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CONSTITUENTS OF *ISERTIA HYPOLEUCA* BENTH. (RUBIACEAE)—I.

ISOLATION AND CHARACTERIZATION OF DIHYDROQUINAMINE FROM THE LEAF MATERIAL

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Abstract—A crystalline alkaloid has been isolated from the leaves of *Isertia hypoleuca* Benth. and characterized as dihydroquinamine (I) by chemical and spectroscopic means.

INTRODUCTION

THE GENUS *Isertia* of the Rubiaceae was first described by Schreb in 1879. The names *Phosanthus*, *Brignolia*, *Bruinsmania*, *Guettarda*, *Iserta* and *Isartia* are found as synonyms of *Isertia*.[1] To date about twenty-nine species have been reported for this genus, most of them occurring in tropical and subtropical South America.[2–4]

I. hypoleuca Benth. has been botanically described on several occasions[1,4–6,9,12] with certain authors placing it among the *Cinchonaceae*[7] and others putting it in the Mussaendeae.[6,8] *I. coccinea* Vahl var. *β-hypoleuca* Schum. is recorded as binomial.[8] It is a shrub or small tree growing to a height up to 8 m. The leaves are opposite, large, obovate-oblong, densely tomentose beneath. The flowers are red in small dense heads. The species is reported to occur in Ecuador,[9] Brazil,[2,8] Colombia,[4,10,12] the Guianas,[1,2] Panama,[11] Peru,[1,4,9] and Venezuela.[12]

A review of the literature failed to disclose any significant work concerned with the chemical aspects of the genus *Isertia* and *I. hypoleuca* in particular. Thus the present investigation is of interest both from a chemical and chemotaxonomic point of view.

[1] C. SCHUMANN, in *Flora Brasiliensis* (edited by C. F. P. de MARTIUS and A. G. EICHLER) **6**, 284 (1889).
[2] *Index Kewensis*, Vol. 1, p. 1235, Clarendon Press, London (1895).
[3] A. H. R. GRISEBACH, *Flora of the West Indian Islands*, p. 320, Lovel Reeve, London (1864).
[4] P. C. STANDLEY, *The Rubiaceae of Colombia*, Field Mus. Nat. Hist., Bot. Ser., Pubcn. 270, **7**, p. 45, Chicago (1930).
[5] G. BENTHAM, *J. Botany* **3**, 220 (1841).
[6] P. C. STANDLEY, *The Rubiaceae of Ecuador*, Field Mus. Nat. Hist., Bot. Ser., Pubcn. 285, **7**, p. 183, Chicago (1931).
[7] A. H. R. GRISEBACH, *lit. cit.*, p. 316.
[8] C. SCHUMANN, *lit. cit.*, p. 286.
[9] P. C. STANDLEY, *The Rubiaceae of Ecuador*, Field Mus. Nat. Hist., Bot. Ser., Pubcn. 285, **7**, p. 211, Chicago (1931).
[10] R. E. SCHULTES, personal communication.
[11] M. D. CORREA a., personal communication.
[12] P. C. STANDLEY, *The Rubiaceae of Venezuela*, Field Mus. Nat. Hist. Bot. Ser., Pubcn. 302, **7**, p. 384, Chicago (1931).

RESULTS AND DISCUSSION

Alkaloid fractions were obtained from *Isertia hypoleuca* leaves according to the scheme shown in Fig. 1.

Alkaloids were detected mainly in fraction C. Fraction A was found to be devoid of alkaloids while fractions B and D contained only traces. The crude alkaloid mixture was chromatographed on an alumina column (grade I) and eluted with chloroform–methanol (1:1). TLC of the resulting fractions on silica gel GF_{254} plates developed with methylene chloride–methanol (9:1), showed two major alkaloids very close to each other at R_f 0·2–0·3. Several other minor alkaloids were also present. Rechromatography of the crude mixtures on silicic acid columns eluted with methylene chloride–methanol mixtures of increasing polarity

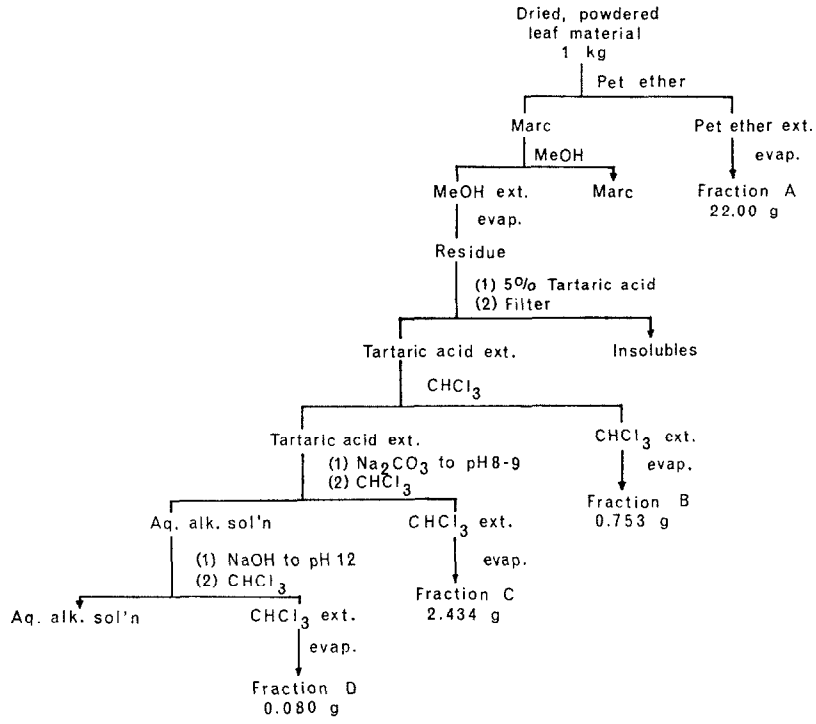


FIG. 1. FLOW SHEET FOR THE ISOLATION OF CRUDE ALKALOID FRACTIONS.

(99:1–95:5) and further purification by preparative TLC yielded a pure alkaloid which was designated alkaloid A. The alkaloid, recrystallized from acetone–water, melted at 154–156° and gave the characteristic violet color of indole alkaloids when the chromatoplates were sprayed with van Urk's reagent. The u.v. spectrum showed absorptions characteristic of dihydroindoles.[13,14] The i.r. spectrum was found to be very similar to that of quinamine.

Furthermore, no significant differences were observed between this substance and an authentic sample of quinamine* on TLC. The empirical formula $C_{19}H_{26}O_2N_2$ (mass spect-

* We wish to thank Dr. B. Witkop, NIH, Bethesda, Maryland, for kindly providing a sample of the pure alkaloid.

[13] A. W. SANGSTER and K. L. STUART, *Chem. Rev.* **65**, 109 (1965).

[14] *Physical Data of Indole and Dihydroindole Alkaloids* (edited by N. NEUSS), Vol. 1, Eli Lilly, Indianapolis, Indiana (1964).

rum, M+314) and the absence of olefinic protons in the NMR spectrum (3–5τ) suggested the presence of a saturated quinuclidine moiety in Alkaloid A. This view was supported by the fact that this alkaloid did not take up hydrogen.

The observation that alkaloid A, on treatment with weak acids, was easily converted to a crystalline substance (II) of empirical formula $C_{19}H_{24}ON_2$ (mass spectrum, M+296) suggested the presence of a labile hydroxyl group which undergoes an elimination reaction with the immediate formation of a double bond. Moreover, II showed a characteristic blue fluorescence under u.v. light resembling the behavior of the naturally occurring 3-alkylidene phthalide, ligustilide, which also shows a strong blue fluorescence in similar circumstances.[15–18] In contrast alkaloid A was devoid of any fluorescence. This behavior can explain the seemingly anomalous result that alkaloid A takes up bromine. The addition of halogen was considered to take place via II to yield the corresponding dibromide III (scheme 1). This was confirmed by treating alkaloid A with mineral acid followed by addition of bromine to yield a product identical in melting point and R_f value with that obtained by addition of bromine to alkaloid A.

TABLE 1. MAJOR FRAGMENT IONS IN THE MASS SPECTRA OF QUINAMINE AND ALKALOID A

Quinamine *m/e*	Alkaloid A *m/e*
108	110
121*	123*
136	138
164	166
281	283
294	296
311	313
312	314
313	315

* Base peak.

Additional evidence for the identity of alkaloid A was provided by mass spectral data. The fragmentation pattern of our alkaloid was found to be very similar to that of quinamine, differing only by two mass units in the major fragment ions. The equivalent fragment ions are indicated in Table 1. The lower mass end of the two spectra are almost identical. In addition to the base peak at *m/e* 121 the spectrum of quinamine shows a very intense *m/e* 136 peak which is most likely due to the splitting of the quinucludine moiety to give ion XI, which itself can generate the *m/e* 121 fragment by H transfer and loss of $\cdot CH_3$ as well as the *m/e* 108 by cleavage of $\cdot C_2H_4$. The spectrum of alkaloid A does not show as great an intensity in its *m/e* 138 peak. However, the same bond ruptures can be presumed to occur, yielding ions at *m/e* 123 and 110. Besides these, other important fragments in the spectrum of alkaloid A can be found at 297 (M+—OH) and 285 (M+—C_2H_5). Possible fragmentation schemes are shown in Fig. 2.

Therefore, alkaloid A must have structure I which is dihydroquinamine and the above reactions result as indicated in Scheme 1.

[15] H. MITSUHASHI, U. NAGAI, T. MURAMATSU and H. TASHIRO, *Chem. Pharm. Bull. (Japan)* **8**, 243 (1960).
[16] H. MITSUHASHI and U. NAGAI, *Tetrahedron* **19**, 1277 (1963).
[17] H. BOHRMANN, E. STAHL and H. MITSUHASHI, *Chem. Pharm. Bull. (Japan)* **15**, 1606 (1967).
[18] E. STAHL and H. BOHRMANN, *Naturwissenschaften* **54**, 118 (1967).

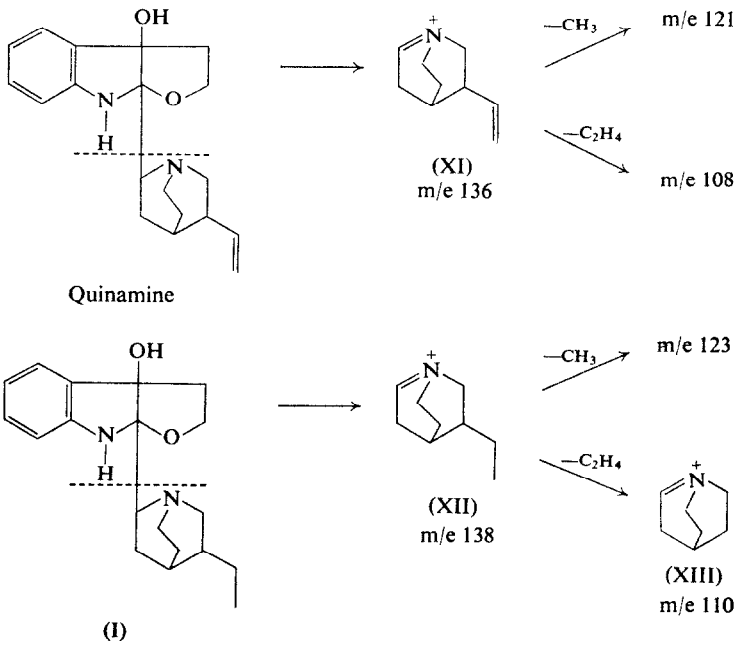
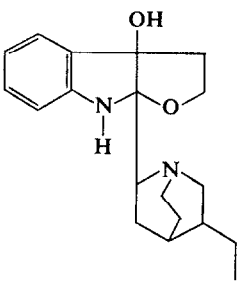
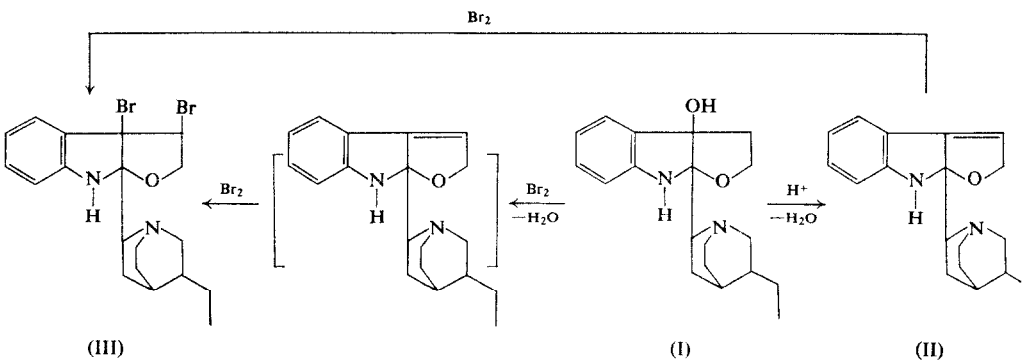
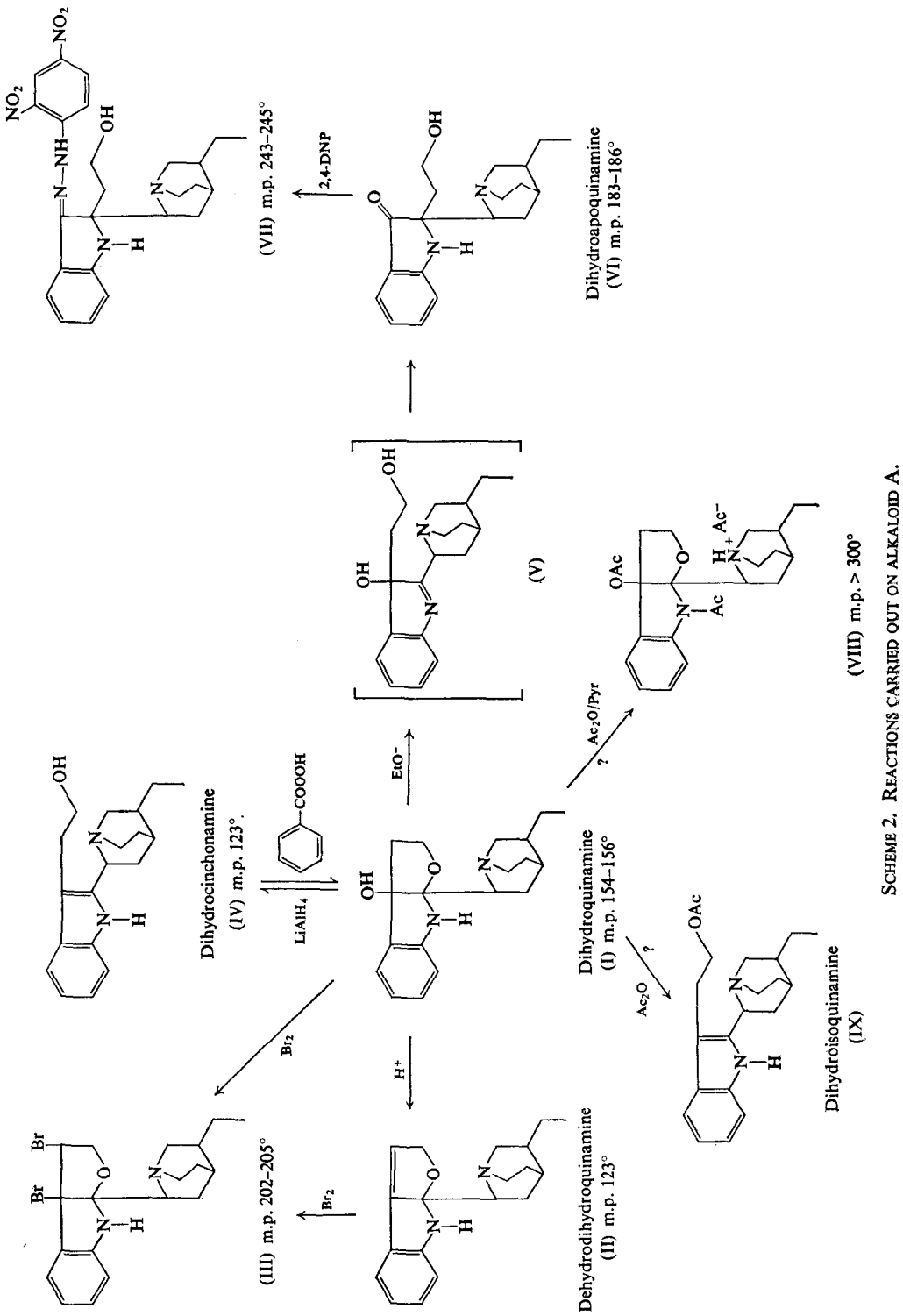


FIG. 2. POSSIBLE FRAGMENTATION PATTERNS OF QUINAMINE AND ALKALOID A.





SCHEME 1. REACTIONS OF DIHYDROQUINAMINE ON TREATMENT WITH ACIDS AND BROMINE.



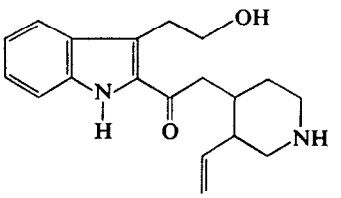
SCHEME 2. REACTIONS CARRIED OUT ON ALKALOID A.

Further investigation of alkaloid A followed methods previously reported for the structure elucidation of quinamine. All of the reactions carried out are summarized in Scheme 2.

Refluxing alkaloid A with sodium ethoxide[19] gave a yellow solution which, after chromatography on a silica gel column, yielded dihydroisoquinamine (VI) as yellow crystals, m.p. 183–186°; dinitrophenylhydrazone, m.p. 143–145°. According to Taylor[20] the reactions proceed via V. It should be noted here that both dihydroquinamine and dihydroisoquinamine have been prepared synthetically by reduction of the respective compounds. However, the synthetic dihydroquinamine has a m.p. of 184–185° and the dihydroisoquinamine a m.p. of 202–204°. This may reflect a different steroisomer for our alkaloid.

Paralleling the reduction of quinamine to cinchonamine described by Goutarel *et al.*,[21] alkaloid A was reduced with $LiAlH_4$ to a product which crystallized as fine colorless needles melting at 123° (h R_f 35). The reverse reaction with peracetic acid reported by Witkop[22] was carried out by spotting the reduction product on a silica gel plate, adding a drop of a methanolic solution of perbenzoic acid, allowing to react for a short time, and developing the plate. The resulting material had the same R_f value as alkaloid A. Therefore, the product with higher R_f must be dihydrocinchonamine (IV).

Hesse[23] reported the preparation of quinamidine (X) from quinamine by allowing the latter to stand for several days with 13% HCl or by refluxing with aqueous tartaric acid at



Quinamidine
(X)

130°. Likewise, Kirby[24] obtained a crude material, "quinamicine", on refluxing quinamine with dilute acetic acid; however, this product was later found to be impure quinamidine.[25] Treatment of alkaloid A with acids did not afford the expected dihydroquinamidine but always dehydrodihydroquinamine (II).

The results of the acetylation experiments carried out with alkaloid A were not comparable to those reported for quinamine. On treatment of the substance with acetic anhydride in pyridine, fine colorless needles melting above 300° were obtained. This compound may be VIII, the salt formation probably taking place at the more basic quinuclidine ring nitrogen. On prolonged refluxing of alkaloid A with pure acetic anhydride, no product equivalent to the apoquinamine of Henry *et al.*[26] was obtained. Instead a mixture of several substances, including dehydrodihydroquinamine as the major component, was detected by TLC. These investigations are still continuing.

[19] K. S. KIRBY, *J. Chem. Soc.* 735 (1949).
[20] W. I. TAYLOR, *Proc. Chem. Soc.* 247 (1962).
[21] R. GOUTAREL, M. M. JANOT, V. PRELOG and W. I. TAYLOR, *Helv. Chim. Acta* **33**, 150 (1950).
[22] B. WITKOP, *J. Am. Chem. Soc.* **72**, 2311 (1950).
[23] O. HESSE, *Berichte* **5**, 265 (1872); **10**, 2152 (1877); *Annalen* **166**, 217 (1873); **199**, 333 (1879); **207**, 288 (1881).
[24] K. S. KIRBY, *J. Chem. Soc.* 528 (1945).
[25] C. C. J. CULVENOR, L. J. GOLDSWORTHY, K. S. KIRBY and R. ROBINSON, *J. Chem. Soc.* 1485 (1950).
[26] T. A. HENRY, K. S. KIRBY and G. E. SHAW, *J. Chem. Soc.* 524 (1945).

To date only three alkaloids of the 2,2'-indolylquinuclidine type are known to occur in nature; namely quinamine, cinchonamine and conquinamine. They have all been reported as minor alkaloids of species of *Cinchona* and *Remijia.*[27] *I. hypoleuca* Benth. is the first plant found to contain a 2,2'-indolylquinuclidine alkaloid as the major alkaloid. Although it may seem premature to derive conclusions from a single observation, these results would appear to favor placement of *I. hypoleuca* Benth. among the Cinchonaceae. Further work on this plant is continuing and has resulted in the isolation of another very similar alkaloid whose structure elucidation is presently in progress.

EXPERIMENTAL

Melting points are uncorrected and were taken on a Kofler hot-stage or on a Fisher Johns melting point apparatus. I.r. spectra were taken in KBr pellets, or in $CHCl_3$ solution on a Beckman IR-8 spectrophotometer. U.v. spectra were measured in methanol on a Cary Model 15 spectrophotometer. NMR spectra were taken on a Varian A-60 spectrophotometer in deuteriochloroform with tetramethylsilane as an internal standard. Mass spectra were determined on a Hitachi-Perkin Elmer Model RMU-6D mass spectrometer. (Analyses performed by Morgan-Schaffer Corporation, Montreal, Canada.)

Isolation of the Alkaloids

1 kg of coarsely ground *I. hypoleuca* leaves was exhaustively defatted with light petroleum (b.p. 30–60°) for 36 hr in a Soxhlet-type extractor. Evaporation of the solvent *in vacuo* left a semi-solid dark-green residue (fraction A; 22·0 g; see Fig. 1). The marc, after air drying, was extracted with MeOH–NH_3 for 48 hr. The extraction was repeated with a fresh charge of solvent for another 24 hr. The combined methanolic extract was evaporated *in vacuo* to a sirupy residue which was then repeatedly extracted with a 5% tartaric acid solution with the aid of a Waring blender until the aqueous solutions did not give a positive test for alkaloids with Dragendorff's and Mayer's reagents. The acid aqueous extract was stored for 2 days at 5°, the insolubles removed by filtration, and extracted with $CHCl_3$ until the organic layer was colorless. Removal of the $CHCl_3$ *in vacuo* left an oily green residue (fraction B; 0·753 g). The aqueous solution was made basic to a pH 8–9 (Na_2CO_3) and extracted several times with $CHCl_3$. The combined $CHCl_3$ extract was dried (Na_2SO_4), filtered, and evaporated to dryness *in vacuo* to a pale-yellow crystalline residue (fraction C; 2·434 g). The pH of the alkaline solution was adjusted to pH 12 (NaOH) and extracted several times with $CHCl_3$. The extract, upon evaporation of the solvent *in vacuo*, gave a brown residue (fraction D; 0·08 g).

Partial purification of the alkaloidal mixtures was achieved by chromatography on Woelm neutral alumina (grade I) using $CHCl_3$–MeOH (1:1) as eluant. The fractions thus obtained were rechromatographed on Merck silicic acid columns with CH_2Cl_2–MeOH mixtures of increasing polarity (99:1 to 95:5) as solvent. Fractions of 20 ml were collected utilizing an automatic fraction collector. The development of the columns was monitored by TLC. Fractions showing similar composition were combined. As none of these fractions was found to contain a single component, further purification and separation was carried out by preparative TLC. Plates were prepared using silica gel GF_{254} as the sorbent and the Desaga apparatus,* according to the standard procedures of Stahl.[28] For preparative work the layer was 500 μ thick; for analytical work 250 μ. The plates were usually developed twice (CH_2Cl_2–MeOH,9:1) to a distance of 15 cm using a saturated chamber. After development, they were dried and viewed under a short wavelength u.v. lamp. Visualization was possible by spraying the chromatograms with either van Urk's[29] or modified Dragendorff's[30] reagents. For preparative separations, 10–20 mg of alkaloidal mixture in CH_2Cl_2 solutions was applied as a narrow band 2 cm from one of the edges of the plate, allowed to dry, and then developed. The separated bands were marked under an u.v. lamp, scraped from the plate, the powder packed in small columns, and eluted with $CHCl_3$–MeOH (1:1, v/v). Evaporation of the solvent *in vacuo* left a semicrystalline, pale-yellow residue which, after recrystallization from acetone–water, gave alkaloid A as silky, white needles melting at 154–156°; $C_{19}H_{26}O_2N_2$ (mass spectrum, M+314); u.v.$_{max}$ 242 and 300 nm (log ϵ 3·932, 3·425) unchanged on addition of 1 N NaOH or HCl in methanol. ν_{max} (KBr) 3333 cm^{-1} (NH or OH), 1613 and 1471 cm^{-1} (aromatic), 1374 cm^{-1} (CH_3), 1111, 1046 and 1020 cm^{-1} (C—O—C), 745 cm^{-1} (four adjacent H on aromatic ring); ν_{max} ($CHCl_3$, NaCl prism) 3413 cm^{-1} (NH

* C. A. Brinkman, Cantiague Road, Westbury, Long Island, N.Y.

[27] W. I. TAYLOR, in *The Alkaloids* (edited by R. H. F. MANSKE), Vol. 8, p. 237, Academic Press, New York (1965).

[28] D. WALDI, in *Thin-Layer Chromatography* (edited by E. STAHL), p. 31, Springer-Verlag, Berlin (1965).

[29] D. WALDI, *lit. cit.*, p. 490.

[30] R. J. BLOCK, E. L. DURRUM and G. A. ZWEIG, *A Manual of Paper Chromatography and Paper Electrophoresis*, 2nd edition, p. 361, Academic Press, New York (1958).

or OH), 1603 and 1460 cm^{-1} (aromatic), 1383 cm^{-1} (CH_3), 1105, 1036 and 1016 cm^{-1} (C—O—C); NMR: 2·5–3·5τ (multiplet, 4H), 5·6τ (singlet, 1H) 7·0τ (quartet, 6H), *ca.* 7·5τ (quartet, 3H), *ca.* 8·4τ (multiplet 9H), 9·15 (triplet, 3H). No (OCH_3) or NCH_3 (Zeisel).

Dehydrodihydroquinamine (II)

A solution of 5 mg of alkaloid A in 1 ml of pure methanol and one drop of 2 N HCl was heated on a steam bath for 5 min. After cooling, the solvent was removed *in vacuo* and the residue taken in Et_2O. The ether extract was washed with $NaHCO_3$, dried (Na_2SO_4), filtered, and evaporated to dryness *in vacuo*. The residue recrystallized from acetone–water gave thick blades gathered in rosettes (3 mg), m.p. 121–123°; m.w.: 296 (mass spec.) corresponding to an empirical formula $C_{19}H_{24}ON_2$; i.r. ν_{max} (KBr) 3195 cm^{-1} (NH), 1650 cm^{-1} (aromatic ring or C=C), 1075 cm^{-1} (C—O—C), 840 cm^{-1} ($\begin{smallmatrix}R\\R\end{smallmatrix}\!\!>\!C=C\!<\!\!\begin{smallmatrix}R\\H\end{smallmatrix}$) and 748 cm^{-1} (4 adjacent hydrogens on aromatic ring); u.v. λ_{max} (MeOH) 236 and 306 mμ (log $E^{1\%}_{1cm}$ 3·88, 3·92) unchanged upon addition of methanolic 1 N NaOH or HCl.

Dibromodehydrodihydroquinamine (III)

A solution of alkaloid A in $CHCl_3$ was treated with a 1% Br_2 in $CHCl_3$ until the color of the solution remained yellowish. Removal of the solvent *in vacuo* left yellowish, thick crystals of the dibromo derivative, m.p. 202–205°; R_f, 0·40. The same compound was obtained by adding Br_2 in $CHCl_3$ to dehydrodihydroquinamine in $CHCl_3$.

Dihydroisoquinamine (VI)

A solution of 0·1 g of KOH in 2 ml of EtOH and 5 mg of alkaloid A was gently boiled for 1 hr. After cooling, the reaction mixture was chromatographed on a Merck silicic acid column and eluted with CH_2Cl_2–MeOH (9:1). After removal of the solvent *in vacuo*, the residue was recrystallized from EtOH to give yellow crystals (3 mg) of dihydroisoquinamine, m.p. 183–186°.

Dinitrophenylhydrazone of Dihydroisoquinamine

A solution of 1 mg of dihydroisoquinamine in 0·5 ml of EtOH and a few drops of a 5% ethanolic solution of 2,4-dinitrophenylhydrazine was warmed on a steam bath for 20 min and, on cooling, the dinitrophenylhydrazone crystallized as yellowish plates which were separated by filtration. The product melted at 243–245°.

Dihydrocinchonamine (IV)

A mixture of 5 mg of alkaloid A and 4 mg of $LiAlH_4$ in 1 ml of Et_2O was allowed to stand at room temperature for 3 hr. The excess reagent was destroyed (H_2O) and the mixture extracted with Et_2O. The ethereal solution was chromatographed on a Woelm neutral alumina column (grade I) and eluted with CH_2Cl_2–MeOH (9:1). Evaporation of the solvent *in vacuo* left colorless needles (2 mg) of dihydrocinchonamine melting at 123°; hR_f = 35.

Perbenzoic Acid Oxidation of Dihydrocinchonamine

About 50 mg of dihydrocinchonamine in EtOH solution was spotted on a silica gel GF254 plate. After evaporation of the solvent, one drop of a 5% solution of perbenzoic acid in EtOH was applied on the same spot and allowed to react for 1 hr. Perbenzoic acid, alkaloid A, and dihydrocinchonamine were spotted on the same plate to serve as references. After development of the chromatogram, it was dried, sprayed with modified Dragendorff's reagent and the R_f values recorded. The R_f values were: alkaloid A (0·20), dihydrocinchonamine (0·35), dihydrocinchonamine + perbenzoic acid (0·20), perbenzoic acid (tailing from 0–0·10).

Acetylation Experiments

(*a*) A mixture of 10 mg of alkaloid A and 1 ml of a solution of acetic anhydride–pyridine (1:2) was heated on a steam bath for 2 hr, allowed to cool, and evaporated to dryness under a jet of air. The residue was dissolved in EtOH, chromatographed on a silicic acid column and eluted with CH_2Cl_2. Removal of the solvent *in vacuo* left a dry residue which was taken in hot EtOH. On cooling, colorless long needles were obtained and separated by filtration (4 mg), m.p. > 300°. (*b*) A mixture of 5 mg of alkaloid A and 1 ml of acetic anhydride was refluxed for several minutes on a steam bath. The reaction mixture was examined by TLC and found to contain at least six compounds, one of them corresponding to dehydrodihydroquinamine (II).

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