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SCHOLARINE: AN INDOLE ALKALOID OF *ALSTONIA SCHOLARIS*

AVIJIT BANERJI and ARUP K. SIDDHANTA

Department of Pure Chemistry, University College of Science, 92, Acharya P. C. Road, Calcutta 700009, India

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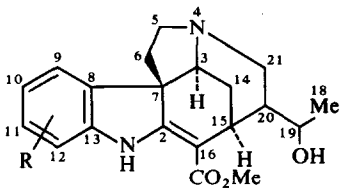
Key Word Index—*Alstonia scholaris*; Apocynaceae; indole alkaloids; scholarine.

Abstract—A new alkaloid designated scholarine was isolated from the leaves of *Alstonia scholaris*. It was (±) 12-methoxyechitamidine as determined by chemical and spectroscopic investigations.

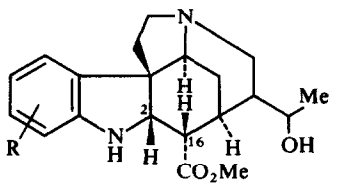
In continuation of our work [1, 2] on the alkaloid constituents of *Alstonia scholaris* R. Br. [3], we isolated another new indole alkaloid. The structure elucidation of this compound, designated scholarine, is described in the present paper.

The new alkaloid was obtained from the C_6H_6–$CHCl_3$ (1:1) eluate of the EtOH extract of the leaves of *Alstonia scholaris*. Scholarine, $C_{21}H_{26}N_2O_4$ (M^+ 370.1885, calc. 370.1892), mp 205–206° (dec.), $[\alpha]_D^{25}$ 0° ($CHCl_3$), exhibited a UV spectrum characteristic of a β-anilinomethacrylate moiety (λ_{max}^{EtOH} 234, 291, 335 nm; log ε 3.98, 3.70, 4.05; no acid shift of the absorption maxima with 1% $HClO_4$). The presence of the >C=C–COOMe grouping was also indicated by its IR spectrum (ν_{max}^{KBr} 1678, 1668 and 1607 cm^{-1}).

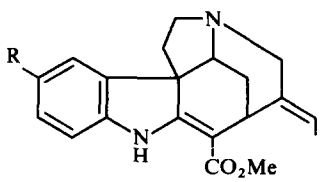
In addition to the M^+, the mass spectrum of scholarine showed prominent peaks at *m/e* 139 and 94 which are diagnostic of the akuammicine-type *Strychnos* alkaloids. A comparison of its mass spectrum with that of echitamidine (**1**) [4] shows their close similarity and reveals that scholarine is probably a methoxyechitamidine derivative (**2**). In agreement with this only three protons were discernible in the aromatic region as a multiplet in its 80 MHz ^1H NMR spectrum ($CDCl_3$). In addition, the ^1H NMR spectrum showed the presence of two OMe groups (3H, *s* each at δ 3.77 and 3.79) and an



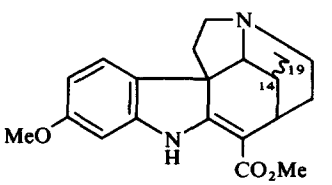
1 R = H, Echitamidine
2 R = OMe, Scholarine



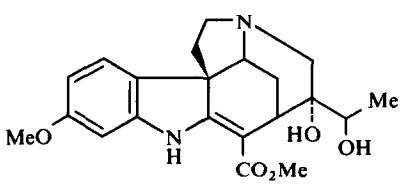
3 R = H
4 R = OMe



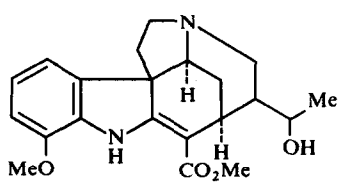
6 R = H, Akuammicine
7 R = OH, Sewarine



8 14,19-Dihydrocondylocarpine



9 Alstovine



10 Scholarine

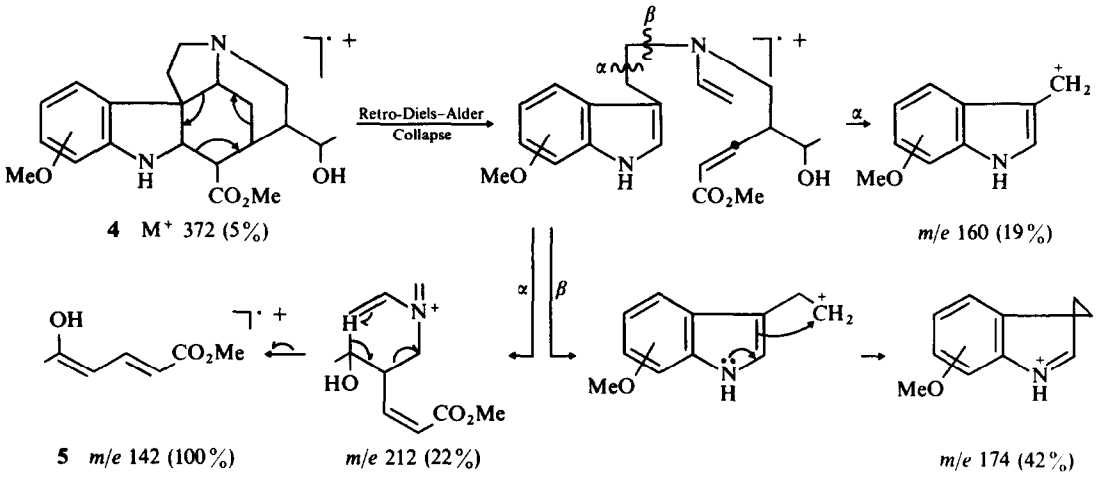
-NH- group (1H, s at δ 8.39, disappears on deuteration). A Me doublet appeared at δ 1.22 (J = 6.2 Hz) showing the presence of a >CH-Me moiety as in echitamidine (**1**). The methine proton of this grouping appeared at δ 4.62 as a multiplet. The above conclusions were supported by the 25.2 MHz ^{13}C NMR ($CDCl_3$) spectrum of scholarine. Three aromatic methine carbons (at 113.2, 122.5 and 111.2 ppm) and two OMe's (at 51.5 and 55.5 ppm) were discernible in the spectrum. The presence of -CH(OH)Me moiety was also confirmed by the appearance of an upfield Me at 20.3 ppm and a downfield methine carbon at 68.8 ppm.

That scholarine is an echitamidine derivative was finally confirmed by its reduction to the dihydro derivative with zinc–methanolic sulphuric acid. The amorphous dihydroscholarine exhibited absorption maxima at 230 and 291 nm in the UV spectrum characteristic of dihydroindoles. Its mass spectrum showed a M^+ at m/e 372 (corresponding to $C_{21}H_{28}N_2O_4$), and the fragmentation pattern was similar to those of 2,16-dihydroechitamidine (**3**) [4] and tetrahydroakuammicine [5, 6]. Dihydroscholarine (**4**) exhibited intense peaks at m/e 212, 174 and 160 corresponding to m/e 212, 144 and 130, respectively, from 2,16-dihydroechitamidine (**3**). The latter two fragments containing the indole moieties are shifted by 30 amu in **4** thereby showing the presence of a OMe group in the benzene ring. The base peak appeared at m/e 142 in **4**. The origin of this fragment, which has been assigned structure **5**, is shown in Scheme 1.

The 80 MHz ^{1}H NMR spectrum ($CDCl_3$) of dihydroscholarine showed the appearance of two new one-proton signals at δ 3.59 and 3.27 each as doublets, which have been attributed to C-2 H and C-16 H (J = 6.5 Hz). The magnitude of the coupling constant indicates the gauche-disposition of the C-2 and C-16 hydrogens. Hence the relative stereochemistry of the dihydro product is probably that shown in **4**.

The only point which remained to be settled was the placement of the OMe group in the aromatic nucleus. A comparison of the UV spectrum of scholarine (**2**) with those of akuamicine (**6**) [7], sewarine (**7**) [8] and 11-methoxy-14,19-dihydrocondylocarpine (**8**) [9] reveals that the OMe in **2** is not situated either at C-10 or C-11. This point was settled from an examination of the 25.2 MHz ^{13}C NMR spectrum ($CDCl_3$). The OMe group is placed at the C-12 position rather than at C-9 from a comparison of the chemical shifts of C-9, C-10, C-11, C-12 of scholarine with those of akuammicine (**6**) (E. Wenkert, personal communication) and alstovine (**9**) [10] (Table 1). The chemical shifts calculated on the basis of additivity relationships [11] agreed closely with those observed only if the OMe group was placed at C-12 (Table 1). Hence the structure of scholarine is (±)-12-methoxyechitamidine (**10**).

The relative stereochemistries at the chiral centres C-3, C-7 and C-15 follow from considerations of molecular geometry. The optical rotations of the alkaloids having the β-anilinoacrylate chromophore are known to be very high [12]. Since the observed rotation is 0°, the compound



Scheme 1. Mass spectral fragmentation of **4**.

Table 1. Comparison of ^{13}C NMR shifts of compounds **2**, **6**, and **9**

Carbon	**6**	**9**	**2**	Calculated values for **10**
C-9	120.3*	120.3	113.2	112.7
C-10	120.5*	105.9	122.5	121.3
C-11	127.3	159.9	111.2	112.8
C-12	109.1	97.2	144.7	140.3

* Values may be interchanged.

is presumably a racemic modification. To our knowledge, this is the second instance in which a racemic akuammicine-type alkaloid has been shown to occur in a plant, the first being the occurrence of pseudoakuammicine [(±)-akuammicine] in *Picralima nitida* [13].

EXPERIMENTAL

Plant material. Leaves of *A. scholaris* R.Br. were collected from the Indian Botanic Garden, Shibpore, Howrah. A voucher specimen (No. AS-L) has been preserved in our laboratory.

Isolation and properties of scholarine. Dried and powdered leaves (6 kg) were extracted with EtOH (25 l.) for 20 days in a percolator at room temp. The EtOH extract was concd to *ca* 1 l. A 500 ml portion of this extract was mixed with 5% aq. citric acid soln (500 ml) for 16 hr. The resulting mixture was filtered through a bed of Celite, the filtrate made alkaline with NH_4OH soln at 0° and extracted with $CHCl_3$ (3 × 1 l.). The $CHCl_3$ extract was washed with H_2O, dried (Na_2SO_4), concd and then chromatographed over Brockmann Al_2O_3. The C_6H_6–$CHCl_3$ (1:1) eluate afforded rhazine and scholarine in consecutive fractions. Rhazine was identified by comparison with an authentic sample (mmp, co-TLC, superimposable IR spectra) [1]. Scholarine was obtained from C_6N_6—$CHCl_3$ (1:1) as a brown amorphous solid (yield: 12 mg, 0.0004%), mp 205–206° (dec.). It produced the following colour reactions: 1% ceric ammonium sulphate in 50% phosphoric acid on TLC plate, deep blue changing to green to green-rimmed yellow to grey colour; concd HNO_3 on TLC plate, bright green colour which disappeared on standing. TLC of scholarine: Si gel G, MeOH, R_f 0.26; Al_2O_3, MeOH–EtOAc (1:9), R_f 0.32. MS *m/e* (rel. int.): 370.1885 (75), M^+ ($C_{21}H_{26}N_2O_4$: calc. 370.1892), 257 (33), 256 (32), 255 (100), 139 (30), 115 (25), 94 (45); IR (KBr) cm^{-1}: 3330 (>NH); 1678, 1668, 1607 (>C=CH–COOMe); 1170, 1115, 1070, 1035, 915, 775, 730, 700 (1,2,3-trisubstituted phenyl ring). ^1H NMR (80 MHz, $CDCl_3$): δ 8.39 (1H, *br. s*, disappears on deuteration, >NH), 6.71–6.91 (3H, *m*, C-9 H, C-10 H, C-11 H), 4.62 (1 H, *m*, C-19 H), 3.79 (3H, *s*, Ar-OCH_3), 3.77 (3H, *s*, >C=CH-CO_2CH_3), 1.22 (3H, *d*, J = 6.2 Hz, C-19 Me).

2,16-*Dihydroscholarine* (**4**). Zn dust (1 g) was added in small portions to a soln of scholarine (**2**) (7 mg) in 10% H_2SO_4 in MeOH (10 ml). The mixture was then refluxed under N_2 for 4 hr. The mixture was filtered, the residue washed with MeOH, and the combined filtrate and washings concd. It was then distributed between an ice-cold aq. $NaHCO_3$ and NH_3 soln and $CHCl_3$. The $CHCl_3$ extract was washed (H_2O), dried (Na_2SO_4) and evapd to give dihydroscholarine as a gummy mass (5 mg) which was then purified by prep. TLC on Al_2O_3 plates using MeOH–EtOAc (1:9). Dihydroscholarine was obtained as an amorphous solid. MS *m/e* (rel. int.): 372 (5), M^+, 212 (22), 174 (42), 160 (19), 142 (100). ^1H NMR (80 MHz, $CDCl_3$): δ 8.30 (1H, *br. s*, disappears on deuteration, indoline NH), 6.97–6.62 (3H, *m*, C-9 H, C-10 H, C-11 H), 4.21 (1H, *m*, C-19 H), 3.78 (3H, *s*, Ar-OCH_3), 3.75 (3H, *s*, CO_2CH_3), 3.59 (1H, *d*, J = 6.5 Hz, C-3 H), 3.27 (1H, *d* with fine splitting, J = 6.5 Hz, C-16 H), 1.17 (3H, *br. d*, C-19 Me).

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