−11° (c 0.227, $CHCl_3$); UV (MeOH) 220, 263 nm (ε 15 900, 6600); IR (KBr) 3530, 1795, 1750, 1720, 1640 cm^{-1}.

Anal. Calcd for $C_{30}H_{42}O_7$: C, 70.01; H, 8.23. Found: C, 69.71; H, 8.25.

The acetate **4b** could, however, be obtained as the major component of a mixture in the following manner.

A solution of chromium trioxide (0.260 g) in acetic acid (20 mL) was added slowly (144 h) to a solution of neriifolin 4′-acetate (**2c**, 0.500 g). The reaction mixture was worked up in the manner described above for neriifolin to give a crude product (0.380 g), which was mainly the acetate **4b** (see Table I for NMR spectrum) contaminated with a small amount of digitoxigenin formate **2g**. The mixture was subjected to column chromatography on neutral alumina (80 g, Fluka, activity I). The enone **6** (0.350 g, 78%) was eluted with hexane–ethyl acetate (9:1 and 17:3).

Digitoxigenin (2e). (A) Hydrolysis of Digitoxigenin Formate (2g). A solution of the formate (2.28 g, 0.00564 mol) in methanol (250 mL) and 0.1 N sulfuric acid (125 mL) was boiled under reflux for 1 h. The solution was neutralized by the addition of dilute sodium bicarbonate solution, the solvent was then removed in vacuo, and the residue was extracted with ethyl acetate. The extract was washed with water, dried over sodium sulfate, and evaporated in vacuo, giving a residue which after crystallization from aqueous methanol and then ethyl acetate–ether gave digitoxigenin (1.56 g): mp 237–238 °C; $[\alpha]_D$ +18° (c 0.348, $CHCl_3$); UV (MeOH) 218 nm (ε 14 500) [lit.[25] mp 249–255 °C; $[\alpha]_D$ +14.6 ± 2° (MeOH)]. Chromatography of the mother liquor on silica gel (50 g) gave digitoxigenin formate [0.326 g, 14% recovery, eluted with hexane–ethyl acetate (2:1)] and a small amount (0.194 g) of digitoxigenin (total yield 1.75 g, 83%).

(B) Hydrolysis of the Enone 5. A solution of the enone (1.2 g, 0.00233 mol) in methanol (60 mL) and 0.1 N sulfuric acid (30 mL) was left at room temperature for 18 h. The reaction was worked up as described above to provide a solid, which was crystallized from ethyl acetate–ether to give digitoxigenin (0.61 g), mp 242–245 °C. The residue obtained on evaporation of the mother liquor was separated by TLC on silica gel using hexane–ethyl acetate as the developing solvent. In this way a further quantity (0.17 g, total yield 89%) of digitoxigenin was isolated as well as the nonpolar methyl glycoside **7** and the γ-pyrone **8**. After crystallization from dichloromethane–ether compound **7**, obtained in 8% yield, had: mp 97–98 °C; $[\alpha]_D$ −64° (c 0.45, $CHCl_3$); UV (MeOH) 255.5 nm (ε 5410); IR (KBr) 1710, 1645 cm^{-1}; NMR ($CDCl_3$) δ 1.39 (d, 3 H, J = 6.8 Hz), 3.48 (s, 3 H), 3.62 (s, 3 H), 4.61 (q, 1 H, J = 6.8 Hz), 5.24 (d, 1 H, J = 4.2 Hz), 5.74 (d, 1 H, J = 4.2 Hz).

Anal. Calcd for $C_8H_{12}O_4$: C, 55.80; H, 7.03. Found: C, 56.06; H, 7.06.

The γ-pyrone **8**, obtained in 23% yield, had mp 155–157 °C [lit.[26] mp 160–162 °C] after crystallization from dichloromethane–ether: $[\alpha]_D$ 0° (c 0.252, $CHCl_3$); UV (MeOH) 277 nm (ε 11 000); IR (KBr) 3265, 1655, 1620, 1563 cm^{-1}.

Anal. Calcd for $C_6H_6O_3$: C, 57.14; H, 4.80. Found: C, 57.16; H, 4.69.

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Registry No.—**7**, 63493-69-6; **8**, 118-71-8.

References and Notes

(1) Contribution No. 481 from the Syntex Institute of Organic Chemistry.
(2) Presented in part at the First Chemical Congress of the North American Continent, Mexico City, Mexico, Nov 30–Dec 5, 1975.
(3) M. Martinez, "Las Plantas Medicinales de Mexico", Quinta Edición, La Impresora Azteca, Mexico, D.F., 1969, pp 73–76.
(4) K. K. Chen and A. L. Chen, *J. Biol. Chem.*, **105**, 231 (1934).
(5) M. Frerejacque, *C. R. Acad. Sci.*, **242**, 2395 (1956); **246**, 459 (1958).
(6) R. Bloch, S. Rangaswami, and O. Schindler, *Helv. Chim. Acta*, **43**, 652 (1960).
(7) (a) H. Helfenberger and T. Reichstein, *Helv. Chim. Acta*, **31**, 1470 (1948); (b) *ibid.*, **31**, 2097 (1948).
(8) Fieser and Fieser[9] have erroneously depicted neriifolin with the equatorial (β) configuration at the anomeric carbon. That the aglycone portion of neriifolin is in the axial (α) configuration is obvious from the magnitude[10] of the coupling constant of the anomeric proton with the 2′-hydrogen ($J_{1',2'}$ = 3.3 Hz, see Table I).
(9) L. F. Fieser and M. Fieser, "Steroids", Reinhold, New York, N.Y., 1959, pp 731–732.
(10) G. Kotowycz and R. U. Lemieux, *Chem. Rev.*, **73**, 669 (1973); see especially p 676.
(11) F. Lauterbach, K. Repke, and D. Nitz, *Naunyn–Schmiedebergs Arch. Exp. Pathol. Pharmakol.*, **239**, 196 (1960).
(12) J. M. Ferland, *Can. J. Chem.*, **52**, 1652 (1974).
(13) Attempts to utilize digitoxigenone as a source of digitoxigenin were abandoned when it was discovered that reduction of this ketone by the method of Henbest[14] gave 14-dehydrodigitoxigenin as the principal product.
(14) Y. M. Y. Haddad, H. B. Henbest, J. Husbands, and T. R. B. Mitchell, *Proc. Chem. Soc.*, 361 (1964); H. B. Henbest and T. R. B. Mitchell, *J. Chem. Soc. C*, 785 (1970); P. A. Browne and D. N. Kirk, *ibid.*, 1653 (1969); E. L. Eliel, T. W. Doyle, R. O. Hutchins, and E. C. Gilbert, *Org. Synth.*, **50**, 13 (1970).
(15) J. C. Collins, W. W. Hess, and F. J. Frank, *Tetrahedron Lett.*, 3363 (1968).
(16) The oxidative processes studied and the **3a/4a** ratios (in parentheses) observed were: chromium trioxide–acetic acid (2.6), chromium trioxide–DMF[17] (0.8), Brown[18] oxidation using dichloromethane instead of ether (3.6), pyridinium chlorochromate[19] (2.0), and Moffatt[20] oxidation (0.9). Two other oxidative processes, e.g., ceric ammonium nitrate [P. Soucy, T-L. Ho, and P. Deslongchamps, *Can. J. Chem.*, **50**, 2047 (1972)] or the thioanisole–chlorine complex [E. J. Corey and C. U. Kim, *J. Org. Chem.*, **38**, 1233 (1973)], left the starting material unaltered.
(17) G. Snatzke, *Chem. Ber.*, **94**, 729 (1961).
(18) H. C. Brown and C. P. Carg, *J. Am. Chem. Soc.*, **83**, 2952 (1961).
(19) E. J. Corey and J. W. Suggs, *Tetrahedron Lett.*, 2647 (1975).
(20) K. E. Pfitzner and J. G. Moffatt, *J. Am. Chem. Soc.*, **87**, 5670 (1965).
(21) See footnote 16 in ref 6.
(22) The structures of these substances are under investigation and a report thereon will be made in due course.
(23) H. P. Sigg, C. Tamm, and T. Reichstein, *Helv. Chim. Acta*, **36**, 985 (1953).
(24) W. Fritsch, W. Haede, K. Radscheit, U. Stache, and H. Ruschig, *Justus Liebigs Ann. Chem.*, 621 (1974).
(25) E. Wyss, H. Jäger, and O. Schindler, *Helv. Chim. Acta*, **43**, 664 (1960).
(26) M. A. Spielman and M. Freifelder, *J. Am. Chem. Soc.*, **69**, 2908 (1947).

Studies Directed toward Synthesis of Quassinoids. 5.[1] Conversion of D-Ring Seco Derivatives of Cholic Acid to δ-Lactones

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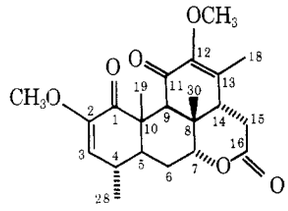
Various δ-lactones, 5,14-*epi*-28,30-dinorquassinoids, were synthesized from D-ring seco derivatives of cholic acid. Chemical and spectral evidence suggests that the δ-lactone ring in these compounds exists in a strained boat conformation.

In pursuit of our goal to convert cholic acid into analogues of quassin (**1**), we had the opportunity to synthesize a number of unique δ-lactones that may be regarded as 5,14-*epi*-28,30-dinorquassinoids. Herein, we describe our results in the lactonization of D-ring seco derivatives of cholic acid.[3]

Results

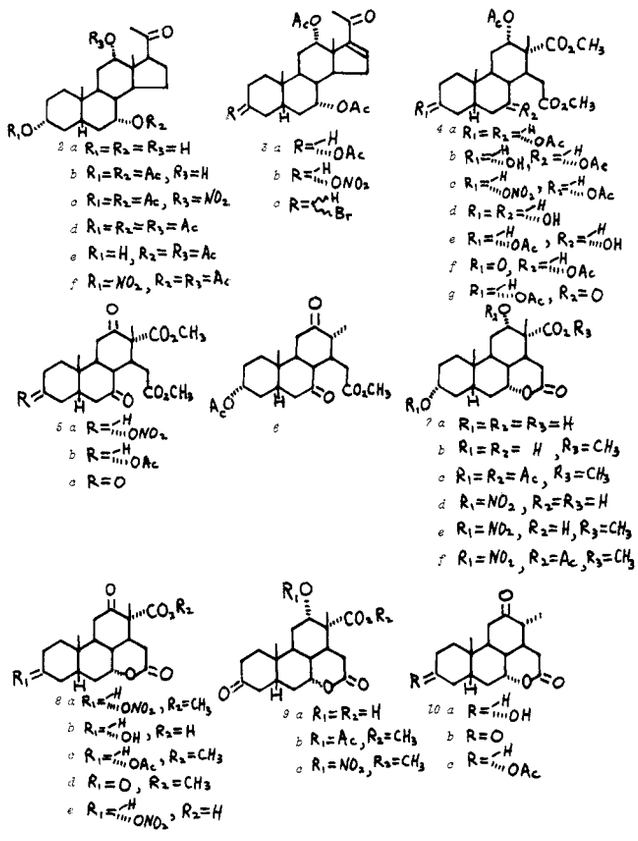
Conversion of the various ketones **2a** to **2f** to 16-en-20-ones for subsequent ozonolysis to give D-ring seco derivatives was explored. The ester ketone **2d** was converted to **2a** by sapon-



1, Quassin

ification in 5% methanolic KOH, and selective acetylation[4] gave the diacetate **2b** which was transformed to **2c** with fuming nitric acid in Ac_2O. Selective hydrolysis of **2d** with methanolic HCl[1] gave diacetate **2e** which was also nitrated with fuming HNO_3 to give **2f**. Nitrate **2f** was treated with Br_2/HOAc and the isolated bromide was heated in HMPT, but did not yield the anticipated **3b**; the product of this conversion turned out to be a mixture of bromides **3c**. A similar bromination and HMPT reaction sequence for **2c** gave a complex mixture that we presume to be C-ring bromide products and was not further investigated.

The lactones were made from the various seco esters by a reaction sequence starting with saponification and subsequent lactone closure with acid treatment. Diester **4a**, formed from enone **3a** by ozonolysis and subsequent esterification, was saponified with methanolic KOH and treated with acid to yield lactone **7a** which yielded lactone **7b** upon esterification with diazomethane and lactone **7c** upon acetylation with acetic anhydride and pyridine; some recovered diester **4a** was also obtained from this sequence of reactions. Jones oxidation



of lactone **7b** gave lactone **8d** and a trace of **5c**. Alternatively, diester **4a** was selectively deacetylated with methanolic HCl to give hydroxy diester **4b** which was nitrated to yield nitroxy diester **4c** or oxidized to yield keto diester **4f**. Controlled saponification of **4c** followed by acid treatment (**7d**) and esterification with diazomethane afforded lactone **7e**. Acetylation or Jones oxidation of **7e** gave either **7f** or nitroxy δ-lactone **8a**, respectively; variable amounts of **5a** and **5c** were coproducts with the latter. Reduction of **8a** with Zn dust in HOAc (**8b**) followed by acetylation provided acetoxy δ-lactone **8c**. Attempts to selectively remove the 3α-acetate group in lactone **7c** with methanolic HCl resulted in concurrent lactone ring opening to yield dihydroxy diester **4d** which could be selectively acetylated with Ac_2O and pyridine to give hydroxy diester **4e**; oxidation of **4e** gave keto diester **4g**. Saponification of **4f** followed by acid treatment gave **9a**. Lactone **9a** was esterified with diazomethane and appropriately transformed to either **9b** or **9c**.

Introduction of the 12-oxo group led to decarboxylation at position 13 under acidic conditions. Diketo diester **5b** and diketo lactone **8d** were decarboxylated in refluxing glacial HOAc containing concentrated HCl to afford diketo ester **6** and δ-lactone **10b**, respectively. Jones oxidation of **7d** followed by Zn dust reduction of the nitroxy group in glacial HOAc gave a product (**8b**) that underwent decarboxylation upon removal of the acetic acid solvent to afford **10a**; acetylation of **10a** gave **10c** containing some precursor acid to ester **6**. Similar decarboxylation of **8a** in glacial HOAc containing concentrated HCl was attended by decomposition of the nitroxy group giving a complex mixture presumed to contain A-ring elimination and chloride products.

Discussion

Since nitrate esters are more resistant to hydrolysis than acetate esters under both acidic and basic conditions but are easily removed through reduction with Zn dust and glacial HOAc, we sought to selectively introduce the nitroxy group at positions 3 or 12 either before or after D-ring cleavage of 16-en-20-one cholic acid derivatives. Introduction before provided nitrate **2f** and **2c** which were subjected to bromination with Br_2/HOAc, but treatment of the corresponding 17α-bromo derivatives with hot HMPT led to **3c**.[5] Selective introduction of the nitroxy group at positions 3 or 12 after D-ring cleavage was easily accomplished on the seco esters **4**.

Conversion of the seco esters **4** to δ-lactones **7** was achieved by saponification of the esters and acid treatment to close the lactone ring. This conversion was never totally complete, for, invariably, starting material or products thereof were also recovered. Treatment of δ-lactone **7c** with methanolic HCl opened the lactone ring faster than hydrolysis of the 3α-acetoxy group, since a brief reaction period (0.5 h) gave both **4d** and **4e** and pure **4d** only after a longer reaction period (>1 h). A distinctive feature in the NMR spectra of the δ-lactones **7** is the wide separation of the angular methyl resonance signals (~18 Hz) as compared with the corresponding diesters (~10 Hz); a major contribution for this wider separation has come from increased shielding of the C-19 methyl group in the δ-lactone. Additionally, the 7β-H NMR signal appears as a hump (δ ~4.6) in the spectra of the δ-lactones but is a downfield peak (δ ~4.9) in the spectra of the precursor diesters, indicating that this proton is in an axial-like orientation in the δ-lactones. These facts may be explained by assuming chair conformations for rings A and C and strained boat conformations for ring B and the lactone ring. The mass spectral loss of ketene from the molecular ion of the 12α-acetoxy- and, to a lesser extent, 12α-nitroxy δ-lactones (**7c, 7f, 9b,** and **9c**) is characteristic of these lactones.

Although it is possible to selectively acetylate the 3α- and 7α-hydroxy groups in **2a**, a similar attempt to selectively acetylate only the 3α-hydroxy group in δ-lactone **7b** was without success, as only **7c** was obtained. However, it was possible to selectively deacetylate the 3α-acetate group in diester **4a** to give **4b**, which was transformed to an intermediate having the 12-oxo group (acid **8b**) permitting easy decarboxylation (to **10a**). Unlike methyl esters **8d** and **5b** which underwent transesterification followed by decarboxylation

in refluxing glacial HOAc containing concentrated HCl without difficulty, methyl esters **8a** and **8c** under these conditions led to product mixtures consisting, presumably, of elimination and chloride-substituted products which was avoided by decarboxylating acid **8b** requiring no prior transesterification and therefore a shorter reaction period. As expected, the NMR spectra of **10b** and **10c** exhibited a doublet (J = 6 Hz) for the C-18 methyl group which was assigned the more stable α orientation.

Experimental Section

General. All melting points were determined with a Fisher-Johns apparatus and are corrected. Infrared data ($\bar{\nu}_{max}$) were obtained in $CHCl_3$ solution against a blank; ^1H NMR data, reported in ppm (δ) form Me_4Si, were determined in $CDCl_3$ with a Varian A-60 or T-60 NMR; mass spectra were obtained at an ionization voltage of 70 eV with a Nuclide 12-90-G single-focusing instrument having a resolution capability of 10 000. C, H, N microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn.

Column chromatography was performed using silica gel (MCB Grade 62), and TLC was performed on silica gel HF_{254} (E. Merck) using hexane–EtOAc as the mobile phase. Visualization of the TLC was effected by spraying with 2% ceric sulfate in 2 N H_2SO_4 followed by brief heating.

3α,7α-Diacetoxy-12α-hydroxy-5β-pregnan-20-one (2b). A solution of triol **2a** (1.0 g) in benzene (50 mL) was reacted with Ac_2O (2 mL) and pyridine (2 mL) at 20 °C for 24 h.[6] Diacetate **2b** (0.8 g) was obtained after workup and crystallization from hexane–acetone: mp 217–219 °C; $\bar{\nu}_{max}$ 3500 (OH), 1740, and 1715 cm^{-1}; ^1H NMR δ 4.90 (peak, 1 H, 7β-H), 4.6 (hump, 1 H, 3β-H), 4.02 (peak, 1 H, 12β-H), 3.2 (t, 1 H, C-17), 2.13 (s, 3 H, C-20), 2.06 and 2.02 (s, 3 H each, 3α,7α-OAc's), 0.93 (s, 3 H, c-19), and 0.65 (s, 3 H, C-18); *m/e* (%) 434 (3, M^+), 374 (100, M − HOAc), 314 (42, M − 2HOAc), 253 (48), and 229 (90).

3α,7α-Diacetoxy-12α-nitroxy-5β-pregnan-20-one (2c). Fuming HNO_3 (1 mL) was added to Ac_2O (3 mL) at −5 °C.[7] To this mixture, a solution of **2b** (0.3 g) in $CHCl_3$ (5 mL) was added dropwise and stirred for 0.5 h. Workup and column chromatography afforded **2c** (0.22 g): $\bar{\nu}_{max}$ 1740 and 1250 (OAc), 1715, and 1630, 1280, 860, and 760 cm^{-1} (NO_3); ^1H NMR δ 5.37 (peak, 1 H, 12β-H), 4.85 (peak, 1 H, 7β-H), 4.5 (hump, 1 H, 3β-H), 2.9 (t, 1 H, C-17), 2.08 (s, 3 H, C-20), 2.06 and 2.02 (s, 3 H each, 3α,7α-OAc's), 0.97 (s, 3 H, C-19), and 0.83 (s, 3 H, C-18); *m/e* (%) 479 (2, M^+), 436 (7, M − CH_3CO), 419 (7, M − HOAc), 373 (3, M − CH_3CO − HNO_3), 359 (63, M − 2HOAc), 313 (33, 359 − NO_2), 295 (57, 359 − HNO_3), 281 (39), 271 (46, 313 − CH_2CO), and 253 (87).

3α-Hydroxy-7α,12α-diacetoxy-5β-pregnan-20-one (2e). A solution of triacetate **2d** (2.0 g) in absolute CH_3OH (20 mL) was reacted with AcCl (1 mL) and allowed to stand at room temperature for 1 h. The organic solid obtained by H_2O precipitation was recrystallized from hexane–ether to yield diacetate **2e** (1.7 g): mp 186–187 °C; $\bar{\nu}_{max}$ 3550 (OH), 1720 (br), and 1250 cm^{-1} (OAc); ^1H NMR δ 5.17 (peak, 1 H, 12β-H), 4.95 (peak, 1 H, 7β-H), 3.5 (hump, 1 H, 3β-H), 2.9 (t, 1 H, C-17), 2.20 (s, 3 H, C-20), 2.10 and 2.03 (s, 3 H each, 7α,12α-OAc's), 0.93 (s, 3 H, C-19), and 0.72 (s, 3 H, C-18); *m/e* (%) 434 (2, M^+), 419 (3, M − CH_3), 392 (38, M − CH_2CO), 374 (7, M − HOAc), 332 (11, M − CH_2CO − HOAc), 314 (100, M − 2HOAc), 299 (40), 296 (84, M − 2HOAc − H_2O), 281 (80), and 253 (80, 296 − CH_3CO).

Anal. Calcd for $C_{25}H_{38}O_6$: C, 69.10; H, 8.81. Found: C, 69.07; H, 8.77.

3α-Nitroxy-7α,12α-diacetoxy-5β-pregnan-20-one (2f). Diester **2e** (1.0 g) was nitrated to yield **2f** (0.8 g): $\bar{\nu}_{max}$ 1740 and 1250 (OAc), and 1630, 1280, 870, and 760 cm^{-1} (NO_3); ^1H NMR δ 5.17 (peak, 1 H, 12β-H), 4.97 (peak, 1 H, 7β-H), 4.8 (hump, 1 H, 3β-H), 3.0 (t, 1 H, C-17), 2.20 (s, 3 H, C-20), 2.09 and 2.03 (s, 3 H each 7α,12α-OAc's), 0.98 (s, 3 H, C-19), and 0.73 (s, 3 H, C-18); *m/e* (%) 479 (2, M^+), 436 (14, M − CH_3CO), 419 (5, M − HOAc), 376 (7, M − CH_3CO − HOAc), 359 (31, M − 2HOAc), 313 (35 359 − NO_2), 295 (55, 313 − H_2O), 253 (56), and 213 (100).

Bromination and Dehydrobromination of 2f. To a solution of nitrate **2f** (1.0 g) in HOAc (30 mL) containing 40% HBr (2 dp) was added Br_2/HOAc (2.1 mL of 1.0 M). After stirring for 15 min, the acetic acid mixture was poured into ice–water. This was then extracted with ether, and the ether layer was washed with H_2O, aqueous $NaHCO_3$, and then H_2O again. The residue obtained from evaporation of the ether was dissolved in HMPT (30 mL) and heated at 100 °C for 1.5 h under a N_2 atmosphere. The cooled reaction mixture was diluted with H_2O which was extracted with EtOAc. Chromatography yielded two bromoenone products (**3c**). The minor and lower R_f component gave the following spectra: $\bar{\nu}_{max}$ 1730 and 1250 (OAc), 1665 and 1600 (C=C–C=O), and 755 cm^{-1} (C–Br); ^1H NMR δ 6.63 (peak, 1 H, C-16), 5.50 (peak, 1 H, 12β-H), 5.02 (peak, 1 H, 7β-H), 4.72 (peak, 1 H, 3α-H), 2.24 (s, 3 H, C-20), 2.07 and 1.97 (s, 3 H each, 7α,12α-OAc's), 1.03 (s, 3 H, C-19), and 0.95 (s, 3 H, C-18); *m/e* (%) 496 and 494 (1 and 1, M^+), 481 and 479 (1 and 1), 453 and 451 (96 and 96, M − CH_3CO), 436 and 434 (11 and 11, M − HOAc), 421 and 419 (2 and 2, M − HOAc − CH_3), 376 and 374 (89 and 89, M − 2HOAc), 361 and 359 (100 and 100, M − 2HOAc − CH_3), and 333 and 331 (21 and 21, M − 2HOAc − CH_3CO). The major component of higher R_f gave the following spectra: $\bar{\nu}_{max}$ 1730 and 1250 (OAc), 1660 and 1600 (C=C–C=O), and 750 cm^{-1} (C–Br); ^1H NMR δ 6.62 (peak, 1 H, C-16), 5.45 (peak, 1 H, 12β-H), 5.00 (peak, 1 H, 7β-H), 3.8 (hump, 1 H, 3β-H), 2.24 (s, 3 H, C-20), 2.10 and 2.00 (s, 3 H each, 7α,12α-OAc's), and 0.96 (s, 6 H, C-18 and C-19); *m/e* 496 and 494 (1 and 1, M^+).

3α,12α-Diacetoxy-13α-carbomethoxy-16-oxo-17-oxa-13,17-seco-7α,17-cyclo-5β-androstane (7c). Diester **4a** (0.5 g) was heated at reflux with 5% KOH/CH_3OH (30 mL) for 12 h, cooled, diluted with H_2O, and concentrated in vacuo to remove most of the CH_3OH. The aqueous mixture was acidified with concentrated HCl and extracted with EtOAc. The residue **7a** left after removal of the EtOAc was dissolved in CH_2Cl_2 and sequentially reacted with diazomethane (**7b**) and Ac_2O and pyridine. The solid obtained after dilution with H_2O was recrystallized from benzene–hexane: mp 233–235 °C; $\bar{\nu}_{max}$ 1740 and 1250 cm^{-1} (OAc); ^1H NMR δ 5.13 (peak, 1 H, 12β-H), 4.6 (hump, 1 H, 3β-H), 4.3 (hump, 1 H, 7β-H), 3.63 (s, 3 H, OCH_3), 2.02 (s, 6 H, 3α, 12α-OAc's), 1.27 (s, 3 H, C-18), and 0.83 (s, 3 H, C-19); *m/e* (%) 450 (3, M^+), 419 (5, M − CH_3O), 408 (42, M − CH_2CO), 390 (7, M − HOAc), 348 (42, M − CH_2CO − HOAc), 330 (48, M − 2HOAc), 298 (29, M − CH_2CO − 2HOAc), 271 (100, M − 2HOAc − CO_2CH_3), and 270 (57).

Anal. CALCD FOR $C_{24}H_{34}O_8$: C, 63.98; H, 7.61. Found: C, 64.36; H, 7.60.

3,12,16-Trioxo-13α-carbomethoxy-17-oxa-13,17-seco-7α,17-cyclo-5β-androstane (8d). δ-Lactone **7b** (0.30 g) obtained as above was dissolved in acetone (20 mL), and Jones reagent was added dropwise while stirring on an ice bath until a brown color was obtained. The reaction was terminated by adding 2-propanol and the Grignard precipitate removed by filtration. The acetone was evaporated off and the residue taken up in EtOAc. This organic layer was washed with H_2O several times and evaporated to dryness. Recrystallization of the residue thus obtained with hexane–benzene gave diketone **8d** (0.07 g): mp 213–215 °C; $\bar{\nu}_{max}$ 1740 and 1720 cm^{-1}; ^1H NMR δ 4.7 (hump, 1 H, 7β-H), 3.80 (s, 3 H, OCH_3), 1.39 (s, 3 H, C-18), and 1.01 (s, 3 H, C-19); *m/e* (%) 362 (100, M^+), 347 (11, M − CH_3), 344 (25, M − H_2O), 331 (18, M − OCH_3), 318 (21, M − CO_2), 305 (63), 290 (28), 277(60), and 259 (48).

Anal. Calcd for $C_{20}H_{26}O_6$: C, 66.28; H, 7.23. Found: C, 66.32; H, 7.37.

Methyl 3α-Hydroxy-7α,12α-diacetoxy-16,17-seco-5β-androstane-16,17-dioate (4b). Triacetate **4a** (1.0 g) was reacted for 0.5 h in CH_3OH (10 mL) containing AcCl (0.5 mL). Diacetate **4b** (0.9 g) was obtained: mp 196–197 °C; $\bar{\nu}_{max}$ 3650 (OH), 1740 and 1250 (OAc), and 1720 cm^{-1} (CO_2CH_3); ^1H NMR δ 5.20 (peak, 1 H, 12β-H), 4.90 (peak, 1 H, 7β-H), 3.66 (s, 6 H, OCH_3), 3.6 (hump, 1 p, 3β-H), 2.62 (m, 2 H, C-15), 2.13 and 2.10 (s, 3H each, 7α,12α-OAc's), 1.18 (s, 3 H, C-18), and 0.93 (s, 3 H, C-19); *m/e* (%) 482 (6, M^+), 451 (5, M − OCH_3), 439 (14, M − CH_3CO), 422 (13, M − HOAc), 407 (82, M − CH_3CO − CH_3OH), 389 (30, 407 − H_2O), 362 (22, M − 2HOAc), 347 (21, 362 − CH_3), 344 (16, M − 2HOAc − H_2O), 330 (53, 362 − CH_3OH), 312 (31, 344 − CH_3OH), and 285 (100, 344 − CO_2CH_3).

Anal. Calcd for $C_{25}H_{38}O_9$: C, 62.22; H, 7.94. Found: C, 62.39; H, 8.05.

Methyl 3α-Nitroxy-7α,12α-diacetoxy-16,17-seco-5β-androstan-16,17-dioate (4c). Diacetate **4b** (1.0 g) was nitrated to give **4c** (0.9 g) as a glassy solid: $\bar{\nu}_{max}$ 1740 and 1250 (OAc), 1630, 1280, 860, and 760 cm^{-1} (NO_3); ^1H NMR δ 5.13 (peak, 1 H, 12β-H), 4.87 (peak, 1 H, 7β-H), 4.8 (hump, 1 H, 3β-H), 3.61 and 3.58 (s, 3 H each, OCH_3's), 2.08 and 2.07 (s, 3H each, 7α,12α-OAc's), 1.17 (s, 3 H, C-18), and 0.97 (s, 3 H, C-19); *m/e* (%) 527 (2, M^+), 484 (6, M − CH_3CO), 467 (12, M − HOAc), 452 (50, M − HOAc − CH_3), 407 (42, M − 2HOAc), and 375 (27).

Methyl 3-Oxo-7α,12α-diacetoxy-16,17-seco-5β-androstane-16,17-dioate (4f). Diacetate **4b** (0.50 g) was oxidized by Jones reagent to give ketone **4f** (0.42 g): mp 218–220 °C; $\bar{\nu}_{max}$ 1740, 1715, and 1250 cm^{-1}; ^1H NMR δ 5.15 (peak, 1 H, 12β-H), 4.92 (peak, 1 H, 7β-H), 3.62 (s, 6 H, OCH_3's), 2.3 (m, 2 H, C-15), 2.08 and 2.05 (s, 3 H each, 7α,12α-OAc's), 1.20 (s, 3 H, C-18), and 1.03 (s, 3 p, C-19); *m/e* 480 (8, M^+), 449 (4, M − OCH_3), 437 (3, M − CH_3CO), 420 (28, M − HOAc),

405 (47, M − CH_3CO − CH_3OH), 387 (15, 405 − H_2O), 360 (33, M − 2HOAc), 345 (28, 360 − CH_3), 328 (38, M − 2HOAc − CH_3OH), 301 (52, M − 2HOAc − CO_2CH_3), 300 (48, 328 − CO), 285 (40, 300 − CH_3), and 241 (100).

Anal. Calcd for $C_{25}H_{36}O_9$: C, 62.49; H, 7.55. Found: C, 62.35; H, 7.57.

3α-Nitroxy-12α-acetoxy-13α-carbomethoxy-16-oxo-17-oxa-13,17-seco-7α,17-cyclo-5β-androstane (7f). A mixture of nitrate **4c** (1.0 g), CH_3OH (50 mL), H_2O (10 mL), and KOH (2 g) was heated at reflux for 12 h. The mixture was concentrated on a rotating evaporator to remove most of the CH_3OH. The aqueous residue was acidified with concentrated HCl, warmed for 0.5 h, and then cooled and extracted with EtOAc. EtOAc was evaporated off, and the residue **7d** was dissolved in CH_2Cl_2 and treated with diazomethane to yield δ-lactone **7e**. Lactone **7e** was acetylated and recrystallized from benzene–chloroform to afford δ-lactone **7f** (0.3 g): mp 224–226 °C; $\bar{\nu}_{max}$ 1740 (br) and 1620, 1280, and 880 cm^{-1} (NO_3); ^1H NMR δ 5.13 (peak, 1 H, 12β-H), 4.9 (hump, 1 H, 3β-H), 4.4 (hump, 1 H, 7β-H), 2.4 (m, 2 H, C-15), 2.03 (s, 3 H, 12α-OAc), 1.28 (s, 3 H, C-18), and 0.89 (s, 3 H, C-19); *m/e* (%) 453 (3, M^+), 422 (4, M − CH_3O), 411 (100, M − CH_2CO), 393 (4, M − HOAc), 383 (25), 352 (10), 348 (11), 347 (12), 330 (13), 329 (14), 315 (19), 287 (40), and 271 (95, M − HOAc − HNO_3 − CO_2CH_3).

Anal. Calcd for $C_{22}H_{31}O_9N$: C, 58.27; H, 6.89; N, 3.09. Found: C, 58.20; H, 6.91; N, 3.01.

3α-Nitroxy-12,16-dioxo-13β-carbomethoxy-17-oxa-13,17-seco-7α,17-cyclo-5β-androstane (8a). δ-Lactone **7e** obtained from 1.0 g of nitrate **4c** was oxidized with Jones reagent to a mixture of the following four compounds isolated by preparative TLC.

The higher R_f component was recrystallized from hexane–EtOAc giving diketone **5a** (0.2 g): mp 177–179 °C; $\bar{\nu}_{max}$ 1740, 1710 and 1620, 1280 and 880 cm^{-1} (NO_3); ^1H NMR δ 4.8 (hump, 1 H, 3β-H), 3.73 (s, 3 H, 13α-CO_2CH_3), 3.58 (s, 3 H, C-16, OCH_3), 1.30 (s, 3 H, C-18), and 1.26 (s, 3 H, C-19); *m/e* (%) 439 (19, M^+), 421 (9, M − H_2O), 408 (55, M − CH_3O), 380 (55, M − CO_2CH_3), 362 (65, M − H_2O − CO_2CH_3), 345 (40), 343 (40), 330 (100), 301 (40), and 283 (85).

Anal. Calcd for $C_{21}H_{29}NO_9$: C, 57.40; H, 6.65. Found: C, 57.40; H, 6.82.

The second most mobile component was recrystallized from hexane–benzene to afford the desired δ-lactone **8a** (0.2 (0.2 g): mp 199–201 °C; $\bar{\nu}_{max}$ 1740, 1710 and1625, 1280, and 860 cm^{-1} (NO_3); ^1H NMR δ 4.9 (hump, 1 H, 3β-H), 4.5 (hump, 1 H, 7β-H), 3.76 (s, 3 H, OCH_3), 1.36 (s, 3 H, C-18), and 0.93 (s, 3 H, C-19); *m/e* (%) 409 (3, M^+), 378 (3, M − CH_3O), 363 (13, M − NO_2), 350 (9, M − CO_2CH_3), 346 (20, M − HNO_3), 287 (25, M − HNO_3 − CO_2CH_3), and 285 (18).

Anal. Calcd for $C_{20}H_{27}O_8N$: C, 58.67; H, 6.65; N, 3.42. Found: C, 59.07; H, 6.92; N, 3.18.

The third most mobile component (30 mg) was triketone **5c**: $\bar{\nu}_{max}$ 1740 and 1715 cm^{-1}; ^1H NMR δ 3.70 (s, 3 H, 13α-CO_2CH_3), 3.59 (s, 3 H, C-16, OCH_3), 1.33 (s, 3 H, C-18), and 1.28 (s, 3 H, C-19); *m/e* (%) 392 (5, M^+), 374 (7, M − H_2O), 360 (10, M − CH_3OH), 333 (9, M − CO_2CH_3), 315 (8, M − CO_2CH_3 − H_2O), 301 (23), 287 (26), and 283 (45).

The most polar component (35 mg) was δ-lactone **8d**.

Methyl 3α-Acetoxy-12,16-dioxo-13β-carbomethoxy-17-oxa-16,17-seco-7α,17-cyclo-5β-androstane (8c). Nitroxy δ-lactone **8a** (0.10 g) was reduced with Zn and acetylated to afford acetoxy δ-lactone **8c** (80 mg) after recrystallization from hexane–benzene: mp 210–212 °C; $\bar{\nu}_{max}$ 1740 and 1710 cm^{-1}; ^1H NMR δ 4.7 (hump, 1 H, 3β-H), 4.5 (hump, 1 H, 7β-H), 3.75 (s, 3 H, OCH_3), 1.36 (s, 3 H, C-18), and 0.90 (s, 3 H, C-19); *m/e* (%) 406 (25, M^+), 346 (92, M − HOAc), 331 (15), 328 (19), 318 (30), 314 (28), 287 (98, M − HOAc − CO_2CH_3), and 259 (100).

Anal. Calcd for $C_{22}H_{30}O_7$: C, 65.01; H, 7.44. Found: C, 65.06; H, 7.30.

Methyl 3α,12α-Diacetoxy-7α-hydroxy-16,17-seco-5β-androstane-16,17-dioate (4e). A solution of δ-lactone **7c** (0.10 g) was reacted for 1 h with CH_3OH containing AcCl at room temperature. Dilution of the reaction mixture with H_2O and subsequent workup yielded diol **4d** which could be acetylated with Ac_2O–pyridine (1:2) at room temperature for 12 h to give diester **4e** (80 mg): mp 158–160 °C; $\bar{\nu}_{max}$ 2450 (OH), 1740, 1715, and 1250 cm^{-1}; ^1H NMR δ 5.14 (peak, 1 H, 12β-H), 4.5 (hump, 1 H, 3β-H), 4.23 (peak, 1 H, 7β-H), 3.74 (s, 3 H, 13α-CO_2CH_3), 3.63 (s, 3 H, C-16, OCH_3), 2.08 and 2.04 (s, 3 H each, 3α,12α-OAc's), 1.19 (s, 3 H, C-18), and 0.98 (s, 3 H, C-19); *m/e* (%) 482 (5, M^+), 464 (4, M − H_2O), 430 (6, M − CH_3OH), 422 (22, M − HOAc), 404 (10, M − H_2O − HOAc), 390 (23, M − CH_3OH − HOAc), 372 (11, 390 − H_2O), 362 (32, M − 2HOAc), 344 (14, 362 − H_2O), 330 (45, 362 − CH_3OH), 312 (20), 302 (27), 285 (70, 344 − CO_2CH_3) and 271 (65).

Anal. Calcd for $C_{25}H_{38}O_9$: C, 62.22; H, 7.94. Found: C, 61.99; H, 8.08.

3α,12α-Diacetoxy-7-oxo-16,17-seco-5β-androstane-13,17-dioate (4g). Hydroxy diacetate **4e** was oxidized with Jones reagent to give ketone **4g** in nearly quantitative yields: mp 154–155 °C; ^1H NMR δ 5.12 (peak, 1 H, 12β-H), 4.5 (hump, 1 H, 3β-H), 3.68 and 365 (s, 3 H each, OCH_3), 2.05 and 2.02 (s, 3 H each, 3α,12α-OAc's), 1.22 (s, 3 H, C-18), and 1.20 (s, 3 H, C-19); *m/e* (%) 480 (11, M^+), 449 (9, M − CH_3O), 448 (10, M − CH_3OH), 420 (18, M − HOAc), 389 (13, M − CH_3O − HOAc), 388 (13, M − CH_3OH − HOAc), 360 (48, M − 2HOAc), 329 (20, 360 − CH_3O), 328 (54, 360 − CH_3OH), 313 (14), 301 (20), 300 (36), 287 (25), 285 (18), and 269 (100).

Anal. Calcd for $C_{25}H_{36}O_9$: C, 62.49; H, 7.55. Found: C, 62.31; H, 7.68.

3,16-Dioxo-12α-acetoxy-13α-carbomethoxy-17-oxa-13,17-seco-7α,17-cyclo-5β-androstane (9b). Ketone **4f** (0.40 g) was saponified and subsequently treated with warm HCl solution to yield δ-lactone **9a**. Treatment of crude δ-lactone **9a** with diazomethane and then Ac_2O–pyridine yielded a product which was purified by TLC. The polar material thus isolated was recrystallized from hexane–benzene to afford δ-lactone **9b** (0.10 g): mp 211–212 °C; $\bar{\nu}_{max}$ 1750, 1730, and 1710 cm^{-1}; ^1H NMR δ 5.17 (peak, 1 H, 12β-H), 4.5 (hump, 1 H, 7β-H), 3.65 (s, 3 H, OCH_3), 2.3 (m, 6 H, C-2, C-4, and C-15), 2.03 (s, 3 H, 12α-OAc), 1.25 (s, 3 H, C-18), and 0.95 (s, 3 H, C-19); *m/e* (%) 406 (8, M^+), 375 (6, M − CH_3O), 364 (55, M − CH_2CO), 346 (10, M − HOAc), 336 (7, M − CH_2CO − CO), 328 (8, M − HOAc − H_2O), 318 (15), 314 (13, M − CH_2CO − HOAc), 305 (10), 304 (11), 300 (11), 287 (31, M − HOAc − CO_2CH_3), and 241 (75).

Anal. Calcd for $C_{22}H_{30}O_7$: C, 65.01; H, 7.44. Found: C, 65.17; H, 7.51.

3,16-Dioxo-12α-nitroxy-13α-carbomethoxy-17-oxa-13,17-seco-7α,17-cyclo-5β-androstane (9c). δ-Lactone **9a** made from ketone **4f** (0.50 g) was nitrated and purified by TLC to yield δ-lactone **9c** (0.10 g) as a glassy solid: $\bar{\nu}_{max}$ 1740 (br) and 1640, 1280, 860, and 760 cm^{-1} (NO_3); ^1H NMR δ 5.22 (peak, 1 H, 12 β-H), 4.6 (hump, 1 H, 7β-H), 3.70 (s, 3 H, OCH_3), 2.4 (m, 6 H), 1.33 (s, 3 H, C-18), and 0.97 (s, 3 H, C-19); *m/e* (%) 409 (8, M^+), 394 (6, M − CH_3), 376 (9, M − CH_3 − H_2O), 334 (8, 376 − CH_2CO), 302 (60), and 287 (45, 302 − CH_3).

Methyl 3α-Acetoxy-7,12-dioxo-16,17-seco-5β-androstane-16,17-dioate (5b). Zn dust reduction of nitrate **5a** (0.20 g) followed by acetylation yielded acetate **5b** (0.19 g): mp 143–144 °C; $\bar{\nu}_{max}$ 1740, 1720, and 1710 cm^{-1}; ^1H NMR δ 4.7 (hump, 1 H, 3β-H), 3.74 and 3.61 (s, 3 H each, OCH_3), 1.99 (s, 3 H, 3α-OAc), 1.32 (s, 3 H, C-18), and 1.24 (s, 3 H, C-19); *m/e* (%) 436 (8, M^+), 418 (8, M − H_2O), 404 (11, M − CH_3OH), 386 (9, M − H_2O − CH_3OH), 377 (25, M − CO_2CH_3), 376 (28, M − HOAc), 359 (34, 377 − H_2O), 345 (63, M − CH_3OH − CO_2CH_3), 327 (30), 316 (26), 299 (39), and 285 (100, M − CH_3OH − HOAc − CO_2CH_3).

Anal. Calcd for $C_{23}H_{32}O_8$: C, 63.29; H, 7.39. Found: C, 63.54; H, 7.46.

Methyl 3α-Acetoxy-7,12-dioxo-13,17-seco-17-nor-5β,13α-androstan-16-oate (6). Acetate **5b** (0.15 g) was heated at reflux in HOAc (2 mL) containing concentrated HCl (0.5 mL) for 4 h. Workup and TLC yielded acetate **6** (0.10 g) as a glassy solid: $\bar{\nu}_{max}$ 1730 and 1710 cm^{-1}; ^1H NMR δ 4.7 (hump, 1 H, 3β-H), 3.62 (s, 3 H, OCH_3), 1.97 (s, 3 H, 3α-OAc), 1.27 (s, 3 H, C-19), and 1.07 (d, J = 6 Hz, 3 H, C-18); *m/e* (%) 378 (12, M^+), 318 (25, M − HOAc), 305 (22, M − $CH_2CO_2CH_3$), 300 (21, M − HOAc − H_2O), 287 (39, 305 − H_2O), 258 (28), and 245 (84, M − HOAc − $CH_2CO_2CH_3$).

3,12,16-Trioxo-17-oxa-13,17-seco-7α,17-cyclo-5β,13α-androstane (10b). A solution of δ-lactone **8d** (50 mg) in glacial HOAc (2 mL) containing concentrated HCl (0.5 mL) was heated at reflux for 4 h. Workup and recrystallization from hexane–benzene afforded δ-lactone **10b** (30 mg): mp 171–173 °C; $\bar{\nu}_{max}$ 1740, 1720, and 1700 cm^{-1}; ^1H NMR δ 4.8 (hump, 1 H, 7β-H), 1.12 (d, J = 6 Hz, 3 H, C-18), and 1.03 (s, 3 H, C-19); *m/e* (%) 304 (65, M^+), 289 (6, M − CH_3), 286 (12, M − H_2O), 277 (20), 278 (17, M − CO), and 260 (17, M − CO_2).

Anal. Calcd for $C_{18}H_{24}O_4$: C, 71.03; H, 7.95. Found: C, 71.03; H, 7.83.

3α-Acetoxy-12,16-dioxo-17-oxa-13,17-seco-7α,17-cyclo-5β,13α-androstane (10c). δ-Lactone **7d** obtained from **4c** (0.8 g) was oxidized with Jones reagent, then treated with Zn dust/HOAc, and finally acetylated to yield acetoxy δ-lactone **10c** (0.2 g): mp 185–187 °C; $\bar{\nu}_{max}$ 1755, 1720, and 1695 cm^{-1}; ^1H NMR δ 4.7 (hump, 1 H, 3β-H), 4.5 (hump, 1 H, 7β-H), 2.00 (s, 3 H, 3α-OAc), 1.06 (d, J = 6 Hz, 3 H, C-18), and 0.90 (s, 3 H, C-19); *m/e* (%) 348 (11, M^+), 288 (100, M − HOAc), 273 (27, M − HOAc − CH_3), 270 (10, M − HOAc − H_2O), 260 (11), 245 (18), 229 (48), 228 (45), and 216 (72).

Anal. Calcd for $C_{20}H_{28}O_5$: C, 68.94; H, 8.10. Found: C, 68.95; H, 8.23.

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Registry No.—**2a,** 601-95-6; **2b,** 63533-72-2; **2c,** 63533-73-3; **2d,** 61543-88-2; **2e,** 63533-74-4; **2f,** 63533-75-5; **3c** isomer 1, 63533-76-6; **3c** isomer 2, 63533-77-7; **4a,** 61543-93-9; **4b,** 63533-78-8; **4c,** 63533-79-9; **4d,** 63533-80-2; **4e,** 63533-81-3; **4f,** 63533-82-4; **4g,** 63533-83-5; **5a,** 63533-84-6; **5b,** 63533-85-7; **5c,** 63533-86-8; **6,** 63533-87-9; **7a,** 63533-88-0; **7b,** 63533-89-1; **7c,** 62251-60-9; **7d,** 63547-45-5; **7e,** 63533-90-4; **7f,** 63533-91-5; **8a,** 63533-92-6; **8c,** 63533-93-7; **8d,** 63533-94-8; **9a,** 63533-95-9; **9b,** 63533-96-0; **9c,** 63533-97-1; **10b,** 63533-98-2; **10c,** 63533-99-3.

References and Notes

(1) For Parts III and IV, refer to J. R. Dias and R. Ramachandra, *Synth. Commun.*, **7**, 293 (1977), and *Org. Prep. Proc. Int.*, **9**, in press.
(2) Postdoctoral Research Associate, 1974–1977.
(3) J. R. Dias and R. Ramachandra, *J. Org. Chem.*, **42**, 1613 (1977).
(4) J. Baker and R. T. Blickenstaff, *J. Org. Chem.*, **40**, 1579 (1975).
(5) Also, we discovered that nitrate esters, like 3β-nitroxy-5α-cholestane, undergo quantitative HNO_3 elimination in hot HMPT which is currently under investigation in our laboratory.
(6) L. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.*, **72**, 5530 (1950).
(7) I. Tanasescu, F. Hodosan, and I. Jude, *Chem. Ber.*, **91**, 799 (1958).

Protoberberine Alkaloids. Structures of Aequaline, Coramine, Discretinine, and Schefferine

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The structures assigned to the protoberberine alkaloids aequaline and coramine were found to be incorrect. Instead, aequaline was shown to be identical with discretamine (**6**), and coramine was identical with coreximine (**13**). Schefferine was found to have the same structure as kikemanine [(−)-corydalmine] (**8**), and discretinine was shown to be corypalmine (**14**) by comparison with authentic samples.

The protoberberine alkaloids are widely distributed in many plant families, mainly as the tetrahydroprotoberberines and the quaternary protoberberine salts.[1-4] They are biosynthesized from benzyltetrahydroisoquinolines[5-7] and, in turn, serve as biosynthetic intermediates for many other alkaloid groups.

The assignment of the substitution pattern of protoberberines isolated from natural sources has often presented considerable problems, especially when insufficient material has been available for chemical degradations. Spectroscopic data can give valuable information,[8] but the final proof of structure comes from chemical synthesis. Several protoberberine alkaloids have been isolated whose structures are still not known in all detail, and there are others which have been assigned incorrect structures.

In 1972 two tetrahydroprotoberberine alkaloids were isolated from the bark of *Schefferomitra subaequalis* and named aequaline and schefferine.[9] Both alkaloids were levorotatory and gave (−)-tetrahydropalmatine (**1**) on methylation with diazomethane, thereby establishing a 2,3,9,10-tetraoxygenated substitution pattern. Elemental analysis of aequaline gave the molecular formula $C_{19}H_{21}NO_4$. The NMR spectrum established the presence of two methoxyl and two hydroxyl groups, and mass spectroscopy showed that both rings A and D each had one hydroxyl and one methoxyl group. A 9-hydroxy-10-methoxy substitution was suggested based on the relative abundances of the fragments. Since aequaline was shown by direct comparison to be different from scoulerine (**2**), the structure of aequaline was proposed to be (−)-3,9-dihydroxy-2,10-dimethoxytetrahydroprotoberberine (**3**).

Microanalysis of the second alkaloid, schefferine, gave a molecular formula $C_{20}H_{23}NO_4$ and a molecular ion peak *m/e* 341 in its mass spectrum indicating the presence of one hydroxyl and three methoxyl groups. Based on the fragmentation pattern, two methoxyl groups could be assigned to ring A. Since monomethylation of aequaline with diazomethane gave schefferine as one of the products, structure **4** was assigned to schefferine.

Recently, mass spectrometric criteria were developed for detecting a methoxyl group in position 9 of protoberberine alkaloids based on the abundance of the $(M - OCH_3)^+$ fragment compared to that of the molecular ion.[8] Compounds with a 9-methoxy substituent give a $(M - OCH_3)^+$ fragment ranging from 12 to 19% of the molecular ion. If the compounds are either unsubstituted in position 9 or have a 9-hydroxy substituent, the relative abundance of the $(M - OCH_3)^+$ fragment is <3% of the molecular ion peak. Preliminary mass spectroscopic studies[10] have indicated that both aequaline and schefferine contain a 9-methoxy substituent. In order to clarify this discrepancy and to establish unequivocally the correct structure of aequaline, compound **3** was synthesized by intramolecular Mannich condensation of (±)-norprotosinomenine (**18b**) with formaldehyde at pH 6.4 and room temperature. Cyclization occurred ortho and para to the phenolic hydroxyl group to give a mixture of (±)-3,9-dihydroxy-2,10-dimethoxytetrahydroprotoberberine (**3**) and (±)-3,11-dihydroxy-2,10-dimethoxytetrahydropseudoberberine (**5**). Spectroscopic comparison (IR, NMR, MS) of aequaline with compound **3** showed that aequaline did not have the structure assigned to it. Two diphenolic 2,3,9,10-substituted isomers of compounds **2** and **3** have been isolated from natural sources and are named discretamine (**6**)[11,12] and stepholidine (**7**).[13,14] Both compounds have recently been synthesized.[15] A comparison of aequaline with discretamine and stepholidine (IR, NMR, mass spectrometry, TLC) showed clearly that aequaline is identical with discretamine. It, therefore, also follows that schefferine must be 9-methoxy-10-hydroxy-substituted,

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