Indian Journal of Chemistry
Vol. 41B, December 2002, pp. 2698-2700

Note

19-Epialstonine from *Amphicome emodi* roots

Biswanath Dinda* & Utpal Chandra De

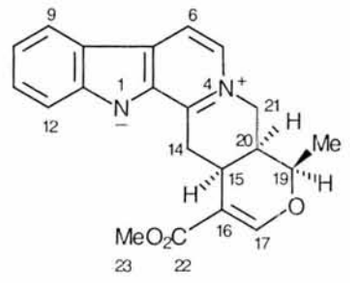
Department of Chemistry, Tripura University, Agartala 799 130, India

Email: dindabtu@rediffmail.com Fax: 0381-37-4801

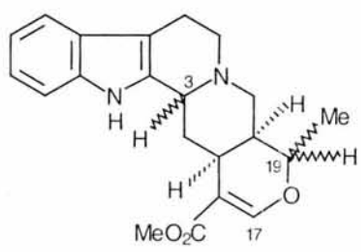
Received 13 June 2001; accepted (revised) 23 November 2001

A new indole alkaloid, 19-epialstonine has been isolated from the ethyl acetate extract of the roots of *Amphicome emodi*. The structure of the alkaloid has been established on the basis of spectroscopic (including 2D NMR experiments) and chemical studies.

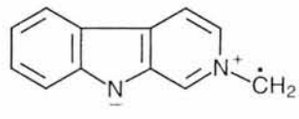
Amphicome emodi Royle ex Lindl syn *Incarvillea emodi* (Royle ex Lindl) Chatterjee (local name : Kaur in Kashmir) has been used for a long time in traditional Indian medicine as febrifuge[1]. In Northern India it is used as a substitute of *Swertia chirata*. Although earlier investigation on roots and leaves of the plant reported[2] the presence of unidentified amorphous alkaloid, the investigation by Kapoor *et al.*[3] reported the presence of mannitol, cinnamic acid, vanillic acid, androsin and amphicoside (iridoid glycoside). We reinvestigated the roots of this plant and isolated an alkaloid designated as 19-epialstonine **1** from the ethyl acetate extract. The present note deals with the results of isolation and characterization of the alkaloid.



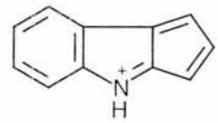
1



2 C-3 β-H; C-19 α-Me, β-H
3 C-3 α-H; C-19 β-Me, α-H
4 C-3 β-H; C-19 β-Me, α-H
5 C-3 α-H; C-19 α-Me, β-H



m/z 182



m/z 154

Results and Discussion

The alkaloid **1**, $C_{21}H_{20}N_2O_3$ (M^+ 348.401), mp 245° (dec) (MeOH), $[\alpha]_D^{21}$ +195.3° (c, 0.38, MeOH) was obtained in brownish granular crystals. It showed positive test with Dragendorff's reagent and in UV spectrum in MeOH it showed absorptions at λ_{max} 250 (log ε, 3.32), 310(3.08) and 370(2.50) nm, characteristic of indole alkaloids[4]. Its IR spectrum in KBr pellet showed absorption bands for >NH (3400 cm^{-1}), >C=N⁺= (1630 cm^{-1}) and β-alkoxy acrylic ester, -C(CO_2Me) =CH-O- (1695, 1610, 1240 and 1185 cm^{-1}) functions[5]. The 400 MHz ^{1}H NMR spectrum of the compound in CD_3OD gave much information about the structure by displaying signals at δ 1.36(3H, d, *J*=6.5 Hz, C-19 Me), 2.65(1H, m, H-20), 3.03(1H, dd, *J*=12.0 and 9.0 Hz, H_a-14), 3.30(1H, m, H-15), 3.84(3H, s, CO_2Me-16), 4.56(1H, dd, *J*=12.0 and 9.0 Hz; H_a-21), 4.61(1H, dd, *J*=12.0 and 5.5 Hz, H_e-14), 4.78(1H, octet, *J*=6.5 and 4.0 Hz, H-19), 4.91(1H, dd, *J*=12.0 and 5.5 Hz, H_e-21), 7.42(1H, t, *J*=7.5 Hz, H-10), 7.71(1H, s, H-17), 7.72(1H, d, *J*=7.5 Hz, H-12), 7.76(1H, t, *J*=7.5 Hz, H-11), 8.26(1H, d, *J*=7.5 Hz, H-9), 8.32(1H, d, *J*=7.5 Hz, H-5), 8.42(1H, d, *J*=7.5 Hz, H-6) and corroborated the structure **1** for the compound. The assignment of the protons was made by decoupling, DEPT, 2D ^{1}H-^{1}H (COSY) and 2D ^{13}C-^{1}H (HMQC) and HMBC NMR experiments (**Table I**). The high chemical shift value of C-19 methyl suggested the *cis* D/E ring junction of the alkaloid[6]. The positive ion FAB MS of the compound also corroborated this structure by recording a base peak at m/z 349 $[M+H]^+$ along with other significant mass peaks at m/z 333$[M-15]^+$ (3%), 307(7), 289(6), 182(3), 154(21) and 136(14). The signals in the ^{13}C NMR spectrum at δ_c 25.94 (C-15) and 38.43

Table I—^{13}C NMR and HMBC spectral data for **1** (in CD_3OD, 100 MHz)

Carbon	δ_c	HMBC	Carbon	δ_c	HMBC
2	132.4(s)	H-6, H-14, H-21	**14**	31.9 (t)	H-15, H-21
3	141.0(s)	H-5, H-14, H-21	**15**	25.9 (d)	H-14, H-20, H-21
5	145.2(d)	H-6, H-21	**16**	107.2 (s)	H-15, H-17, H-19
6	116.9(d)	H-5, H-9	**17**	156.3 (d)	H-19
7	107.2(s)	H-5, H-9	**19**	73.0 (d)	H-21, H-24
8	121.2(s)	H-9, H-10	**20**	38.4 (d)	H-15, H-21, H-24
9	123.2(d)	H-10, H-6	**21**	57.6 (t)	H-14, H-20, H-24
10	124.0(d)	H-9, H-11, H-12	**22**	168.4 (s)	H-17, H-23
11	132.9(d)	H-9, H-10, H-12	**23**	52.0 (q)	
12	114.0(d)	H-10, H-11	**24**	14.4 (q)	H-20, H-21
13	135.6(s)	H-11, H-12			

(C-20) were very similar to that of akuammigine **2** having *cis* D/E ring junction (values are 25.7 and 37.2 ppm, respectively)[7].

Reduction of the alkaloid with methanolic sodium borohydride afforded two tetrahydroderivatives – 19-epitetrahydroalstonine **3**, $C_{21}H_{24}N_2O_3$ (M^+ 352.432), mp 143° ($CHCl_3$) and 19-epiakuammigine **4**, $C_{21}H_{24}N_2O_3$ (M^+ 352.432), amorph. (MeOH-$CHCl_3$). Therefore, the structure of the parent alkaloid was assigned as 19-epialstonine **1**. This is the first report of indole alkaloid from the family, Bignoniaceae. The X-ray crystallographic and biological study of this alkaloid will be carried out later on.

Experimental Section

General. All solvents were distilled before use. Silica gels (Merck) were used for column and thin layer chromatography. UV, IR, MS and NMR spectra were recorded on spectronic 21D, Perkin-Elmer 577, Jeol SX 102 and Bruker AM 400 spectrometers, respectively. Optical rotations were recorded on a Perkin-Elmer 141 polarimeter.

Plant material. The roots of *Amphicome emodi* were supplied by M/S United Chemical and Allied Products, Calcutta in October 1998. Herbarium sample was preserved in Shibpur Botanical Garden, Howrah, Calcutta, India.

Extraction and isolation. Air-dried roots (250g) of *A. emodi* were extracted with EtOAc in a Soxhlet apparatus for 48 hr. The extract was concentrated under reduced pressure to a semi-solid residue (4.5g) and was subjected to CC over silica gel (60-120 mesh). Elution of the column with CH_2Cl_2-MeOH (9:1) afforded a brownish solid which on repeated CC over silica gel gave brownish granular crystals of **1** (yield 0.04g, 1.6×10^{-2} %), mp 245° (dec.) (MeOH), (R_f 0.53 in $CHCl_3$-MeOH; 4:1 mixture on silica gel G). Anal. Found: C, 71.32; H, 5.76; N, 7.90. Calc. for $C_{21}H_{20}N_2O_3$: C, 72.40; H, 5.78; N, 8.04 %. ^{13}C NMR HMBC and HMQC data (see **Table I**).

Borohydride reduction of 1. Compound **1** (15mg) was dissolved in 3 mL of MeOH in an Erlenmeyer flask. To it $NaBH_4$ (30 mg) was added and the mixture was stirred at 30-40° for 4 hr. The solvent from the mixture was removed under reduced pressure, the residue was diluted with H_2O (10 mL) and extracted with $CHCl_3$ (10 mL × 3). The $CHCl_3$ extract was dried over anhydrous Na_2SO_4, concentrated and subjected to CC over silica gel. C_6H_6-$CHCl_3$ (1:1) eluate on removal of solvent gave pale yellowish granular crystals of **3** (7 mg). Anal. Found: C, 71.50; H, 6.78; N, 7.88. Calc. for $C_{21}H_{24}N_2O_3$: C, 71.57; H, 6.86; N, 7.95%. UV (MeOH): 228(log ε, 3.80), 250 sh(3.22) and 292(2.24) nm; IR (KBr): 3400(NH), 1700(C=O), 1630(C=C) cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$): δ 1.48(3H, d, *J*=7.0 Hz, C-19-Me), 3.49(1H, m, H-3), 3.74(3H, s, MeO_2C-16), 4.43(1H, octet, H-19), 7.05-7.45(4H, m, H-9-12), 7.52(1H, s, H-17), 7.93(1H, br s, exchangeable with D_2O, HN-1). $CHCl_3$-MeOH (9.5:0.5) eluate gave colourless amorphous solid of **4** (3mg), HR EI-MS *m/z* (rel. int.): 352.4320(100%), ($C_{21}H_{24}N_2O_3$, calculated 352.4322), ^1H NMR (300 MHz, $CDCl_3$): δ 1.38(3H, d, *J*=6.5 Hz, Me-C-19), 3.13 (1H, m, H-3), 3.55 (3H, s, MeO_2C-16), 3.96 (1H, m, H-19), 7.05-7.48(4H, m, H-9-12), 7.61(1H, s, H-17), 7.90(1H, br s, exchangeable with D_2O, HN-1).

The ^{1}H NMR spectral data were similar to those reported for tetrahydroalstonine **5** and akuammigine **2** except for chemical shift values of H-19 and methyl C-19[8].

Acknowledgement

The authors thank the RSIC, CDRI, Lucknow for MS and elemental analysis. The authors are indebted to Prof. S R Jensen, Department of Organic Chemistry, Technical University of Denmark, Denmark for the measurement of 2D NMR and MS. One of the authors (U C D) is thankful to the CSIR, New Delhi, India for the award of a Senior Research Fellowship.

References

1 Chopra R N, Nayar S L & Chopra I C, *Glossary of Indian medicinal Plants*, (CSIR, New Delhi), **1956**, p 16.
2 Bhatnagar J K, Bhatla M S & Jolly S S, *Indian J Pharm*, 28, **1965**, 160.
3 Kapoor S K, Kohli J M & Zaman A, *Tetrahedron Lett*, **1971**, 2839.
4 Neuss N, *Physical data of indole and dihydroindole alkaloids*, **1962**.
5 Sainsbury M & Webb B, *Phytochemistry*, 11, **1972**, 2337.
6 Phillipson J D & Supavita N, *Phytochemistry*, 22, **1983**, 1809.
7 Wenkert E, Chang C-J, Chawla H P S, Cochran D W, Hagaman E W, King J C & Orito K, *J Am Chem Soc*, 98, **1976**, 3645.
8 Gutzwiller J, Pizzolato G & Uskokovie M, *J Am Chem Soc*, 93, **1971**, 5907.