Aspidospermidose: A New Dihydroindole Alkaloid from the Leaves of *Rhazya stricta*

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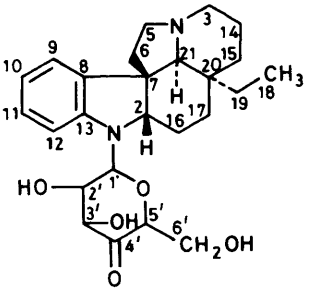
A new dihydroindole alkaloidal glycoside, aspidospermidose, has been isolated from the leaves of *Rhazya stricta*, and has been assigned structure (**1**) on the basis of spectral studies.

Rhazya stricta Decaisne (Apocynaceae) is a small, glabrous, erect shrub which is widely distributed in Western Asia and Pakistan. It has long been used for the treatment of various diseases;[1-4] the anti-cancer activity of some of its alkaloids has also been reported.[5-7] Continuing our investigations on the leaves of *R. stricta*, we report here the isolation and structure determination of a new dihydroindole alkaloid (**1**)

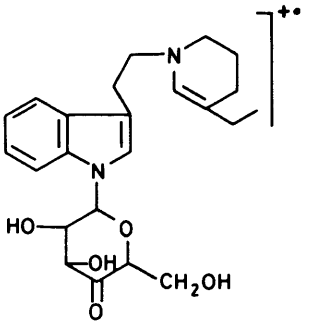


(**1**)

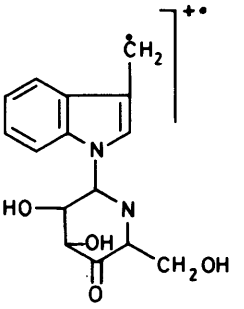
which bears a glycosidic linkage (with the sugar in an oxidised state on N_a). Alkaloidal glycosides with oxidised sugar units have not, to our knowledge, been previously reported in Nature. The structure of aspidospermidose has been elucidated by extensive n.m.r. studies, including 2D-n.m.r.,[8-10] nuclear Overhauser enhancement (n.O.e.) measurements, homodecoupling, and ^{13}C-n.m.r. [broad band and gated spin echo (GASPE)] experiments.

Results and Discussion

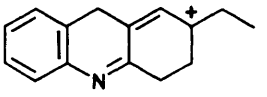
Aspidospermidose was isolated by separation on the basis of differential basicity of the crude alkaloids followed by preparative t.l.c. The u.v. spectrum of aspidospermidose was characteristic for the dihydroindole chromophore. The i.r. spectrum of the alkaloid showed the presence of a carbonyl function. The high resolution mass spectrum of the alkaloid showed the molecular ion peak at *m/z* 442.2444, corresponding to the molecular formula $C_{25}H_{34}N_2O_5$ and indicating ten double bond equivalents in the molecule. Other prominent peaks were found to occur at *m/z* 425, 414 (ion I), 290 (ion II), 281, 210 (ion III) and 124 (ion IV). The mass fragmentation pattern of the alkaloid was similar to that of aspidospermidine and related compounds.[11-14] The peak at *m/z* 281 ($C_{19}H_{25}N_2$) corresponded to the loss of 161 m.u. ($C_6H_9O_5$, a glycosidic unit) from the molecular ion while the highly oxygenated fragment at *m/z* 290 ($C_{15}H_{16}NO_5$) (ion II) suggested the attachment of the glycosidic linkage with the indole part of the molecule. When the compound was treated with D_2O and the mass spectrum re-recorded, M^+ was found to be shifted by 3 m.u. to *m/z* 445. The peaks at *m/z* 414 and 290 also shifted to *m/z* 417 and 293 respectively, thereby suggesting the presence of three exchangeable hydrogen atoms (OH groups?) in the molecule. The molecular ion was confirmed by mass spectrometry using f.a.b. and f.d. sources. Linked scan measurements of metastable transitions were carried out to establish ion fragmentation pathways. These showed that the ions at *m/z* 425, 414, 290, and 281 arose directly from the parent M^+ (442). The ion with *m/z* 290 was thus also shown to arise from the ions at *m/z* 442 and 414 but did not fragment to the ions at *m/z* 210 and 124 or other ions formed from the dihydroindole moiety; this suggests that *m/z* 290 is independent of the aspidosperma part of the molecule.



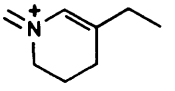
Ion I



Ion II



Ion III



Ion IV

The ^{1}H n.m.r. spectrum of aspidospermidose (300 MHz, $CDCl_3$), showed a 3 H triplet at δ 0.62 ($J_{18,19\alpha} = J_{18,19\beta} = 7.4$ Hz) whilst the 19α- and 19β-H signals appeared at δ 0.89 (m) and 1.54 (m) respectively. These results indicated the non-equivalence of the methylene protons of the C-ethyl group, a result of their prochiral nature hindrance; this phenomenon has been observed previously in Aspidosperma-type alkaloids.[15,16] A signal at δ 2.34 (br s) was assigned to 21-H, its upfield value suggesting α-stereochemistry.[16] The 2-H signal appeared at δ 3.84 (dd, $J_{2,16\beta} = 10.4$ Hz, $J_{2,16\alpha}$ 4.8 Hz), its upfield chemical shift suggesting β-stereochemistry.[14] The upfield chemical shift value of 12-H (δ 6.50) indicated the presence of an N_a-glycoside linkage. The presence of a $C_6H_9O_5$ sugar unit was evident from the 1′-H signal at δ 4.91 (br s), the chemical shift being consistent with this type of system. The coupling constant may be lowered due to the 1′-H bond angle being modified by interaction with the adjacent electron-pairs of the α-oxygen and nitrogen atoms.[17,18] Steric interactions with 16-H protons may also be responsible for modification in the conformation of the sugar ring, resulting in the virtual disappearance of the

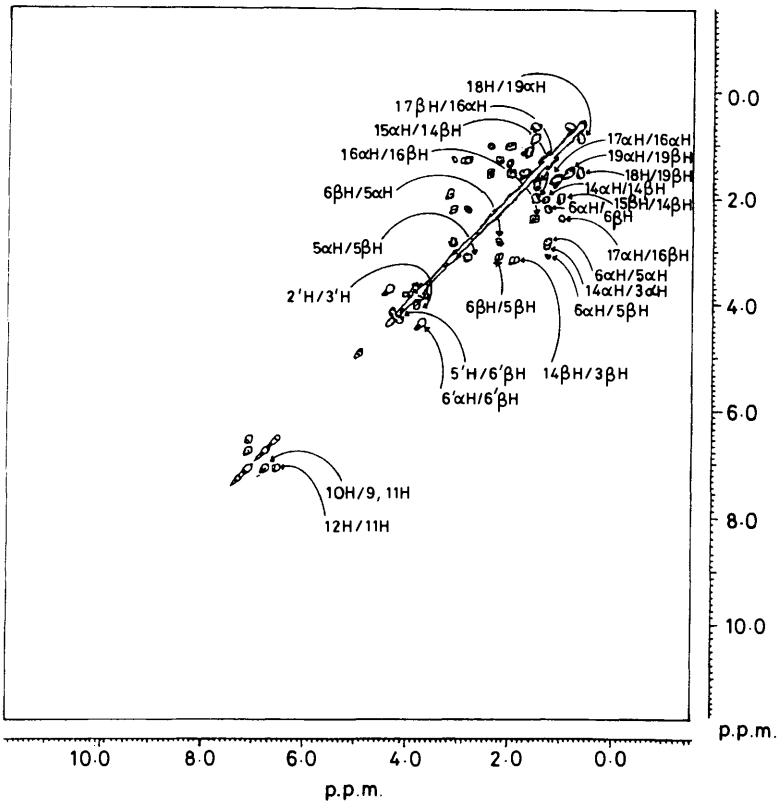


Figure 1. COSY-45 Spectrum of aspidospermidose. The *first* proton mentioned in each case is that lying directly *above* the cross peak.

vicinal coupling with 1-H. The 5'-H signal appeared at δ 3.94, the downfield chemical shift value reflecting the presence of the carbonyl function α-to this proton. The downfield chemical shift value for 2'-H (δ 3.71) and 3'-H (δ 3.78) are also due to the deshielding effect of the adjacent carbonyl group.

Two-dimensional n.m.r. measurements were carried out to verify the assignments. The coupling interactions were established through the co-related spectroscopy-45 (COSY-45) spectrum while the multiplicity of the overlapping proton signals was determined from the 2D-*J*-resolved spectrum.[8-10] The assignment for the 18-H protons at δ 0.62 could thus be confirmed by the COSY-45 spectrum, which showed strong cross peaks with the signals at δ 0.89 for 19α-H and at δ 1.54 for 19β-H. Both these protons exhibited geminal and vicinal couplings.[15] 5'-H Showed cross peaks with 6'-H but not with any other proton. This suggested that no protons were present at C-4'. The COSY-45 spectrum of aspidospermidose is presented in Figure 1 with the important interactions indicated.

The nuclear Overhauser enhancement spectroscopy (NOESY) spectrum served to establish the spatial proximities. The stereochemistry of 21-H at δ 2.34 could be confirmed from the NOESY spectrum since it showed a strong cross peak with the signal at δ 7.08 for 9-H. This could only arise if 21-H possessed α-stereochemistry, which would also result in the C(6)–C(7) bond having a β-configuration. The signal at δ 6.50 for 12-H showed a strong cross peak in the NOESY spectrum with that for 1'-H, and a weak cross peak with the signal at δ 3.71 for 2'-H. This served to establish the attachment of the sugar unit to N_a.

In order to confirm the relative stereochemistry at the various asymmetric centres, and to record subtle n.O.e. effects not visible in the NOESY spectrum, n.O.e. difference measurements were carried out (Table 1). Irradiation at δ 0.62 (18-H) resulted in 2.5% n.O.e. at δ 1.75 (15α-H) thus establishing that these

Table 1.

Proton irradiated (δ)	Proton enhanced (δ)	% N.O.e.
21-H (2.34)	19β-H (1.54)	2.9
	9-H (7.08)	6.9
19β-H (1.54)	18-H (0.62)	2.8
	21-H (2.34)	3.7
18-H (0.62)	15α-H (1.75)	2.5
17β-H (1.66)	15β-H (1.28)	2.3
16β-H (2.38)	14β-H (1.90)	3.6
15α-H (1.75)	18-H (0.62)	2.9
	3α-H (2.86)	1.8
15β-H (1.28)	17β-H (1.66)	1.7
14β-H (1.90)	16β-H (2.38)	3.2
	5β-H (2.79)	2.4
12-H (6.50)	1'-H (4.91)	6.7
	2'-H (3.71)	2.3
	11-H (7.06)	10.5
11-H (7.06)	10-H (6.76)	12.6
	12-H (6.50)	9.6
10-H (6.76)	9-H (7.08)	9.7
	11-H (7.06)	15.1
9-H (7.08)	6α-H (1.25)	6.2
	10-H (6.76)	10.6
	21-H (2.34)	7.6
6α-H (1.25)	9-H (7.08)	6.2
6β-H (2.21)	2-H (3.84)	3.7
5β-H (2.79)	14β-H (1.90)	3.1
3α-H (2.86)	15α-H (1.75)	2.4
2-H (3.84)	6β-H (2.21)	4.1
1'-H (4.9)	2'-H (3.71)	5.7
	12-H (6.50)	8.3
2'-H (3.71)	1'-H (4.91)	5.3
	12-H (6.50)	1.9
3'-H (3.78)	5'-H (3.94)	4.3
5'-H (3.94)	3'-H (3.78)	4.8

protons are close. Irradiation at δ 1.75 (15α-H) resulted in 2.9% n.O.e. at δ 0.62 (18-H) and 1.8% n.O.e. at δ 2.86 (3α-H). This served to establish the preferred conformation of ring D which causes 3α-H and 15α-H to lie close to each other. Irradiation at δ 1.90 (14β-H) resulted in 3.2% n.O.e. at δ 2.38 (16β-H) and 2.4% n.O.e. at δ 2.79 (5β-H). These interactions indicate the close proximities of 16β-H and 5β-H to 14β-H. Irradiation at δ 4.91 (1′-H) resulted in 8.3% n.O.e. at δ 6.50 (12-H) and 5.7% n.O.e. at δ 3.71 (2′-H). These strong n.O.e. interactions showed that 1′-H lies close to 12-H, and thus confirmed the presence of the glycosidic linkage on N_a. Corresponding interactions were also observed on irradiation at δ 6.50 (12-H) which resulted in 6.7% n.O.e. at δ 4.91 (1′-H). Irradiation at δ 7.08 (9-H) caused 10.6% n.O.e. at δ 6.76 (10-H) and 7.6% n.O.e. at δ 2.34 (21-H). The n.O.e. interaction between 9-H and 21-H is only possible when 21-H has α-stereochemistry. The n.O.e. difference measurements and 2D-NOESY spectrum thus served to establish the structure of aspidospermidose. The n.O.e. results are summarized in Table 1.

The ^{13}C-n.m.r. spectrum (75 MHz, $CDCl_3$, Table 2), showed the presence of 25 carbon atoms. The multiplicity assignments were made by carrying out GASPE experiments. The methyl carbon of the ethyl side chain resonated at δ 6.84 while the methylene carbon appeared at δ 29.32. The signal at δ 66.62 was assigned to C-21, its upfield value reflecting α-stereochemistry at this centre.[19] The C-3′ signal appeared at δ 80.41, its downfield chemical shift value reflecting the presence of carbonyl function α to this carbon whilst the C-1′ carbon resonated at δ 80.61, suggesting the presence of a *N*-glycoside linkage (rather than an *O*-glycosidic one) to heteroaromatic aglycone.[20] The C-12 signal at δ 105.56, shifted upfield from the normal value (*ca.* 110 p.p.m.), again reflected the presence of an N_a-glycoside. A weak signal at δ 205.80 was assigned to C-4′.[21]

Experimental

The u.v. spectrum was recorded on a Shimadzu UV-240 spectrophotometer; the i.r. spectrum was recorded on JASCO A-302 i.r. spectrophotometer; the mass spectra were recorded on Finnigan MAT-312 mass spectrometer connected to a PDP 11/34 (DEC) computer system. The ^{1}H n.m.r. spectra were recorded at 300 MHz, and the ^{13}C n.m.r. spectra at 75 MHz on a Bruker AM-300 n.m.r. spectrometer. Optical rotation was recorded on Polartronic Universal Australian standard K-157 digital polarimeter. T.l.c. experiments were performed on silica gel (GF-254, 0.2 mm) precoated plates (E-Merck). The plant material was collected from a small village near Karachi and was identified by Prof. S. I. Ali, at the Botany Department of Karachi University where a voucher specimen is deposited.

Isolation of Aspidospermidose.—The EtOH extract of the fresh leaves (67 kg) of *Rhazya stricta* was concentrated, acidified with 5% HCl, filtered, and basified with NH_3 (conc.) to *ca.* pH 9. The solution thus obtained was extracted with $CHCl_3$, dried (anhydrous Na_2SO_4), filtered, and evaporated to dryness (317 g). The crude alkaloidal extract thus obtained was dissolved in 10% acetic acid. The solution was subjected to gradient pH separation after stepwise basification with NH_3 (conc.). The fraction obtained at pH 6 was dried (Na_2SO_4) filtered, and evaporated to dryness (17 g). A portion (6.5 g) was subjected to preparative t.l.c. on silica gel (GF-254) pre-coated plates using light petroleum (40—60 °C)–acetone–diethylamine (6.0:3.5:0.5) as the solvent system to afford the alkaloid. This was further purified on silica gel plates with light petroleum (40—60 °C)–acetone–diethylamine (7.5:2.0:0.5) as the solvent system, to give a pure alkaloid (8 mg, 1.19 × 10^{-5}%), (R_F 0.15), $[\alpha]_D^{24}$ + 203° (*c* 24 mg/100 ml MeOH) which gave a pink colour reaction with ceric sulphate solution and a characteristic colour reaction with Dragendorff's reagent. $\lambda_{max.}$(MeOH) 211 (log ε 4.27), 250 (3.62), and 303 nm (3.47). $\lambda_{min.}$ 227 (4.02) and 272 (3.07); $\nu_{max.}$(KBr) 3 400—3 450 (OH), 1 740 (C=O), 1 605 (C=C), 1 050 (CO), and 750 cm^{-1} (Ar C-H); h.r.m.s. 442.2444 ($C_{25}H_{34}N_2O_5$, 6%), 425.2432 ($C_{25}H_{33}N_2O_4$, 1), 414.2154 ($C_{23}H_{30}N_2O_5$, 7), 290.1016 ($C_{15}H_{16}NO_5$, 9), 281.1978 ($C_{19}H_{25}N_2$, 4), 210.1184 ($C_{15}H_{16}N$, 1), and 124.1129 ($C_8H_{14}N$, 100%); m.s. (D_2O) *m/z* 445, 417, 293, 281, 210, and 124; δ (300 MHz; $CDCl_3$) 0.62 (3 H, t, $J_{18,19\alpha}$ $J_{18,19\beta}$ 7.4 Hz, 18-H), 0.89 (1 H, m, 19α-H), 1.54 (1 H, m, 19β-H), 1.12 (1 H, dd, $J_{17\alpha,17\beta}$ 14.3 Hz, $J_{17\beta,16\alpha}$ 5.5 Hz, 17α-H), 1.66 (1 H, m, 17β-H), 1.25 (1 H, m, 6α-H), 2.21 (1 H, m, 6β-H), 1.28 (1 H, m, 15β-H), 1.75 (1 H, m, 15α-H), 1.50 (1 H, m, 14α-H), 1.90 (1 H, m, 14β-H), 1.57 (1 H, m, 16α-H), 2.38 (1 H, m, 16β-H), 2.34 (1 H, br s, 21-H), 3.06 (1 H, dd, $J_{5\alpha,6\alpha}$ 8.9 Hz, $J_{5\alpha,6\beta}$ 3.2 Hz, 5α-H), 2.79 (1 H, m, 5β-H), 2.86 (1 H, dd, $J_{3\alpha,3\beta}$ 14.5 Hz, $J_{3\alpha,14\alpha}$ 7.2 Hz, 3α-H), 3.20 (1 H, m, 3β-H), 3.84 (1 H, dd, $J_{2,16\beta}$ 10.1 Hz, $J_{2,16\alpha}$ 4.8 Hz, 2-H), 6.50 (1 H, d, $J_{12,11}$ 7.7 Hz, 12-H), 6.76 (1 H, split dd, $J_{10,9}$ 7.2 Hz, $J_{10,11}$ 7.2 Hz, $J_{10,12}$ < 1 Hz, 10-H), 7.06 (1 H, split dd, $J_{11,10}$ 7.2 Hz, $J_{11,12}$ 7.7 Hz, $J_{11,9}$ 1.8 Hz, 11-H), 7.08 (1 H, d, $J_{9,10}$ 7.2 Hz, 9-H), 4.91 (1 H, m, 1′-H), 4.33 (1 H, d, $J_{6'\beta,6'\alpha}$ 12.24 Hz, 6′β-H), 3.68 (1 H, m, 6′-αH), 3.94 (1 H, m, 5′-H), 3.78 (1 H, m, 3′-H), and 3.71 (1 H, m, 2′-H); ^{13}C n.m.r. (75 MHz, $CDCl_3$) see Table 2.

Table 2.

Carbon No.	δ	Carbon No.	δ
2	71.45[c]	15	31.98
3	52.68[a]	16	25.09
5	53.91[a]	17	29.69[b]
6	34.57	18	6.84
7	56.00	19	29.32[b]
8	135.67	20	35.58
9	122.92	21	66.62
10	119.48	1′	80.61
11	127.58	2′	70.45[c]
12	105.56	3′	80.61
13	152.43[d]	4′	205.80[d]
14	21.86	5′	84.90[d]
		6′	64.75

Values are in δ (p.p.m.). [a, b] and [c] These assignments are interchangeable. [d] Weak signals.

Identification of the Glycone/Aglycone Moieties.—Aspidospermidose (**1**) (5 mg) was hydrolysed under acidic conditions (1M HCl; 0.2 ml) at 80 °C for 2 h. The material thus obtained was basified with NH_3 (conc.) and extracted with ethyl acetate (3 × 5 ml). The combined organic extracts were dried (Na_2SO_4), and evaporated under reduced pressure to afford a gum (2.7 mg). The mass spectrum of the product showed prominent peaks at *m/z* 282, 254, 210, and 124. The fragmentation pattern was identical to that of aspidospermidine.[13] The sugar derivative (R_F 0.34) present in the aqueous layer was checked for purity by t.l.c. using EtOAc–MeOH–AcOH–H_2O (65:20:15:15) as solvent. It was then reduced with $NaBH_4$ and its mass spectrum was recorded. It showed M^+ at *m/z* 164 consistent with the molecular formula $C_6H_{12}O_6$. Comparison of the glycone with standard sugars on silica gel (GF-254) plates with EtOAc–MeOH–AcOH–H_2O (65:20:15:15) as the solvent system (R_F 0.28) showed that the sugar was inseparable from glucose on co-chromatography.

Acknowledgements

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