Structure and Relative Stereochemistry of a New Polycyclic Alkaloid, Asparagamine A, showing Anti-oxytocin Activity, Isolated from *Asparagus racemosus*

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The relative stereochemistry of a new cage-type alkaloid, asparagamine A **1**, isolated from the roots of *Asparagus racemosus* Willd. (Liliaceae), has been elucidated by spectroscopic, chemical and single-crystal X-ray analyses. This novel polycyclic pyrrolizidine derivative is both the first alkaloid to be isolated from this genus and also the first pyrrolizidine derivative with carbon substituents at C-5 and C-8. The compound showed anti-oxytocin activity *in vitro* in a dose of 10^{-5}–10^{-6} mg cm^{-3}.

The dried, decorticated roots of *Asparagus racemosus* Willd. (Liliaceae), a small woody plant growing in Southeast Asia, India, Australia and Africa, have earlier been used in Ayurvedic medicine.[1] As a part of our study on the bioactive products in Asian tropical medicinal plants and crude drugs,[2–6] we reported the isolation of a new alkaloid named asparagamine A **1** from the roots of *A. racemosus* in a preliminary communication.[7] We now describe the isolation, structural elucidation and also the biological activity of this new alkaloid.

Results and Discussion

Isolation and Structural Elucidation of **1**.—From the 75% EtOH extracts of air-dried roots of *A. racemosus*, compound **1** was isolated as a major compound (0.13% of dry wt.) by chromatographic (silica gel) separation.[7] It was positive to Dragendorff's and I_2-platinate reagents and had the molecular formula $C_{22}H_{27}NO_5$; it was calculated to have ten degrees of unsaturation. The IR (ν_{max}/cm^{-1} 1750, 1620 and 1015) and UV (λ_{max}/nm 294.4) spectral data indicated the presence of an α,β-unsaturated lactone structure in the molecule. The ^1H NMR spectrum of **1** exhibited signals for three methyl groups [δ 0.93 (t, 23-H), 1.32 (d, 17-H) and 2.01 (s, 19-H)], one methoxy group [δ 4.08 (s, 18-H)] and a set of olefinic hydrogens [δ 5.44 (d, 20-H) and 5.71 (dt, 21-H)]. The ^{13}C NMR spectrum of **1** showed signals for 22 carbons including one ester carbonyl (δ 169.7, C-16), one ketal carbon (δ 112.7, C-9), and three sets of olefinic carbons [δ 126.4 (C-20)/133.2 (C-21), 148.3 (C-10)/127.7 (C-13) and 162.8 (C-14)/98.3 (C-15)] together with a substituted pyrrolizidine ring. At this stage, six remaining degrees of unsaturation were accounted for by six rings, no other double bond being indicated by the spectral data. In order to distinguish the signals of the carbons adjacent to the nitrogen atom, a ^{13}C NMR spectrum of **1** was compared with that of its *N*-oxide prepared by the treatment of **1** with *meta*-chloroperbenzoic acid. In the ^{13}C NMR spectrum of the *N*-oxide, the signals of C-3 (δ 63.7), C-5 (δ 76.8) and C-8 (δ 91.3) were shifted in the range of +8–+16 ppm compared to those of **1** (Table 1). Such substituent effects arising from the *N*-oxide have also been reported in several pyrrolizidine[8] and quinolizidine[9] alkaloids. Remarkable down- and up-field shifts at C-21 (+3.5 ppm) and C-20 (−6.8 ppm), respectively, were ascribable to the anisotropic effect of the *N*-oxide bond.

Finally the planar structure of **1** was deduced from the results of several two-dimensional (2D)-NMR (HH-COSY, CH-COSY, HOHAHA etc.) analyses in conjunction with the foregoing *N*-oxidation, decoupling experiments, and chemical-shift considerations (Fig. 1).

Table 1 ^{13}C NMR data for asparagamine A **1** and its *N*-oxide

Carbon no.	**1** δ	*N*-oxide δ	Δ (*N*-oxide-**1**)
1	51.1	48.0	−3.1
2	26.8	21.6	−5.2
3	47.9	63.7	15.8
5	60.7	76.8	16.1
6	32.8	31.5	−1.3
7	80.6	79.9	−0.7
8	82.9	91.3	8.4
9	112.7	110.7	−2.0
10	148.3	147.1	−1.2
11	34.5	34.5	0
12	47.5	48.3	0.8
13	127.7	127.9	0.2
14	162.8	162.2	−0.4
15	98.3	98.6	0.3
16	169.7	169.1	−0.6
17	18.2	17.7	−0.5
18	58.8	58.7	−0.1
19	9.0	8.8	−0.2
20	126.4	119.6	−6.8
21	133.2	136.7	3.5
22	25.2	25.5	0.3
23	13.4	12.8	−0.6

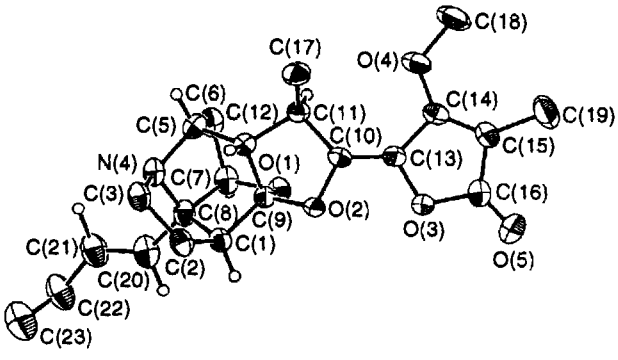


Fig. 1 X-Ray crystallographic numbering system for compound **1**

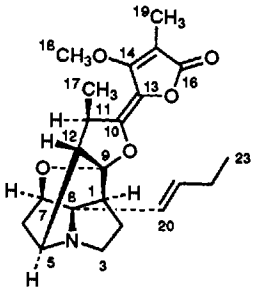
The significant C–H long-range correlations observed in the

Table 2 HMBC data for **1**

Position	^{13}C	HMBC (^1H)
1	51.1 (CH)	H-20
5	60.7 (CH)	H-3, 7, 12
7	80.6 (CH)	H-20
8	82.9 (C)	H-3, 5, 6, 20
9	112.7 (C)	H-1, 5, 7, 12
10	148.3 (C)	H-17
11	34.5 (CH)	H-12
12	47.5 (CH)	H-17
14	162.8 (C)	H-18, 19
15	98.3 (C)	H-19
16	169.7 (C)	H-19

HMBC spectra of **1** are summarized in Table 2. The presence of the ether bridge between C-7 and C-9 was evident from a three-bond correlation between 7-H and C-9, and from their chemical shifts. An *E* configuration was ascribed to the double bond between C-20 and C-21 on the basis of the large coupling constant (*J* 15.5 Hz) of its olefinic hydrogens (20-H and 21-H). NOESY and ROESY interactions between 17-H and 18-H confirmed *Z* stereochemistry for a second double bond between C-10 and C-13.

In order to establish the stereochemical configurations of each chiral centre in the polycyclic rings of **1**, a single-crystal X-ray diffraction analysis was undertaken on a crystal obtained by recrystallization from ether. The molecular structure and relative stereochemistry of **1** was unequivocally established as shown in Fig. 1. The X-ray structure showed the basic pyrrolizidine skeleton with methine bridge between C-5 and C-9 and an ether bridge between C-7 and C-9. In particular, the presence of butenyl group at C-8 and the substituent at C-5 are uncommon in pyrrolizidine alkaloids so far found.



1

Although several steroidal[10] and sugar[11] constituents had been isolated from *A. racemosus*, this is the first alkaloid to be isolated from the genus *Asparagus*. Also, although numerous macrocyclic types of pyrrolizidine alkaloid have been isolated from several pyrrolizidine-bearing plants,[12] to the best of our knowledge, asparagamine A **1** is the first example that has a complex polycyclic structure fused with a pyrrolizidine ring at C-5 and C-8. Its cage-type structure which seems to be quite unique, makes it of both biosynthetic and pharmacological interest. In respect of the former, the carbon skeleton of **1** may be derived from a condensation of two molecules of ornithine, a monoterpene unit and two molecules of acetic acid with a loss of one carbon unit.

Biological Activity of **1**.—Although many pyrrolizidine alkaloids are hepatotoxic as a result of their metabolites,[13] it was thought that **1** might be atypical because of its major structural differences. Nevertheless, **1** shows an inhibitory effect on oxytocin-induced contracture of rat diestrus uterus (inhibition values were 67.4 and 38.8% at 10^{-5} and 10^{-6} mg cm^{-3} for oxytocin at 10^{-3} IU cm^{-3}) and antitumour activity *in vitro* (10–100 μg cm^{-3} in a dose-dependent manner: IC_{50}s were 79.8, 55.5, 79.8 and 65.5 μg cm^{-3} to L1210, L1210/5FU^R, L1210/CDDP^R and Kato-III cells, respectively, while those of adriamycin were 0.2, 0.07, 0.2 and 0.06 μg cm^{-3}, respectively). The compound had no antimicrobial activity with *Trichophyton mentagrophytes*, *T. rubrum*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*. Since **1** is a major component of *A. racemosus*, it is thought to be responsible for a significant portion of the anti-abortifacient activity of this Ayurvedic crude drug, together a second active principle.[10a]

Experimental

M.p.s were determined by using a Yanagimoto micro-melting point apparatus and were uncorrected. IR spectra were measured on a HITACHI 260-10 spectrometer. UV spectra were recorded on a HITACHI U-3200 spectrophotometer. NMR spectra were obtained with a JEOL JNM-ALPHA 500 (500 MHz) spectrometer for $CDCl_3$ or C_6D_6 solutions. The chemical shifts are given as δ (ppm) values with TMS as an internal standard. *J* Values are given in Hz. EI-MS and FAB-MS were obtained with a JEOL HX-110 spectrometer. Optical rotations were recorded with a JASCO DIP 140 polarimeter using a 10 cm sample tube; values given as 10^{-1} cm^2 g^{-1}. Column chromatography was carried out on Kieselgel 60 (70-230 and 230-400 mesh, Merck). X-ray analysis was carried out with a Rigaku AFC6S diffractometer.

Isolation of **1**.—Air-dried roots (130 g) of *A. racemosus* were ground and extracted with 75% EtOH (500 cm^3 × 3). The combined extracts, concentrated in suspension, were partitioned between water (400 cm^3) and AcOEt (400 cm^3 × 3). The AcOEt fractions were combined, dried (Na_2SO_4) and evaporated to dryness to give a residue (1.5 g). A portion (1.0 g) of this residue was subjected to silica-gel column chromatography with AcOEt–MeOH (99 : 1, v/v) as eluent to yield **1** (110 mg, 0.13%/dry wt.) consisting of colourless prisms, m.p. 180 °C; $[\alpha]_D$ +202.5 (*c* 1.08 in MeOH); λ_{max}(MeOH)/nm 294.4 (log ε 4.26); ν_{max}(KBr)/cm^{-1} 2950, 1750, 1620, 1465, 1370, 1150, 1015 and 970; *m/z* (HR-EI) 385.1895 (Calc. for $C_{22}H_{27}NO_5$; 385.1889); *m/z* (EI) (rel. int.) 385 (M^+, 64.7%), 370 (5.7), 356 (13.0), 231 (14.4), 230 (17.5), 203 (12.3), 202 (44.6), 188 (6.5), 174 (6.8), 162 (92.9), 161 (38.1), 160 (100), 134 (14.7), 120 (11.5), 106 (12.1), 83 (10.1), 69 (8.0) and 55 (7.0); δ_H 5.71 (1 H, dt, *J* 15.5, 6.4, 21-H), 5.44 (1 H, d, *J* 15.5, 20-H), 4.14 (1 H, br s, 7-H), 4.08 (3 H, s, 18-H), 3.45 (1 H, br s, 5-H), 3.04 (1 H, m, 11-H), 3.03 (1 H, m, 3-H), 2.93 (1 H, m, 3-H), 2.79 (1 H, d, *J* 5.9, 1-H), 2.01 (3 H, s, 19-H), 2.01 (2 H, m, 22-H), 1.89 (1 H, d, *J* 12.2, 6-H), 1.82 (1 H, m, 2-H), 1.77 (1 H, m, 12-H), 1.74 (1 H, m, 6-H), 1.73 (1 H, m, 2-H), 1.32 (3 H, d, *J* 6.4, 17-H) and 0.93 (3 H, t, *J* 7.5, 23-H); ^{13}C NMR see Table 1.

N-*Oxidation of* **1** *with* m-*Chloroperbenzoic Acid*.—To a stirred solution of **1** (50 mg) in CH_2Cl_2 (20 cm^3) a solution of *m*-chloroperbenzoic acid (24.7 mg, 1.1 equiv.) in CH_2Cl_2 (10 cm^3) was added at room temperature. After 1 h, the solvent was evaporated and the residue was subjected to silica gel column chromatography with AcOEt–MeOH mixture as eluent to give the *N*-oxide (35 mg, 70%) as a colourless amorphous solid, $[\alpha]_D$ +146.5 (*c* 0.84 in MeOH); λ_{max}(MeOH)/nm 291.4 (log ε 4.23); ν_{max}($CHCl_3$)/cm^{-1} 2960, 2930, 1750, 1630, 1465, 1400, 1370, 1155, 1000 and 955; *m/z* (HR-EI) 402.1919 (Calc. for $C_{22}H_{28}NO_6$; 402.1917); δ_H 6.08 (1 H, d, *J* 15.9, 20-H), 5.82 (1 H, dt, *J* 15.9, 6.4, 21-H), 4.28 (1 H, br s, 7-H), 4.11 (3 H, s, 18-H), 4.01 (1 H, br s, 5-H), 3.97 (1 H, m, 3-H), 3.65 (1 H, ddd, *J* 5.5, 12.9, 12.9, 3-H), 3.19 (1 H, m, 11-H), 3.12 (1 H, d, *J* 7.4, 1-H), 2.99 (1 H,

d, *J* 12.7, 6-H), 2.25 (2 H, m, 2-H, 12-H), 2.15 (2 H, m, 22-H), 2.07 (1 H, d, *J* 13.4, 6-H), 2.01 (3 H, s, 19-H), 1.85 (1 H, m, 2-H), 1.38 (3 H, d, *J* 6.7, 17-H), 0.99 (3 H, t, *J* 7.3, 23-H); ^{13}C NMR see Table 1.

Crystallographic Analysis of **1**.—*Crystal data*. Empirical formula: $C_{22}H_{27}NO_5$, formula weight: 385.46, crystal system: orthorhombic, space group: $P2_12_12_1$, a = 9.845(1), b = 27.065(1), c = 7.768(1) Å, V = 2069.8(4) Å^3, Z = 4, D_c = 1.237 g cm^{-3}. A colourless prism of *ca.* 0.35 × 0.20 × 0.15 mm in size was mounted with graphite monochromated Cu-Kα radiation at 23 ± 1 °C, μ(Cu-Kα) = 7.14 cm^{-1}. A total of 1827 reflections was collected. The intensities of three representative reflections were measured after every 150 reflections. No decay correction was applied. The structure was solved by direct method[14] and expanded using Fourier techniques.[15] Full listings of the crystallographic results have been deposited with the Cambridge Crystallographic Data Centre.*

Biological Activity Assays of **1**.—The inhibitory effect of **1** on oxytocin-induced contracture of rat (SD) diestrus uterus was assayed at concentrations of 10^{-5} and 10^{-6} mg cm^{-3} when the contracture was induced by oxytocin at 10^{-3} and 3 × 10^{-3} IU cm^{-3} according to the modified method of Ichida.[16] The cytotoxic activities of **1** were determined at concentrations of 1–100 μg cm^{-3} by MTT assay[17] using mouse cell lines (L1210, L1210/5FU^R, L1210/CDDP^R) and human cell lines (Kato-III, MKN-28, MKN-45). The antimicrobial activities of **1** were tested by the disc agar diffusion method as described previously.[18]

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* For details of this system, see *Instructions for Authors* (1995), *J. Chem. Soc., Perkin Trans. 1*, 1995, issue 1.

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