chloroform–methanol, 99:1). An analytical sample of **6a** melted at 171–173°; uv, λ_{max}^{EtOH} 227 mμ (ε 45,100), 279 (10,400). *Anal.* Calcd for $C_{29}H_{37}N_3O_3$: C, 73.26; H, 7.84; N, 8.84. Found: C, 72.98; H, 8.08; N, 8.66.

3-Ethyl-1,2,3,4,6,7,12,12b-octahydro-2-(3′,4′-dihydro-6′,7′-dimethoxyl-1-isoquinolinyl)methylindolo[2,3-*a*]quinolizine Dihydrochloride (7a).—The amide **6a** (5 g, 0.001 mol) in 100 ml of 1,2-dichloroethane was refluxed for 3.5 hr with 10 ml of phosphorus oxychloride, then cooled to 4°. The excess phosphorus oxychloride was decomposed with water, and 10% dilute ammonium hydroxide solution was added until the solution was basic. The organic layer was washed with saturated sodium chloride solution, dried over sodium sulfate, and concentrated. The residue in chloroform was chromatographed on Florisil. Fractions eluted with chloroform–methanol (99:1) crystallized from methylene chloride–ether. The product **7a** (3.1 g, 64% yield) melted at 180–183°. A perfect analysis for the base could not be obtained. It was converted into the hydrochloride on treatment with methanolic HCl and the product was recrystallized from methanol–ethyl acetate. The analytical sample melted at 260–262°; uv, λ_{max}^{EtOH} 220 mμ (ε 44,600), 248 (15,200), 273 (9600), 282 (10,300), 290 (10,200), 306 (8500), 360 (7400). *Anal.* Calcd for $C_{29}H_{35}N_3O_2 \cdot 2HCl \cdot 2H_2O$: C, 61.47; H, 7.29; N, 7.42; mol wt (free base), 457.6. Found: C, 61.55; H, 7.14; N, 7.56; mol wt (mass spectrum), 457.

3-Ethyl-1,2,3,4,6,7,12,12b-octahydro-2-(1′,2′,3′,4′-tetrahydro-6′,7′-dimethoxy-1-isoquinolinyl)methylindolo[2,3-*a*]quinolizine Dihydrochloride (8a).—The base **7a** (3 g, 0.0066 mol) in 120 ml of methanol and 5.0 ml of water was reduced with 0.6 g of sodium borohydride for 0.5 hr at reflux and 1 hr at 25°. Excess hydride was destroyed with acetic acid, the methanol was evaporated, and the residue was made basic with 10% ammonium hydroxide solution. The chloroform extracts were washed with saturated sodium chloride solution, dried over sodium sulfate, and concentrated. The amorphous residue was converted into the hydrochloride by treatment with methanolic HCl, and recrystallized from methanol to yield 0.25 g of one isomer of **8a** (7%). Further recrystallization from methanol–ethyl acetate gave an analytical sample with mp 305–307°. *Anal.* Calcd for $C_{29}H_{37}N_3O_2 \cdot 2HCl \cdot 0.5H_2O$: C, 64.32; H, 7.44; N, 7.75; mol wt (free base), 459.6. Found: C, 64.54; H, 7.59; N, 7.80; mol wt (mass spectrum), 459.

Another isomer (2.35 g, 62.5% yield) was obtained when the mother liquors were crystallized from methanol–ethyl acetate. The analytical sample melted at 266–268°; uv, λ_{max}^{EtOH} 223 mμ (ε 44,600), 281 (11,400), 288 (sh) (9300). *Anal.* Calcd for $C_{29}H_{37}N_3O_2 \cdot 2HCl \cdot 2H_2O$: C, 61.26; H, 7.61; N, 7.39. Found: C, 61.56; H, 7.61; N, 7.26.

3-Ethyl-1,2,3,4,6,7,12,12b-octahydro-2-(1′,2′,3′,4′-tetrahydro 6′,7′-dimethoxy-2′-methyl-1-isoquinolinyl)methylindolo[2,3-*a*]-quinolizine Dihydrochloride (9a).—The major isomer **8a** (1.97 g) was liberated from its hydrochloride with dilute ammonium hydroxide and extracted into chloroform. The solution was washed with saturated sodium chloride, dried over sodium sulfate, and concentrated. The residue in 30 ml of ethyl formate was heated for 16 hr on a steam bath under 40 psi pressure. The mixture was cooled, the solution was evaporated, and the residue was partitioned between chloroform and 10% ammonium hydroxide solution. The chloroform layer was washed with saturated sodium chloride solution and dried over sodium sulfate. On concentration to dryness, 1.86 g of amorphous formyl compound was obtained.

A solution of the formyl compound (1.7 g) in 30 ml of tetrahydrofuran was added to a solution of 1 g (0.03 mol) of lithium aluminum hydride in 100 ml of tetrahydrofuran, and heated at reflux for 6 hr. The mixture was cooled, excess hydride was destroyed with water, and the mixture was filtered. The filtrate was acidified with 10% sulfuric acid, washed with ether, made basic with 10% ammonium hydroxide solution, and extracted with chloroform. The extract was washed with saturated sodium chloride solution, dried over sodium sulfate, and concentrated. The residue was converted into the dihydrochloride of **9a**, which was crystallized from methanol–ethyl acetate. It melted above 295°; uv, λ_{max}^{EtOH} 220 mμ (ε 54,200), 273 (sh) (12,600), 279 (12,900), 288 (11,900). *Anal.* Calcd for $C_{30}H_{39}N_3O_2 \cdot 2HCl \cdot 0.5H_2O$: C, 64.85; H, 7.62; N, 7.56; mol wt (free base), 473.6. Found: C, 65.01; H, 7.82; N, 7.47; mol wt (mass spectrum), 473.

Registry No.—**6a**, 19202-96-1; **6b**, 19203-01-1; **7a**, 19202-97-2; **7b**, 19203-02-2; **8a** (α), 19202-98-3; **8a** (β), 19203-03-3; **8b**, 19203-04-4; **9a**, 19202-99-4; **9b**, 19233-86-4; **10a**, 19203-05-5; **10b**, 19203-06-6; **11a**, 19203-07-7; **11b**, 19203-08-8.

Structure Elucidation and Chemistry of *Catharanthus* Alkaloids. IV.[1,2] Structures of Horhammericine and Horhammerinine

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Several α-methyleneindoline bases have been reported isolated and characterized from *Catharanthus*, as well as from other species of plants. None of the monomeric α-methyleneindoline alkaloids from *Catharanthus* species have been shown to elicit antitumor activity; however, we have shown that lochnerinine exhibits significant cytotoxicity in cell culture against Eagle's 9 KB carcinoma of the nasopharynx.[3]

The purpose of this report is to present our evidence for the structures of two new α-methyleneindoline bases which we have recently isolated and characterized from the apocynaceous plant *Catharanthus lanceus*, namely, horhammericine (**1**) and horhammerinine (**2**).[4,5]

Both **1** and **2** could be analyzed in comparison with three other alkaloids of known structure, *i.e.*, lochnericine (**3**),[6–9] lochnerinine (**4**),[6–9] and minovincinine (**5**).[10,11]

The mass spectral fragmentations of **3** and **4** are presented in Scheme I.[6,9] It can readily be seen that

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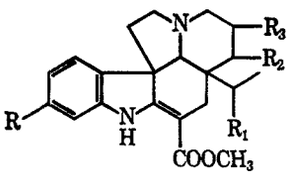
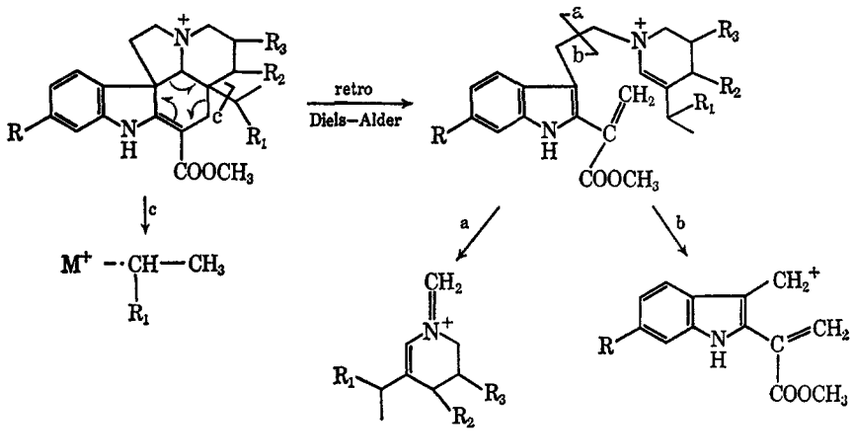


Alkaloid	R	R_1	R_2		R_3
Horhammericine (1)	H	OH		–O–	
Horhammerinine (2)	CH_3O	OH		–O–	
Lochnericine (3)	H	H		–O–	
Lochnerinine (4)	CH_3O	H		–O–	
Minovincinine (5)	H	OH	H		H

gave a peak at *m/e* 336 ($M^+ - 18$). The other analytical techniques such as nmr and ir also verified the presented structures of **3**, **4**, and **5**.[6-11]

The ultraviolet spectra of **1**[5] and **2**,[4] as well as **3**,[6-9] are compared in Table I. Lochnericine (**3**) and horhammericine (**1**) are comparable, as are lochnerinine (**4**) and horhammerinine (**2**), all being of the α-methyleneindoline type. The main mass spectral fragmentation peaks of horhammericine and horhammerinine are also summarized in Table I. It can readily be seen from Scheme I that the combination of a hydroxy side chain

SCHEME I



the typical primary fragmentation of the α-methyleneindolines is a retro-Diels–Alder reaction giving fragments according to pathway a or b. Fragmentation a shows the presence of the epoxy moiety in both **3** and **4** at *m/e* 138, whereas fragmentation b indicates that **4** has a methoxyl group on the aromatic nucleus, which raises the *m/e* value by 30 (*m/e* 244) over that obtained in **3** at *m/e* 214.

Loss of the ethyl side chain is indicated (pathway c) by the $M^+ - 29$ peak at *m/e* 323 in compound **3**, and at *m/e* 353 in compound **4**.

A look at the mass spectral fragmentation pattern of **5** (Scheme I) shows the same type of degradation as seen in **3** and **4**.[10] Fragmentation pathway a gives *m/e* 140, consistent with the structure given, and pathway b shows the ion *m/e* 214 as observed in **3**. Loss of the hydroxy ethyl side chain gave a peak at *m/e* 309 ($M^+ - 45$), and loss of the hydroxy group as water

TABLE I

UV AND MASS SPECTRAL COMPARISONS

R	Alkaloid	Uv
H	Lochnericine	226, 297, 327
OCH_3	Lochnerinine	247, 326
H	Horhammericine	228, 299, 327
OCH_3	Horhammerinine	245, 325

Horhammericine ($C_{21}H_{24}N_2O_4$) M^+	Horhammerinine ($C_{22}H_{26}N_2O_5$) M^+
368	398
350 ($-H_2O$)	380 ($-H_2O$)
323 ($M^+ - 45$)	353 ($M^+ - 45$)
214	244
154	154

of **5** with the presence of the epoxide group of **3** and **4** would lead to the proposed structures **1** and **2**. Both **1** and **2** lose water ($M^+ - 18$) and the hydroxy side chain ($M^+ - 45$) by pathway c. The basic aromatic fragments at *m/e* 214 and 244, pathway b, are the same as those exhibited in the spectra for **3**[6,9] and **4**.[6,9] The ion at *m/e* 154 can then be accounted for as shown by pathway a, both containing the epoxide and hydroxy side chain.

The nmr spectra of the aromatic regions of **1** and **3** were comparable, as were the aromatic regions of **2** and **4**. The methyl signal of the hydroxy side chain in both **1** and **2** was split into a doublet at σ 1.0, as would be expected. The ir spectra of **1** and **2** contained the hydroxyl absorption at *ca.* 3.0 μ.

Deuterium oxide was injected into the mass spectrometer with compound **2**, which resulted in a shift of the molecular ion peak to *m/e* 399. The deuterated analog showed a large $M^+ - 19$ peak which indicated that the hydroxyl group of the side chain did indeed exchange deuterium for hydrogen as expected. A comparison of the peak at *m/e* 155 in both charts also shows that the deuterium-enriched sample exchanged deuterium for hydrogen of the hydroxyl group. The fact that there was an increase in intensity of the *m/e* 245 and 259 peaks in the deuterated sample also indicate some NH to ND exchange. The rest of the deuterated spectrum compared very closely with that obtained prior to deuterium introduction.

Registry No.—Horhammericine, 19459-04-2; horhammerinine, 19459-05-3; lochnericine, 2447-58-7; lochnerinine, 2579-65-9.