The Isolation and Structural Elucidation of Voacristine Hydroxyindolenine

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Earlier reports have described the isolation from Ervatamia dichotoma (Roxb.) Blatter of coronaridine[4] and heyneanine.[5] We

(4) S. M. Kupchan, A. Bright, and E. Macko, J. Pharm. Sci., 52, 598 (1963).

(5) S. M. Kupchan, J. M. Cassady, and S. A. Telang, Tetrahedron Letters, 1251 (1966).

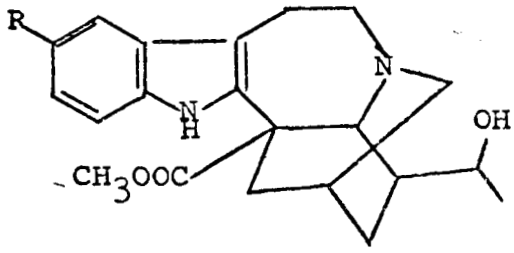
report herewith the isolation and characterization of voacristine hydroxyindolenine, and its synthesis from voacristine.

Partition of the ethanol extract of E. dichotoma root bark between ether and 4% hydrochloric acid gave a crude alkaloid fraction upon neutralization of the acid and extraction with ether. The ether extract was washed with 1% sodium hydroxide to remove the phenolic bases, and the non-phenolic bases were chromatographed on neutral alumina. Elution with benzene-chloroform (1:1) yielded a fraction which was further fractionated by partition chromatography.[6] Six purple bands were visible on

(6) K. S. Brown, Jr. and S. M. Kupchan, J. Chromatog., 9, 71 (1962).

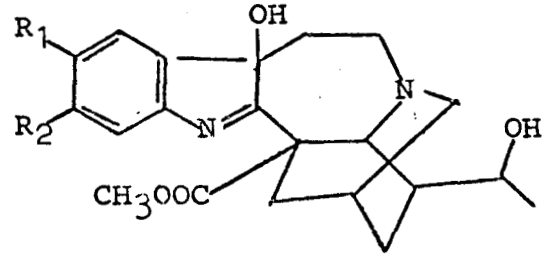
the partition column. After elution, the third band yielded heyneanine (I).[5] The fourth band was rechromatographed on a partition column, and yielded four bands. Treatment of the third

band with benzene-Skellysolve B gave a crystalline material. Recrystallization from the same solvent system yielded colorless crystals, $C_{22}H_{28}N_2O_5$ (by high resolution mass spectrometry), m.p. 176-179° dec., $[\alpha]_D^{26}$ - 22° (c 0.51, chf.). The ultraviolet spectrum (λ_{max}^{EtOH} 229.5, 268, 291, 300 (sh), 314; 12,380, 4,400, 4,780, 4,410, 3,810) and infrared spectrum suggested an indole-type alkaloid bearing substituents in the aromatic ring. The infrared spectrum showed broad bands at 2.80 μ (w) and 3.10 μ (m), indicative of the presence of hydroxyl groups, and a band at 5.76 μ (s), indicative of the presence of a carbomethoxy group. The nmr spectrum contained a doublet (1H) centered at τ 2.66 (J_{Ortho} = 8 cps), a doublet of doublets (1H) centered at τ 3.24 (J_{Ortho} = 8 cps, J_{Meta} = 2.5 cps) and a doublet (1H) centered at τ 3.12 (J_{Meta} = 2.5 cps). This pattern is consistent with a 1,2,4-trisubstituted benzene ring. In addition, the nmr spectrum showed sharp singlets at τ 6.18 (3H) and τ 6.30 (3H), confirming the presence of a methoxyl and carbomethoxy group, respectively, and a doublet (3H) centered at τ 8.93 (J = 6.5 cps) and a broad multiplet (1H) centered at τ 5.97, indicative of the presence of the $-CHOHCH_3$ group.



I, R = H

III, R = OCH_3



IIa, R_1 = OCH_3, R_2 = H

b, R_1 = H, R_2 = OCH_3

The conventional and high resolution mass spectral data (Figure 1) strongly supported the assignment of a hydroxyindolenine structure of the iboga-type to the alkaloid: Peaks at m/e 383 (M-OH), 365 (M-OH-H_2O), 355 (M-C_2H_5O) and 341 (M-$COOCH_3$) confirmed the presence of two hydroxy functions, a hydroxy-substituted C_2-side chain and the carbomethoxy grouping. This pattern, together with the peaks at m/e 260, 218, 190, 176 and 162 (Figure 1), is entirely analogous to that of other hydroxyindolenines[7] of the iboga-series. Since the position

(7) a) D. W. Thomas, Ph.D. Thesis, MIT, 1966.

b) D. W. Thomas and K. Biemann, manuscript in preparation. The interesting mass spectral fragmentation of these hydroxyindolenines is discussed in detail in these reports.

of the methoxy grouping was limited to C-12 or C-13 by the characteristic coupling constants observed in the nmr spectrum (see above), the mass spectral pattern established that the alkaloid should be represented by structures IIa or IIb. Structure IIa for the alkaloid was proven unambiguously by its synthesis[8] from voacristine (III): Passage of oxygen through

(8) See reference 7a for the preparation of other hydroxyindolenines in this series.

an irradiated solution of III in benzene, followed by chromatography on alumina, yielded an amorphous product with spectral properties essentially identical to those of the natural product. Further purification by partition chromatography furnished a product which, upon crystallization from benzene-Skellysolve B, proved identical to the hydroxy-indolenine isolated from the alkaloid mixture.[9]

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(9) In view of the relatively facile autoxidation of indole alkaloids of the iboga series, the isolation of these products from the plant does not necessarily prove their natural occurrence.

Experimental

Melting points were determined with a Thomas-Hoover Unimelt apparatus, and are corrected. IR spectra were measured in $CHCl_3$ solution (10%) on a Beckman double beam recording spectrophotometer, model IR-5A. Nmr spectra were determined in $CDCl_3$ at 60 Mc on a Varian Associates A-60 recording spectrophotometer using TMS as the internal standard, and were electronically integrated. The UV spectrum was determined in 95% EtOH on a Beckman recording spectrophotometer model DK2A. The optical rotation was determined in $CHCl_3$ solution with a Zeiss-Winkel polarimeter and is approximated to the nearest degree. High resolution mass spectra were

obtained on a C.E.C. 21-110 instrument, with photoplate recording. Elemental compositions for all ions were determined; low resolution mass spectra were obtained both on a C.E.C. 21-103 and a C.E.C. 21-110 instrument. Skellysolve B refers to petroleum ether distilling at 60-68°. The solvent system used for partition chromatography consisted of ethylene chloride-Skellysolve B-methanol-water (2.5:15:2:0.3), using Bromcresol Purple as the indicator in the stationary (lower) phase. We are grateful to Smith Kline and French Laboratories for the alcohol extract of E. dichotoma.

Extraction and Preliminary Fractionation.—The dried ground root bark (4.6 kg) was extracted with ethanol after preliminary extraction with petroleum ether. The ethanol extract was concentrated and the residue was taken up in dilute HCl. The insoluble material was removed and the filtrate brought to pH 7. The solid that separated was collected and dried (crude alkaloid fraction; 50 g). A portion (18.2 g) of the crude alkaloid fraction was partitioned between ether (125 ml) and four 80 ml portions of 4% HCl. The combined acid solutions were treated with $(NH_4)_2CO_3$ until alkaline and thoroughly extracted with ether (4 x 200 ml). The combined ether solutions were extracted with 4% HCl (4 x 100 ml). The combined acid solutions were again treated with $(NH_4)_2CO_3$ until alkaline and extracted with ether (5 x 100 ml). The combined ether solutions were washed with 1% NaOH (5 x 20 ml)

to remove the phenolic bases. The ether solution was washed with water, dried over fused K_2CO_3 and concentrated to yield the nonphenolic bases (8.8 g).

Isolation of Alkaloids.—The nonphenolic bases (8.8 g) were treated with benzene-Skellysolve B (4:1) and filtered to remove insoluble material (0.1 g). The filtrate was added to a column of neutral Woelm grade I alumina (210 g). Elution with benzene-Skellysolve B (1:1; 3000 ml) followed by benzene-Skellysolve B (3:1; 3000 ml) yielded a clear syrup (fraction 1; 1.51 g). Elution with benzene (3000 ml) yielded fraction 2 (0.51 g). Elution with benzene-chloroform (1:1; 3000 ml) yielded a solid yellow foam (fraction 3; 5.42 g). Partition chromatography of the third fraction (5.42 g) on a Celite 545 column (495 g) yielded six bands. Treatment of band 3 (1.33 g) with Skellysolve B yielded heyneanine.[5] Rechromatography of band 4 (460 mg) on a Celite 545 partition column (495 g) yielded four bands. Treatment of band 3 (145 mg) with benzene-Skellysolve B yielded a colorless crystalline material. Recrystallization from the same solvent system yielded voacristine hydroxyindolenine (IIa; 53 mg), $C_{22}H_{28}N_2O_5$, mp 176-179° dec.; $[\alpha]_D^{26}$ - 22° (c 0.51, chf.); $\lambda_{max}^{CHCl_3}$ 2.80 (w), 3.10 (m), 5.76 (s), 5.90 (m), 6.25 (m), 6.45 (w), 6.78 (s), 6.98 μ(s); λ_{max}^{EtOH} 229.5, 268, 291, 300 (sh), 314 mμ (ε 12,380, 4,400, 4,780, 3,810, 4,410); nmr τ 2.66 (one proton, doublet, J_{Ortho} = 8 cps), 3.12 (one proton, doublet, J_{meta} = 2.5 cps),

(one proton, doublet of doublet, J_{Ortho} = 8 cps, J_{Meta} = 2.5 cps), 5.97 (one proton, broad multiplet), 6.18 (three protons, $-OCH_3$), 6.30 (three protons, $-CO_2CH_3$), and 8.93 (three protons, doublet, J = 6.5 cps); mass spectrum Figure 1.

Synthesis of Voacristine Hydroxyindolenine from Voacristine.—Voacristine (III, 500 mg) in 5 ml of benzene was illuminated by an ultraviolet lamp ("Blak-Ray", UVL-22, U.V. Products, Inc., San Gabriel, Calif.) while oxygen was slowly bubbled through the solution. Benzene was periodically added to maintain the volume. After 8 hr the product was chromatographed on 50 g of Woelm alumina III. Benzene/chloroform eluted unchanged voacristine, and chloroform eluted a 150 mg fraction containing the hydroxyindolenine together with a small amount of voacristine. Purification by tlc on silica gel H provided 116 mg of amorphous material which gave infrared, mass, and nmr spectra identical with data from the isolated product IIa. A portion of this material (16.4 mg) was subjected to partition chromatography on a Celite 545 column (5 g) which yielded a colorless solid (11.4 mg). Crystallization from benzene-Skellysolve B yielded colorless crystals (6.7 mg), mp 179-179° dec. This material was shown to be identical to the isolated material (mixture tlc, mixture mp).

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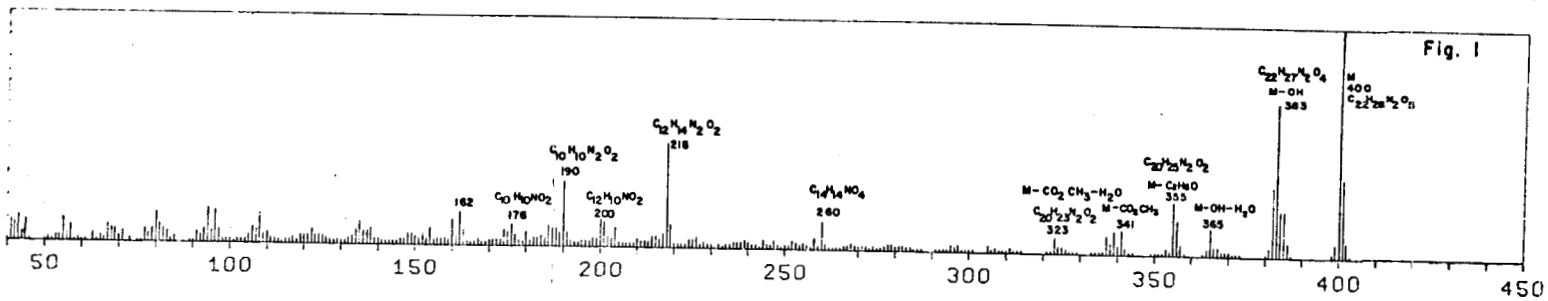
Fig. 1
$C_{22}H_{27}N_2O_4$
M-OH
383
M
400
$C_{22}H_{28}N_2O_5$
$C_{12}H_{14}N_2O_2$
218
$C_{10}H_{10}N_2O_2$
190
$C_{10}H_{10}NO_2$
176
$C_{12}H_{10}NO_2$
200
162
$C_{14}H_{14}NO_4$
260
$C_{20}H_{23}N_2O_2$
323
M-CO_2CH_3-H_2O
M-CO_2CH_3
341
$C_{21}H_{23}N_2O_2$
M-C_2H_3O
355
M-OH-H_2O
365
50
100
150
200
250
300
350
400
450

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