Anal. Calcd. for $C_{20}H_{26}N_2O$: C, 77.38; H, 8.44; N, 9.03. Found: C, 77.51; H, 8.70; N, 9.13.

Ibogaine Hydrochloride.—To a stirred solution of 100 g. of ibogaine in 1000 ml. of acetone, 51 ml. of aqueous HCl (1:1) was added dropwise. Crystalline ibogaine hydrochloride separated immediately. The product was filtered and washed with acetone; yield 108.5 g., m.p. 299–300°, $[\alpha]D$ −63° (ethanol), −49° (water).

Anal. Calcd. for $C_{20}H_{26}N_2O \cdot HCl$: Cl, 10.22. Found: Cl, 10.27.

Chromatography of Ibogaine Mother Liquor.—From 717 g. of the dark amorphous mother liquor material, 320 g. of a light tan powder was obtained by leaching with a total of 12 l. of cyclohexane. A solution of 300 g. in 5 l. of cyclohexane was chromatographed on 15 kg. of neutral alumina (Activity III). Eighteen 12-l. fractions were taken with cyclohexane (1–18), the next nine with benzene (19–27), six with methylene chloride (28–33), two with chloroform (34–35), six with chloroform containing 1% methanol (36–41), and finally three with chloroform–methanol, 1:1 (42–44).

Ibogamine and its Hydroxyindolenine Derivative (Fract. 8–18).—Concentration of the cyclohexane eluate yielded a total of 70 g. of crystalline ibogamine melting at 154–160°. An additional 6.5 g. was recovered from the filtrate. A small sample was recrystallized four times from methanol; m.p. 162–163°, $[\alpha]^{26}D$ −36.4° (chloroform).

Anal. Calcd. for $C_{19}H_{24}N_2$: C, 81.38; H, 8.63; N, 9.99. Found: C, 81.23; H, 8.67; N, 9.94.

The filtrate from the isolation of crude ibogamine was

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(8) The extraction and preparation of the crude ibogaine was carried out by Mr. E. Solook of our Pilot Plant Section.

evaporated to dryness. The residue (5.2 g.) was dissolved in benzene and chromatographed on 156 g. of basic alumina. Benzene (875 ml.) eluted 1.4 g. of ibogamine. Methylene chloride (1800 ml.) and chloroform (900 ml.) eluted the hydroxyindolenine derivative of ibogamine which crystallized readily from acetone; yield 1.5 g., m.p. 161–163°. A small sample, recrystallized from acetone, melted indistinctly about 100°, solidified and then remelted at 168–172°, $[\alpha]^{25}D$ +82.5° (95% ethanol); ultraviolet absorption: plateau, 217–219 mμ (18,800); max., 222 mμ (19,800); shoulder, 228 mμ (13,700); max., 253–254 mμ; (3910); max., 281 mμ (3,200); shoulder, 292 mμ (3,020). The infrared spectrum showed a carbonyl function as an impurity consistent with acetone of solvation.

Anal. Calcd. for $C_{19}H_{24}N_2O \cdot 0.5CH_3COCH_3$: C, 75.66; H, 8.36; N, 8.61. Found: C, 75.34; H, 8.46; N, 8.61.

Tabernanthine (Fract. 19–21).—Crystallization of the combined fractions (102 g.) from ethanol yielded 76 g. of crude ibogaine, m.p. 140–150°, containing about 4% tabernanthine (estimated from a paper chromatogram). The mixture (56 g.) in 1 l. of acetone was titrated with 30.5 ml. of 1:1 aqueous hydrochloric acid. The insoluble ibogaine hydrochloride was filtered and washed with acetone. The filtrate was made basic with ammonium hydroxide, filtered and evaporated to dryness. The residue in 200 ml. of methylene chloride was washed with water and dried over sodium sulfate. Evaporation of the solvent and crystallization of the residue (4.7 g.) from ethanol yielded 1.5 g. of tabernanthine melting at 207–211°.

A small sample was recrystallized from ethanol and sublimed at 160° at 0.005 mm., m.p. 213.5–215°.

Anal. Calcd. for $C_{20}H_{26}N_2O$: C, 77.38; H, 8.44; N, 9.03. Found: C, 77.09; H, 8.37; N, 8.99.

Tabernanthine Hydrochloride.—To a solution of tabernanthine (0.45 g.) in 5 ml. of methylene chloride, 0.30 ml. of 1:1 aqueous hydrochloric acid was added. The mixture was chilled for two hours and then filtered. The product was recrystallized from water; yield 0.42 g., m.p. 275–277°. Another recrystallization from water failed to raise the melting point; $[\alpha]^{25.5}D$ −66° (methanol).

Anal. Calcd. for $C_{20}H_{26}N_2O \cdot HCl$: C, 69.25; H, 7.85; N, 8.08; Cl, 10.22. Found: C, 69.60; H, 7.86; N, 8.06; Cl, 10.33.

Hydroxyindolenine Derivative of Ibogaine and Iboquine.—A benzene solution of 5 g. of mother liquor residue from the crystallization of the ibogaine–tabernanthine mixture was chromatographed on 150 g. of basic alumina. Benzene eluted 2.1 g. of ibogaine while methylene chloride and methylene chloride containing 1% methanol afforded the hydroxyindolenine derivative of ibogaine. Following crystallization from 95% ethanol the compound melted at 123–124°, $[\alpha]D$ +74° (ethanol); ultraviolet absorption: max., 223 mμ (13,760); shoulder 260 mμ; max., 283 mμ (5,900); shoulder, 290 mμ; max., 312–313 mμ (3,530).

Anal. Calcd. for $C_{20}H_{26}N_2O_2 \cdot H_2O$: C, 69.74; H, 8.19; N, 8.13. Found: C, 69.64; H, 8.21; N, 8.24.

Recrystallization from acetone or from benzene gave a product melting sharply at 147–149°.

The latter fractions eluted with methylene chloride containing 1% methanol gave crystals from acetone, 0.04 g., which after recrystallization from methanol–ether melted at 268–271°. The ultraviolet and infrared absorption were identical with those of iboquine.

Gabonine (Fract. 24).—A 0.5-g. sample of the amorphous fraction (5.4 g.) was rechromatographed on 15 g. of basic alumina. Methylene chloride eluted 0.16 g. of gabonine which was crystallized from ethanol–water, m.p. 211–215°. After three recrystallizations from methylene chloride–ethanol and drying *in vacuo* the compound melted at 223–226°, $[\alpha]^{24}D$ +65° (chloroform); ultraviolet absorption: max. at 253 mμ (25,400), 287–288 mμ (6,860) and 355–359 mμ (5,850). The infrared spectrum showed strong absorption at 1620 cm.$^{-1}$, medium at 1672, and very weak absorption in the OH, NH region. The compound may contain an amide or conjugated carbonyl grouping.

Anal. Calcd. for $C_{21}H_{28}N_2O_4$: C, 67.72; H, 7.58; N, 7.52; CH_3O, 16.4. Found: C, 67.65, 67.81; H, 7.78, 7.53; N, 7.71, 7.86; CH_3O, 16.42.

Kisantine (Fract. 25–29).—Of the 4.1 g. of material eluted, 3.5 g. was rechromatographed on 175 g. of basic alumina (Activity III). A crystalline compound, m.p. 130–135°, was obtained from the benzene eluate in too small an amount to permit positive identification. Continued elution of the column with methylene chloride afforded gabonine, 0.06 g. Methylene chloride containing 1% methanol eluted kisantine which crystallized from ethanol, m.p. 236–238°, $[\alpha]^{26}D$ −15° (chloroform); ultraviolet absorption: max., 213 mμ (31,700) and 270–276 mμ (6,270); shoulder, 296 mμ (4,710). The infrared spectrum showed a medium intensity band at 1670 cm.$^{-1}$ and a strong band at 1630 cm.$^{-1}$ in the carbonyl region. The compound was sublimed before analysis.

Anal. Calcd. for $C_{21}H_{28}N_2O_3$: C, 70.76; H, 7.92; N, 7.86; $CH_3O(2)$, 17.4. Found: C, 70.57, 70.68; H, 7.67, 7.92; N, 7.96; CH_3O, 16.25; no N-methyl.

Desmethoxyiboluteine (Fract. 37).—Chromatography of 1.2 g. of the amorphous fraction in benzene on 139 g. of basic alumina (Activity III) yielded the hydroxyindolenine derivative of ibogamine, m.p. 157–162°. Methylene chloride containing 0.25% ethanol eluted desmethoxyiboluteine, 0.55 g., which after one crystallization from benzene and five from methanol melted sharply at 141°; ultraviolet absorption: max., 230–231 mμ (23,470); plateau, 250–252 mμ (6,430); shoulder, 256 mμ (6,260); max., 400–401 mμ (3,320). Paper chromatography and infrared absorption confirm the identity of the compound as desmethoxyiboluteine.

Iboluteine (Fract. 39).—Paper chromatography showed this fraction (9.5 g.) to consist almost entirely of iboluteine. A solution of 1.6 g. in benzene was rechromatographed on 100 g. of basic alumina (Activity III). Benzene eluted a mixture of iboluteine with small amounts of desmethoxyiboluteine while later fractions eluted with methylene chloride contained pure iboluteine. For analysis the product was crystallized from methanol and sublimed, m.p. 142°, $[\alpha]^{25}D$ −114° (chloroform).

Anal. Calcd. for $C_{20}H_{26}N_2O_2$: C, 73.59; H, 8.03; N, 8.58. Found: C, 73.51; H, 8.01; N, 8.62.

Voacangine.—From another sample of *Tabernanthe* root (4 kg.), ibogaine was obtained as before on direct crystallization of the total alkaloids. The mother liquor material in benzene was chromatographed on 1500 g. of neutral alumina (Activity II–III). The first 3000-ml. fraction eluted 27 g. of amorphous material which was rechromatographed on 2500 g. of neutral alumina (Activity II–III). Following ibogamine and ibogaine, voacangine, 0.65 g., m.p. 125–130°, was eluted with benzene–ether (1:1). Recrystallization from ethanol raised the m.p. to 136–137°, $[\alpha]^{26}D$ −34° (chloroform), pK_a' 7.4 (40% aqueous methanol); ultraviolet absorption: max., 226 mμ (28,400) and 288–293 mμ (9,140); shoulder, 300 mμ (8,600).

Anal. Calcd. for $C_{22}H_{28}N_2O_3$: C, 71.71; H, 7.66; N, 7.60; $CH_3O(2)$, 16.85. Found: C, 71.50, 72.14; H, 7.68, 7.85; N, 7.67; CH_3O, 16.18.

The isolated compound gave an undepressed mixed melting point with voacangine[9] and had an identical infrared spectrum and X-ray powder diagram.[10] Further elution with ether–methanol mixtures yielded kisantine and the hydroxyindolenine derivative of ibogaine.

Kimvuline.—The mother liquor material (29.7 g.) from the first recrystallization of 206 g. of crude ibogaine was chromatographed on 750 g. of neutral alumina (Activity III). Kimvuline was eluted with benzene following the ibogaine fractions. After one crystallization from methanol the m.p. was 228–230°, 0.26 g. Recrystallization from benzene raised the m.p. to 231–232.5°, $[\alpha]D$ +3.7° (chloroform); ultraviolet absorption: max., 227 mμ (24,800), 288–291 mμ (8,400) and 297 mμ (8,300).

Anal. Calcd. for $C_{20}H_{26}N_2O_2$: C, 73.59; H, 8.03; N, 8.58; CH_3O, 9.07. Found: C, 73.62; H, 8.25; N, 8.57; CH_3O, 8.92, 8.96.

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(9) Supplied through the courtesy of Dr. N. Neuss, Lilly Research Laboratories.

(10) Measured by Prof. G. A. Jeffery, Sarah Mellon Scaife Radiation Laboratory, University of Pittsburgh.