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ECHITOSERPIDINE: A NEW ALKALOID OF THE FRUITS OF *ALSTONIA VENENATA*

PRIYA L. MAJUMDER and BISWANATH N. DINDA

Department of Pure Chemistry, University College of Science, Calcutta-9, India

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Key Word Index—*Alstonia venenata*; Apocynaceae; indole alkaloid; echitoserpidine.

Abstract—The structure of echitoserpidine, a new alkaloid of the fruits of *Alstonia venenata* has been established as (**1**) on the basis of spectral and chemical evidence.

INTRODUCTION

THE STRUCTURE elucidation of 10 new indole alkaloids, besides kopsinine, reserpine and a monoterpenoid pyridine base, venoterpine, isolated from different parts of *Alstonia venenata* R.Br. (syn. *Echites venenata* Roxb.) were reported[1–7] previously. These alkaloids represent three distinct structural patterns, typified[8] by yohimbine, aspidofractinine and vincadifformine. The fruits of this plant are unique in that they were found to elaborate only the latter type of alkaloid. Our continued search for alkaloidal principles from the same source has now resulted in the isolation of one more new alkaloid, designated as echitoserpidine. The present communication discusses the isolation and structure determination of this alkaloid.

RESULTS AND DISCUSSIONS

Echitoserpidine, $C_{30}H_{32}N_2O_7$ (M^+ 532), m.p. 110°, $[\alpha]_D$ −427° ($CHCl_3$) was isolated in poor yield from the petrol extract of the fruits of *A. venenata*. The UV spectrum of the alkaloid, λ_{max}^{EtOH} 220, 295 and 326 (log ϵ, 4·43, 4·23 and 4·20) nm is unique and finds no analogy among any of the known indole alkaloid systems. But the alkaloidal colour reactions and the high specific rotation of the base suggest the presence of a β-anilinomethacrylate chromophore as is present in vincadifformine[8,9] or akuammicine.[8,9] This is further substantiated by the IR spectrum of the alkaloid which shows peaks at ν3300(NH), 1670 and 1600 (Ph–N–C=C–CO_2R) cm^{-1}. The spectrum also reveals the presence of an additional ester carbonyl function (1700 cm^{-1}) and a methylenedioxy group (1360, 1250, 1040, 927

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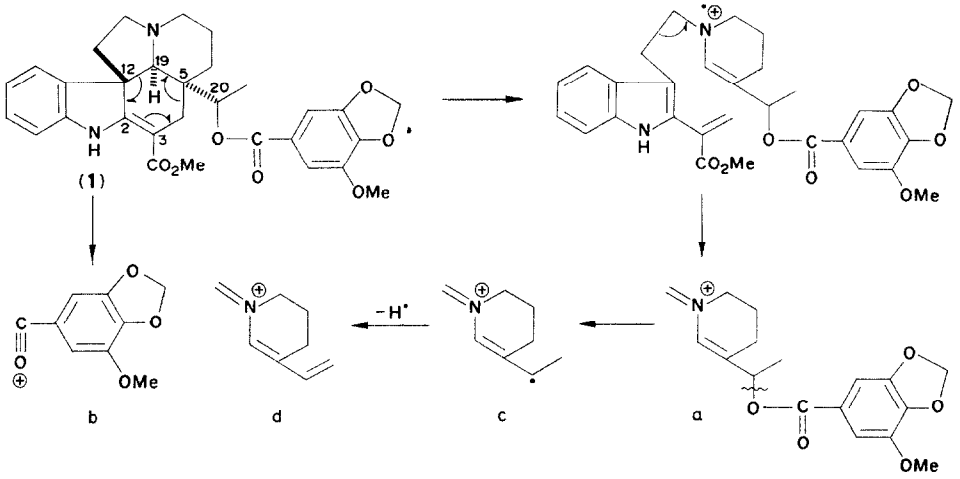
and 724 cm^{-1}). The 60 M Hz PMR spectrum of the alkaloid displays signals which not only corroborate the presence of the β-anilinomethacrylate chromophore but also indicate the existence of an additional benzene ring associated with an ester function in the system, $\geq C{=}CH(O{-}CO{-}Ar){-}Me$.



SCHEME 1. MASS FRAGMENTATION OF ECHITOSERPIDINE.

The precise nature of the structural pattern of echitoserpidine was largely elicited from the MS of the alkaloid. Besides the molecular ion peak at *m/e* 532, the spectrum shows significant peaks at *m/e* 337 (*M*-195), 336 (*M*-196), 318 (*a*, base peak), 179(*b*), 123(*c*) and 122(*d*)which can be best rationalized(Scheme 1)in terms of a vincadifformine-like[10] structure (**1**) for the alkaloid with a substituent of composition $C_9H_7O_5$ (195 m u) on the ethyl side chain. Moreover, the appearance of the peak at *m/e* 179 [$C_9H_7O_4$ (195-16)] suggest that this substituent is probably present as an ester function and is located at C-20 position as indicated by the PMR spectrum of the alkaloid. This and the foregoing evidence also suggest that the above substituent is in all probability a myristicinyloxy group similar to that present in veneserpine,[6] a bark alkaloid of *A. venenata*.

In conformity with the above observations, echitoserpidine on sealed-tube acid-catalyzed hydrolysis, a well-documented reaction[11] for alkaloids of the vincadifformine or akuammicine type, afforded, besides an indolenine base, $C_{19}H_{24}N_2O$ (M^+ 296), m.p. 180°, λ_{max}^{EtOH} 223 and 265 (log ε, 4·35 and 3·71) nm, an acid, $C_9H_8O_5$ (M^+ 196), m.p. 208° (Scheme 2). The acid showed a positive Labat test for the methylenedioxy group and was finally identified as myristicinic acid (**2**). Reduction of the indolenine base with $NaBH_4$ furnished an indolic compound, $C_{19}H_{26}N_2O$ (M^+ 298), λ_{max}^{EtOH} 229, 284 and 290 (log ε, 4·42, 3·80 and 3·79) nm. The structure of the latter was established as (**4**) and consequently that of the indolenine base as (**3**) on the basis of their mass and other spectral data and also from their derivation from echitovenidine,[4] the C-20 β,β-dimethyl-acryloxy analogue of (**1**), under identical reaction conditions.

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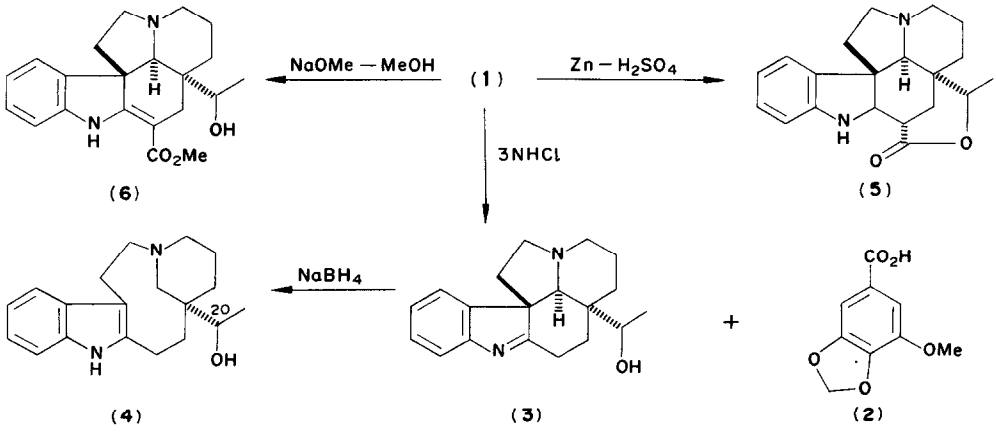

SCHEME 2. TRANSFORMATION REACTIONS OF ECHITOSERPIDINE.

Further confirmation of structure (**1**) for echitoserpidine has been secured from the following chemical correlations with echitovenidine (Scheme 2). Thus, treatment of echitoserpidine with Zn and 10% methanolic H_2SO_4 followed by heating the reaction mixture with 2 N H_2SO_4 acid gave, besides myristicinic acid, another compound, $C_{20}H_{24}N_2O_2$ (M^+ 324) which from its UV, IR and MS data corresponds to the δ-lactone (**5**). This is also obtainable from echitovenidine following the same sequence of reactions as above. The facile formation of this δ-lactone is a clear indication of the attachment of the myristicinyloxy group at C-20 position of echitoserpidine. Moreover, echitoserpidine on methanolysis with sodium methoxide in dry methanol afforded a neutral and a basic compound. The neutral component on further alkaline hydrolysis gave myristicinic acid. The basic compound, $C_{21}H_{26}N_2O_3$ (M^+ 354), m.p. 134°, $[\alpha]_D$ −570° (EtOH), shows typical vincadifformine-type mass fragmentation, the piperidine-bearing peak of (**1**) at *m/e* 318(a) now appearing at *m/e* 140. These observations, thus, strongly suggest structure (**6**) for the basic compound which was finally confirmed by establishing its identity with (−)-minovincinine obtained from echitovenidine under similar reaction conditions.

The unusual UV absorption of echitoserpidine is now explicable as being modified by the myristicinyloxy group since the UV spectrum of (**6**), λ_{max}^{EtOH} 225, 298 and 328 (log ϵ, 4·01, 4·00 and 4·06) nm and the differential UV spectrum of (**1**) with myristicinic acid, λ_{max}^{EtOH} 231, 301 and 329 (log ϵ, 4·10, 4·20 and 4·21) nm are typical of the vincadifformine group of alkaloids. The stereochemistry assigned to (**1**) follows from that of (**6**) and also from the positive specific rotation[12] of the quebrachamine derivative (**4**).

EXPERIMENTAL

M.ps were determined on a Köfler block and are uncorrected. Brockmann alumina was used for column chromatography and silica gel G for TLC. UV spectra were measured using 95% aldehyde-free EtOH and IR spectra were run in Nujol mulls unless otherwise stated. Anhyd. Na_2SO_4 was used for drying solvents. Petrol. used has the b.p. 60–80°.

Isolation of echitoserpidine (**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 exhaustively extracted

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with C_6H_6 and the C_6H_6 extract was washed with NH_4OH, then with H_2O, dried, concentrated and chromatographed. Petrol.–C_6H_6 (1:1) eluate on evaporation gave an oily residue, which on repeated chromatography afforded echitoserpidine, (yield, 0·0035%) crystallised from MeOH, m.p. 110°, $[\alpha]_D$ −427° ($CHCl_3$), produced blue colouration with ceric ammonium sulphate reagent (Found: C, 67·79; H, 5·92; N, 5·19. $C_{30}H_{32}N_2O_7$ requires: C, 67·67; H, 6·01; N, 5·26%). PMR (60 M Hz, $CDCl_3$): δ, 9·0 (1 H, *bs* disappeared on deuteration: >N<u>H</u>), 6·61–7·29 (6 H, *m*, Ar–<u>H</u>), 6·01 (2 H, *s*, =O–C<u>H</u>$_2$–O–), 4·93 [1 H, *q*, *J* 6·5 Hz: >C–C<u>H</u>(O·COAr)Me], 3·90 (3 H, *s*, Ar–OC<u>H</u>$_3$), 3·45 (3 H, *s*, shielded –$CO_2C\underline{H}_3$) and 1·04 [3 H, *d*, *J* 6·5 Hz, –CH(OCOAr)C<u>H</u>$_3$]. MS: *m/e* (%) 532 (M^+, 56), 531 (M-1, 20), 338 (12), 337 (49), 336 (14), 318 (100), 196 (11), 179 (35), 124 (20), 123 (9) and 122 (18).

Sealed-tube acid-catalyzed hydrolysis of echitoserpidine (**1**) *and isolation of myristicinic acid* (**2**) *and the indolenine* (**3**). A solution of echitoserpidine (0·35 g) in 3(N) HCl (25 ml) was heated in an evacuated sealed-tube in a glycerine bath at 110° for 6·5 hr. The seal was broken and the reaction mixture was extracted with Et_2O. The Et_2O layer was washed with H_2O, dried and evaporated when a solid residue was obtained which on repeated crystallizations from MeOH gave myristicinic acid (0·025 g), m.p. 208°, identical (superimposable IR and CO-TLC) with a synthetic sample prepared by the action of bromochloromethane[13] on gallic acid, followed by methylation with Me_2SO_4 and alkali. The aq. fraction was basified with NH_4OH, extracted with ether, dried, concentrated and chromatographed. The benzene eluate on evaporation gave a residue (0·12 g) which on repeated crystallization from petrol. (C_6H_6 (1:8) mixture afforded the indolenine (**3**) in fine needles, m.p. 180° (Found: C, 77·29; H, 7·99; N, 9·35. $C_{19}H_{24}N_2O$ requires: C, 77·02; H, 8·11; N, 9·46%). UV: λ_{max} 223 and 265 (log ϵ, 4·35 and 3·71) nm. IR: ν_{max} 3220 (–OH), 1610 (aromatic band) and 1585 (>C=N–) cm^{-1}.

Sodium borohydride reduction of the indolenine (**3**) *to 20-hydroxyquebrachamine* (**4**). The indolenine (**3**) (0·10 g) was reduced with excess of $NaBH_4$ (0·13 g) in 1 N methanolic KOH (25 ml) by refluxing over boiling H_2O bath for 3 hr. MeOH was then removed under reduced pressure and the residue was treated with H_2O, extracted with Et_2O, dried, concentrated and chromatographed. The C_6H_6–$CHCl_3$ (4:1) eluate on evaporation gave a solid (0·06 g). It was taken in MeOH (3 ml) and was treated with a methanolic soln of picric acid. Within a few min deep yellow crystals of 20-hydroxyquebrachamine picrate separated out. It was filtered and crystallized from MeOH, m.p. 208° (dec.) (Found: N, 13·11. $C_{25}H_{29}N_5O_8$ requires: N, 13·28%). A suspension of the picrate in H_2O was triturated with a strong soln of NaOH and the liberated base was extracted with Et_2O, washed thoroughly with H_2O, dried and the solvent removed when a white solid was obtained. Crystallization from MeOH gave (**4**) in fine plates, m.p. 112°, $[\alpha]_D$ + 145° ($CHCl_3$) (Found: C, 76·36; H, 8·35; N, 9·45. $C_{19}H_{26}N_2O$ requires: C, 76·50; H, 8·73; N, 9·40%). UV: λ_{max} 229, 284 and 290 (log ϵ, 4·42, 3·80 and 3·79) nm. IR: ν_{max} 3400(NH), 3230(OH) and 1602 (aromatic band). Treatment of echitovenidine in the same manner as above gave the same indolenine (**3**) and the indolic base (**4**).

Reduction of echitoserpidine with zinc and methanolic sulphuric acid and isolation of the δ-lactone (**5**). A solution of echitoserpidine (0·10 g) in 10% methanolic H_2SO_4 (15 ml) was reduced with an excess of Zn dust (0·30 g) by refluxing over boiling H_2O bath for 8 hr. Unreacted Zn was filtered off and MeOH from the filtrate was removed under reduced pressure. The residue was diluted with H_2O, made 2 N with respect to H_2SO_4 and refluxed for 10 hr. The soln was extracted with Et_2O, dried and evaporated. The residue on crystallization from MeOH gave myristicinic acid (0·004 g). The aq. acid soln was neutralized with NH_4OH, extracted with Et_2O, dried, concentrated and chromatographed. The petrol.–EtOAc (3:1) eluate gave a white solid which on crystallization from petrol. EtOAc (2:1) mixture gave needle-shaped crystals of the lactone (**5**), m.p. 153°. The same lactone (**5**) was obtained from echitovenidine following the above sequence of reactions (Found: C, 74·28; H, 7·47; N, 8·52. $C_{20}H_{24}N_2O_2$ requires: C, 74·08; H, 7·41; N, 8·64%). UV: λ_{max} 242 and 300 (log ϵ, 3·71 and 3·46) nm. IR: ν_{max} 3250 (NH), 1710 (δ-lactone) cm^{-1}.

Methanolysis of echitoserpidine (**1**) *and isolation of* (−)*-minovincinine* (**6**). Na (0·06 g) was added protion-wise to dry MeOH (20 ml) when the metal gradually dissolved to give a clear soln. Echitoserpidine (0·10 g) was then added to this soln and the mixture was heated under reflux for 8 hr in N_2. MeOH was removed from the reaction mixture under reduced pressure. The residue was treated with H_2O (30 ml) acidified with (1:1) aq. HCl and extracted with Et_2O. The ether extract on evaporation gave an oily residue which was refluxed with 5% methanolic KOH (10 ml) for 2 hr. MeOH was removed under reduced pressure, the soln was acidified with HCl, extracted with Et_2O, dried and the solvent removed. The residue was crystallized from MeOH to give myristicinic acid (0·01 g). The aq. acid soln containing the basic part was neutralized with NH_4OH, extracted with $CHCl_3$, dried, concentrated and chromatographed. The C_6H_6–$CHCl_3$ (3:1) eluate on evaporation gave (−)-minovincinine (0·03 g), m.p. 134° (ether), $[\alpha]_D$ −570° (EtOH) which was identical with that obtained from echitovenidine under identical reaction conditions (Found: C, 71·35; H, 7·25; N, 7·78. $C_{21}H_{26}H_2O_3$ requires: C, 71·19; H, 7·34; N, 7·91%).

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