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STRUCTURE OF ECHITOSERPINE, A NEW ALKALOID OF THE FRUITS OF *ALSTONIA VENENATA*[a]

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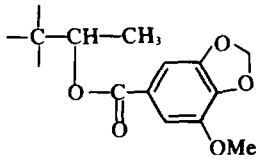
Abstract—Echitoserpine, a new 20-aroyloxy-16-methoxy vincadifformine alkaloid, has been isolated from the fruits of *Alstonia venenata* R.Br. and is shown to possess structure 1 on the basis of spectral and chemical evidence.

The isolation and structure elucidation of thirteen indole alkaloids[1-7] besides a biogenetically important pyridine base, venoterpine[8,9] from different parts of *Alstonia venenata* R.Br. were reported in earlier publications. Classified on the basis of skeletal patterns these alkaloids represent three distinct structural types viz. yohimbine, refractine-pleiocarpine, and vincadifformine. Our continued search for the alkaloidal principles from the fruits of this plant species have now resulted in the isolation of yet another new base which we call echitoserpine. Spectral and chemical evidence leading to structure 1 for the alkaloid are reported in the present communication.

Echitoserpine, $C_{31}H_{34}N_2O_8$ (M^+ 562), m.p. 154°, was isolated from the petrol extract of the fruits of *A. venenata* in poor yield. The UV spectrum of the alkaloid, λ_{max}^{EtOH} 205 (log ϵ, 4·60), 220 sh (4·50), 252 (4·16), 301 (4·19), 325 (4·24) nm coupled with its high specific rotation, $[\alpha]_D$ − 444·5° (c = 0·3, $CHCl_3$) and characteristic IR absorptions (ν_{max} 3400, 1685, 1640 and 1620 cm^{-1}) indicate the presence of a β-anilinomethacrylate chromophore in the base. The IR spectrum also indicates the presence of an additional ester carbonyl (ν_{max} 1720 cm^{-1}) and a methylene dioxy group[10] (ν_{max} 1490, 1370, 1250, 1102, 1035 and 927 cm^{-1}). The presence of the β-anilinomethacrylate chromophore, presumably containing an aromatic methoxyl group in echitoserpine is further justified by the PMR signals of the alkaloid [δ (ppm), 9·0 (br.s., disappearing with D_2O, NH); 3·73 (3H, s, $ArOCH_3$), 3·50 (3H, s, shielded CO_2CH_3) and 6·20–7·13 (5H, m, Ar-H)]. The presence of five aromatic protons further demands the existence of at least two benzene rings in the alkaloid. The PMR spectrum of the alkaloid also displays signals [δ (ppm), 1·05 (3H, d, J = 7 Hz), 4·97 (1H, q, J = 7 Hz), 3·93 (3H, s), 6·05 (2H, s)] which can be attributed to the system,



in echitoserpine.

The precise nature of the structure of the alkaloid was largely elicited from its mass spectrum. Besides the molecular ion peak, the spectrum shows significant peaks at *m/e* 367 (M-195), 366 (M–196), 318 (M-244, base peak), 123, 122 and 179 (195-16) which can be best rationalized (see Scheme 1) in terms of a methoxyvincadifformine-like[11] structure 1 for the alkaloid with an ester function of composition, $C_9H_7O_5$ probably at C-20 position on the ethyl side chain as indicated by the IR and PMR spectra of the alkaloid. The location of the aromatic methoxyl group at C-16 position has been settled by subsequent observations.

Echitoserpine on sealed-tube acid-catalyzed hydrolysis afforded an acid, $C_9H_8O_5$ (M^+ 196), m.p. 208°, identical in all respects with myristicinic acid 2 and an indolenine base, $C_{20}H_{26}N_2O_2$ (M^+ 326), m.p. 199°, λ_{max}^{EtOH} 230 (log ϵ, 3·93), 255 (3·53), 282 (3·49) nm. The latter shows mass spectral fragmentation [significant peaks at *m/e* 281 (M-45) and 256 (M-70)] typical of 1,2-dehydro deacetyl aspidospermine.[12] The reduction of the above indolenine base with sodium borohydride gave an indolic compound, $C_{20}H_{28}N_2O_2$ (M^+ 328), $[\alpha]_D$ + 125·6° (c = 0·39, $CHCl_3$), λ_{max}^{EtOH} 230 (log ϵ, 4·37), 270 (3·64) and 300 (3·64) nm together with a trace of isomeric indoline, λ_{max}^{EtOH} 242 inf (log ϵ, 3·73) and 300 (3·56) nm. The UV spectrum of the indolic base is typical of 6-methoxy-2,3-disubstituted indole chromophore[13,14] and that of the indoline suggests a 7-methoxy hexahydrocarbazole system.[13] The isomeric indole and indoline bases exhibit typical quebrachamine[12,15] [significant peaks at *m/e* 283 (M-45), 187, 174, 173, 140 and 110] and

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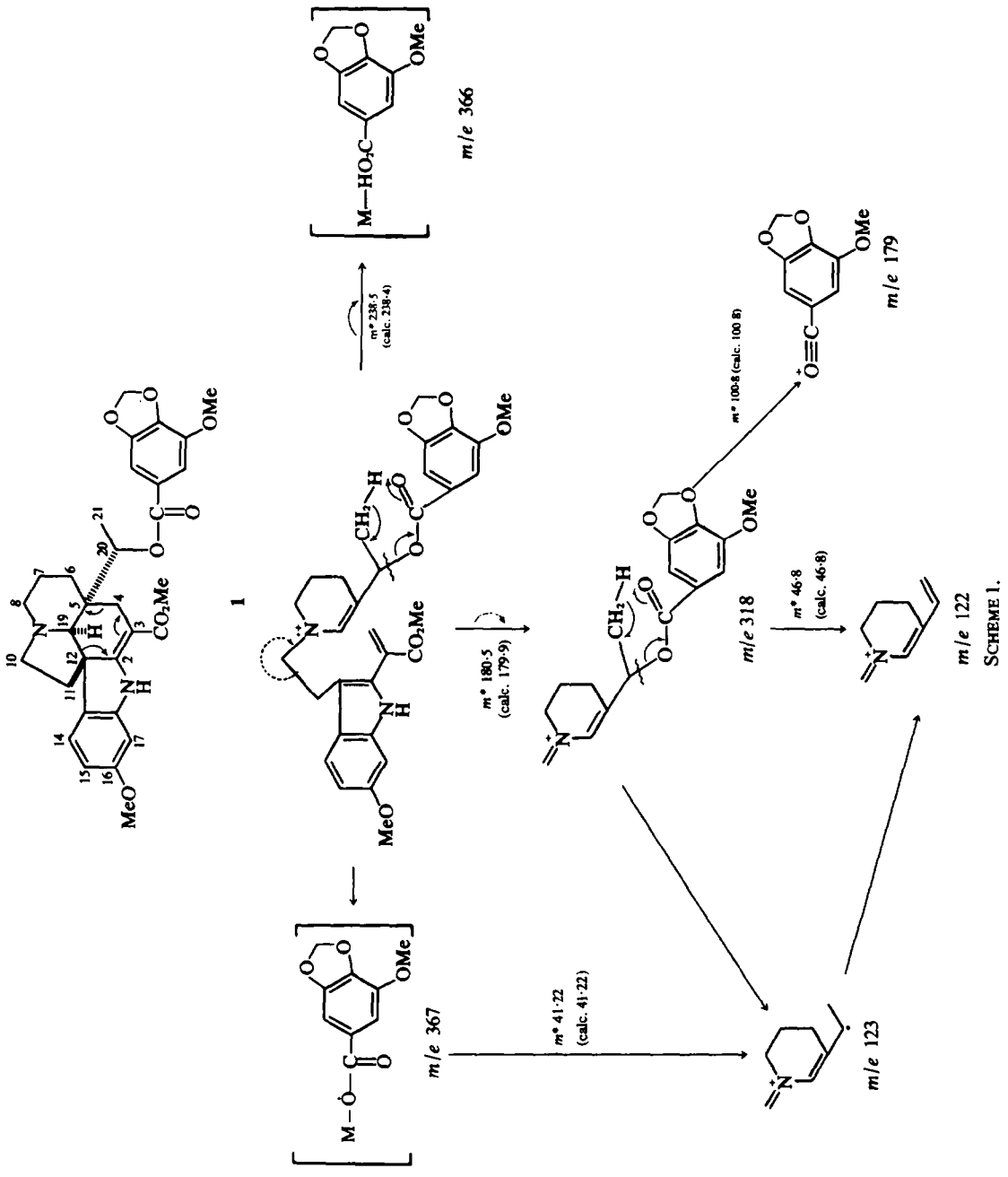
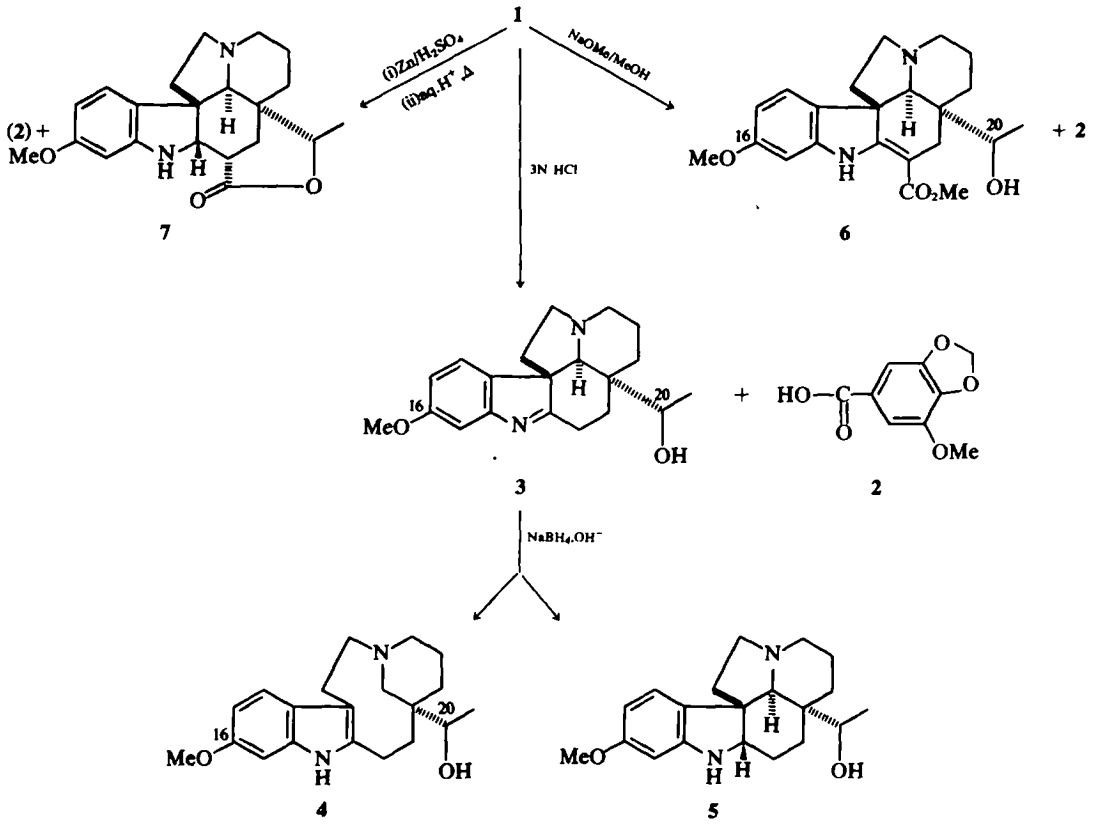

1
m/e 367
m/e 366
m/e 318
m/e 179
m/e 123
m/e 122
m* 41·22 (calc. 41·22)
m* 180·5 (calc. 179·9)
m* 238·5 (calc. 238·4)
m* 46·8 (calc. 46·8)
m* 100·8 (calc. 100·8)
M—HO₂C
M—O—C
MeO
OMe
CO₂Me

SCHEME 1.



SCHEME 2.

aspidospermine[16] [characteristic peaks at *m/e* 300 (M-28), 283 (M-45), 168 and 140] type mass spectral fragmentation, respectively. These observations thus suggest structures **4** and **5** for the indolic and the indoline bases, respectively. The structure of the indolenine, therefore, follows as **3** (see Scheme 2). This evidence thus not only affirms the position of the aromatic methoxyl group at C-16 position in the parent alkaloid, but also justify the structure **1** for the base.

This is also supported by the fact that echitoserpine on methanolysis with sodium methoxide in dry methanol gave, besides myristicinic acid (obtained by further alkaline hydrolysis of the neutral component), a basic compound, $C_{22}H_{28}N_2O_4$ (M^+ 384), $[\alpha]_D - 412°$ (c = 0·15, $CHCl_3$), λ_{max}^{EtOH} 246 (log ϵ, 3·90) and 327 (4·03) nm, the physical constants of which compare excellently with those reported for 16-methoxyminovincinine **6**.[17] It shows typical vincadifformine-like mass spectral fragmentation, the base peak in the mass spectrum of the parent alkaloid at *m/e* 318 is shifted to *m/e* 140 in this compound.

Moreover, the alkaloid on reduction with zinc and 10% methanolic sulphuric acid afforded a lactonic compound, $C_{21}H_{26}N_2O_3$ (M^+ 354), λ_{max}^{EtOH} 245 inf (log ϵ, 3·62) and 302 (3·58) nm. The above spectral data together with its typical mass spectral fragmentation[3,4] (significant peaks at *m/e* 283, 194, 123 and 122) are consistent with the structure **7** for the compound.

All the foregoing evidence, thus, suggests the 16-methoxy-20-myristicinyloxy vincadifformine structure **1** for echitoserpine and the stereochemistry assigned in this formulation follows from the positive specific rotation[18] of **4** as also from its correlation with (−)16-methoxyminovincinine **6**. The presence of a rather uncommon aryoloxy function in this base and its congener alkaloid, echitoserpidine[7] and the bark alkaloid, veneserpine,[5] reveals an interesting biogenetic feature.

EXPERIMENTAL

M.ps determined on a Köfler block, are uncorrected. IR spectra were run in Nujol mulls and UV spectra were taken in 95% EtOH (aldehyde-free). Petrol had b.p. 60–80°. All analytical samples were tested for purity by TLC and mass spectrometry.

Isolation of echitoserpine **1**. Air-dried powdered fruit (1 kg) of *A. venenata* was extracted with petrol. The extract was concentrated, churned with 5% aq citric acid and filtered. The filtrate was extracted with C_6H_6, the extract was washed with NH_4OH, then with H_2O, dried,

concentrated and chromatographed. The petrol-C_6H_6 (1:1) eluate on evaporation gave an oily residue, which on repeated chromatography afforded **1** (yield 0·005%). It was crystallized from MeOH. (Found: C, 66·02; H, 5·97; N, 4·93. $C_{31}H_{34}N_2O_8$ requires: C, 66·19; H, 6·05; N, 4·98%). *m/e* (abundance %): 562 (M^+, 45), 532 (4), 367 (53), 366 (58), 339 (6), 318 (100), 307 (5), 258 (10), 257 (11), 198 (8), 196 (8), 180 (11), 179 (50), 124 (8), 123 (52), 122 (24).

Myristicinic acid **2** *and indolenine* **3** *from* **1**. A solution of **1** (0·35 g) in 3N HCl (22 ml) was heated in an evacuated sealed-tube at about 110° for 6½ h. The reaction mixture was extracted with ether, washed with H_2O, dried and evaporated when a solid residue was obtained which on crystallizations from MeOH gave an acid (0·05 g), identical (m.m.p., co-TLC and IR) with myristicinic acid **2** prepared by the action of CH_2BrCl[19] on gallic acid, followed by methylation with Me_2SO_4 and alkali. The aq fraction was basified with NH_4OH, extracted with ether, dried and concentrated when a crystalline solid was obtained. The solid on crystallizations from ether furnished pure **3** (0·15 g). (Found: C, 73·51; H, 7·92; N, 8·52. $C_{20}H_{26}N_2O_2$ requires: C, 73·62, H, 7·98; N, 8·59%). $[\alpha]_D$ − 213° (c = 0·32, EtOH); ν_{max} cm^{-1}: 3180 (OH), 1590 (>C=N—); *m/e* (abundance %): 326 (M^+, 100), 282 (41), 281 (89), 256 (4), 224 (17), 188 (13), 163 (M^{++}, 3), 109 (54).

16-Methoxy-20-hydroxyquebrachamine **4** *and indoline* **5** *from* **3**. The indolenine **3** (0·1 g) was reduced with an excess of $NaBH_4$ (0·13 g) in 1N MeOH-KOH (25 ml) by refluxing for 3 h. MeOH was then removed under reduced pressure. The residue was diluted with H_2O, extracted with ether, dried and concentrated when a solid residue was obtained which was separated by p-TLC to give **4** (0·06 g) and **5** (0·015 g).

4 (Found: C, 73·03; H, 8·47; N, 8·46. $C_{20}H_{28}N_2O_2$ requires: C, 73·16; H, 8·54; N, 8·54%). ν_{max} cm^{-1}: 3300 (NH, OH); m/e (abundance %): 328 (M^+, 92), 283 (36), 187 (24), 174 (19), 173 (13), 164 (M^{2+}, 13), 142 (18·4), 141 (20·5), 140 (34), 126 (8), 124 (16), 110 (100), 108 (18·4), 96 (23).

5. (Found: C, 73·0; H, 8·49; N, 8·47. $C_{20}H_{28}N_2O_2$ requires: C, 73·16; H, 8·54; N, 8·54%). $[\alpha]_D$−19° (c = 0·53, $CHCl_3$); m/e (abundance %): 328 (M^+, 41·6), 300 (10), 283 (13), 206 (7), 182 (10), 174 (5·3), 173 (3), 168 (15), 160 (7), 149 (6), 141 (13·3), 140 (100), 122 (7·5), 110 (8·3).

16-Methoxyminovincinine **6** *and* **2** *from* **1**. Metallic Na (0·08 g) was added portionwise to dry MeOH (25 ml). To the clear soln, **1** (0·15 g) was added and the mixture was heated under reflux for 8 h in an atmosphere of N_2. The residue after removal of MeOH was treated with 4N aq HCl, and extracted with ether. The ether extract on evaporation gave an oily residue which warefluxed with 5% MeOH–KOH (12 ml) for 2 h. MeOH was removed under reduced pressure, the soln was acidified with HCl, extracted with ether, dried and evaporated to a solid residue which on crystallizations from MeOH gave **2** (0·025 g).

The aq acid soln containing the basic constituents was neutralized with NH_4OH, extracted with $CHCl_3$, dried, concentrated and chromatographed. The benzene–chloroform (3:1) eluate on evaporation yielded **6** (0·05 g). (Found: C, 68·56; H, 7·22; N, 7·23. Calc. for $C_{22}H_{28}N_2O_4$: C, 68·76; H, 7·29; N, 7·29%).

δ(delta)-Lactone **7** *and* **2** *from* **1**. A soln of **1** (0·3 g) in 10% MeOH-H_2SO_4 (30 ml) was reduced with an excess of Zn dust (0·7 g) by refluxing for 8 h. Unreacted Zn was filtered off and MeOH from the filtrate was removed under reduced pressure. The residue was treated with H_2O, made 10% with respect to H_2SO_4 and refluxed for 10 h. The soln was extracted with ether, dried and evaporated when a solid was obtained which on crystallizations from MeOH gave pure **2** (0·04 g). The aq acid part was neutralized with NH_4OH, extracted with ether, dried, concentrated and chromatographed. The petrol–EtOAc (3:1) eluate gave pure **7** (0·13 g). (Found: C, 71·02;H, 7·28; N, 7·83. $C_{21}H_{26}N_2O_3$ requires: C, 71·19; H, 7·35; N, 7·91%). $[\alpha]_D$ + 15·4° (c = 0·78, EtOH); ν_{max} cm^{-1}: 3300 (NH), 1710 (δ-lactone); *m/e* (abundance %): 354 (M^+, 62·2), 283 (17·7), 282 (9), 195 (15·5), 194 (100), 134 (17·7), 133 (17·7), 131 (22·2), 123 (28·8), 122 (9·6).

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