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STRUCTURE OF AURICULARINE, A BIS-INDOLE ALKALOID FROM *HEDYOTIS AURICULARIA*

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Key Word Index—*Hedyotis auricularia*; Rubiaceae; bis-indole alkaloid; auricularine.

Abstract—Auricularine, an alkaloid isolated from *Hedyotis auricularia* has been assigned structure **1** by spectroscopic methods.

The structure of a novel group of bis-indole alkaloids, borreverine [1,2] and isoborreverine have recently been established by X-ray crystallography and high resolution mass spectrometry. In the present communication we report the chemistry of yet another alkaloid, auricularine (**1**), belonging to the same group.

Isolation of auricularine was first reported by Ratnagiriswaran and Venkatachalam [3] who assigned it the molecular formula $C_{42}H_{55}ON_5$. The product we obtained by the same procedure of earlier workers was a mixture of four alkaloids from which auricularine was isolated by preparative-TLC in low yield (0.00005%, major spot). Pure auricularine, mp 196–200° (decomp.); $[\alpha]_D$ 41 ± 1°, ($c = 0.027$, $CHCl_3$); γ_{max}^{KBr} 3400 cm^{-1}, NH; λ_{max}^{EtOH} 228 (log ε: 4.56); 251 (3.99); 285 (4.05) and 293 (4.05) nm, is assigned the correct molecular formula $C_{33}H_{42}N_4$; M^+: 494.3413 and was found to be sensitive to reagents. From a solution of the alkaloid in 3 M HCl kept overnight recovery was only 50% of the pure alkaloid, the rest accounted for by other spots. With $CDCl_3$ and $CHCl_3$ the same phenomenon was observed and the NMR spectrum was recorded soon after preparation of the solution and the compound recovered immediately.

The functionality of auricularine was revealed by the NMR spectrum. The notable features were the presence of two C-Me groups at δ0.32 (3H, *s*) and 0.92 (3H, *s*), a vinylic methyl at 1.72 (3H, *s*), a diMe amino group at 2.58 (6H, *s*) and a tertiary N-Me at 2.60 (3H, *s*). There was a broad absorption signal at 4.23 (1H) which was easily exchanged with D_2O. The presence of a single olefinic proton was observed at 5.55. From 6.2 to 7.6 there were eight aromatic protons. These spectroscopic data were strikingly similar to those reported for borreverine. Auricularine differs from borreverine in its molecular formula by CH_7 which is accounted by the presence of an extra N-diMe group in the form of a N-Me amino group. The D_2O-exchangeable proton present in borreverine at 1.42 is absent in auricularine.

Further confirmatory evidence was obtained by analysis of the high resolution mass spectrum and comparison with that of borreverine. The most important fragments of auricularine are *m/e* 494.3413 (M^+)†, 436.2769 ($C_{30}H_{34}N_3$), 264.1758 ($C_{19}H_{22}N$), 197.1182 ($C_{14}H_{15}N$), 172.1000 ($C_{11}H_{12}N_2$) and 58.0665 (C_3H_8N; 100%). The base peak 58.0665 (C_3H_8N) results from the loss of $CH_2{=}N(Me)_2$ by α-cleavage, resulting in the formation of the fragment $C_{30}H_{34}N_3$ (*m/e* 436) which is identical with the M − 44 peak of borreverine. Thereafter the fragmentation follows the same pattern as in borreverine which is illustrated in Scheme 1.

As expected auricularine gave a positive Otto colour test and a negative Adamkiewicz reaction.

EXPERIMENTAL

All mps are uncorr. Chemical shifts are expressed in δ ppm.

Isolation of auricularine. H. auricularia L. was collected in July and August, when in flower from the Trichur district, Kerala, India and immediately air-dried. Extraction was carried out within 1–2 weeks of collection. Storage for more than 1 month was found to reduce the alkaloidal content.

Dried and coarsely powdered plant (25 kg) was exhaustively extracted with 90% EtOH. The EtOH was removed under red. pres. and the residue (500 g) treated with C_6H_6 to remove lipids. The C_6H_6-insoluble residue was repeatedly shaken with 2% H_2SO_4 until the acid extract ceased to give an alkaloid reaction. The C_6H_6-soluble portion was treated in the same way, the acidic portions combined and then extracted with Et_2O to remove non-basic material. The aq. acid soln was neutralised with 5% Na_2CO_3, taking care that the soln remained distinctly acid to litmus. Excess strong NH_3 was then added and the soln repeatedly extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and dried (Na_2SO_4). A brownish yellow residue (15 g) was obtained on removal of the $CHCl_3$. This was dissolved in 10% aq. HOAc (100 ml) and a 50% soln of KNO_3 (10–12 ml) gradually added until the precipitation of a tarry material was complete and was set aside overnight in a refrigerator and filtered. To the filtrate, excess NH_3 was added and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and dried (Na_2SO_4). The residue obtained after removal of $CHCl_3$ was extracted with C_6H_6 in a Soxhlet. The C_6H_6 soln was concd and chromatographed over basic Al_2O_3 (BDH). The C_6H_6–$CHCl_3$ and $CHCl_3$ eluates upon concn gave a gummy material which was subjected to prep. TLC on Si gel using $CHCl_3$–MeOH (5:1) and the material obtained was

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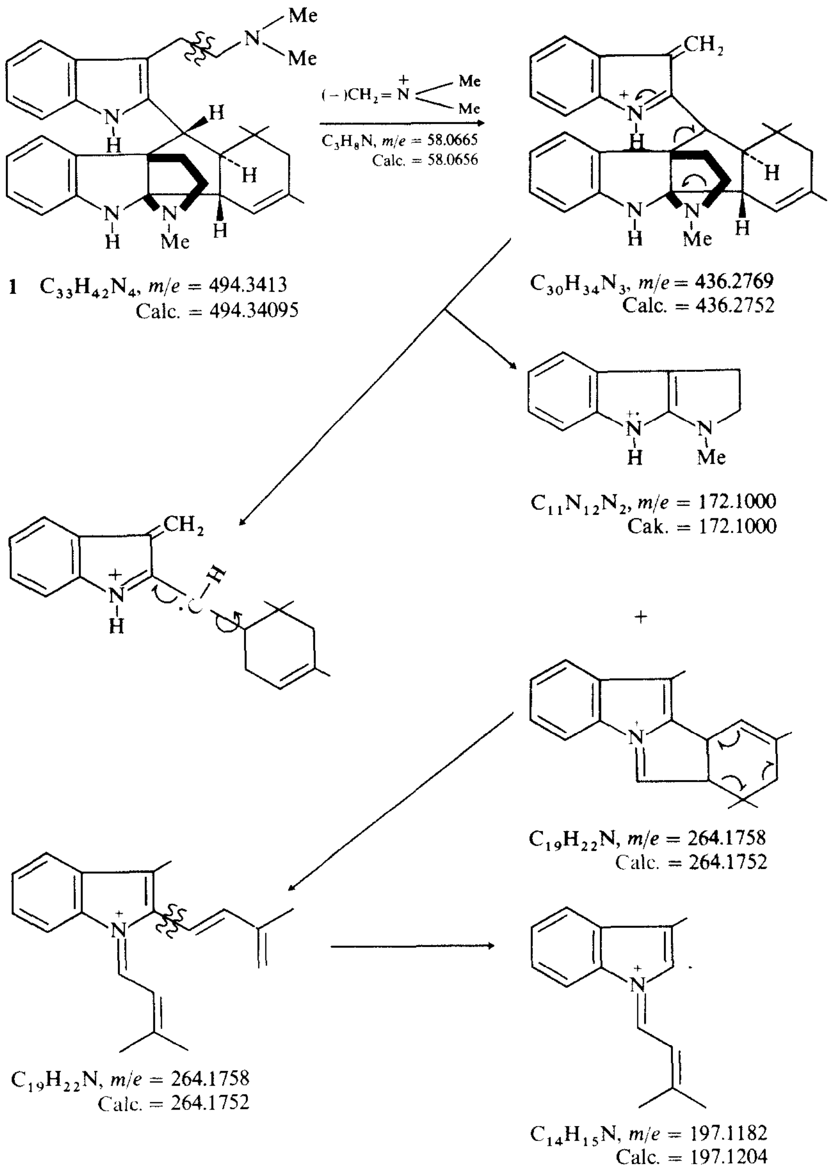
† Analysed by D.S.50 MS data system.



Scheme 1.

crystallized from hexane–Et_2O (12.5 mg) to yield auricularine, mp 196–200° (decomp). (Found: C, 78.63; H, 8.58; N, 11.19. $C_{33}H_{42}N_4{}^1/2H_2O$ requires: C, 78.72; H, 8.55; N, 11.13%); Found: M^+, 494.3413, $C_{33}H_{42}N_4$ requires: 494.34095. The extraction was carried out 4 times to obtain 50 mg of alkaloid.

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