STRUCTURE OF ASPARAGAMINE A, A NOVEL POLYCYCLIC ALKALOID FROM *ASPARAGUS RACEMOSUS*

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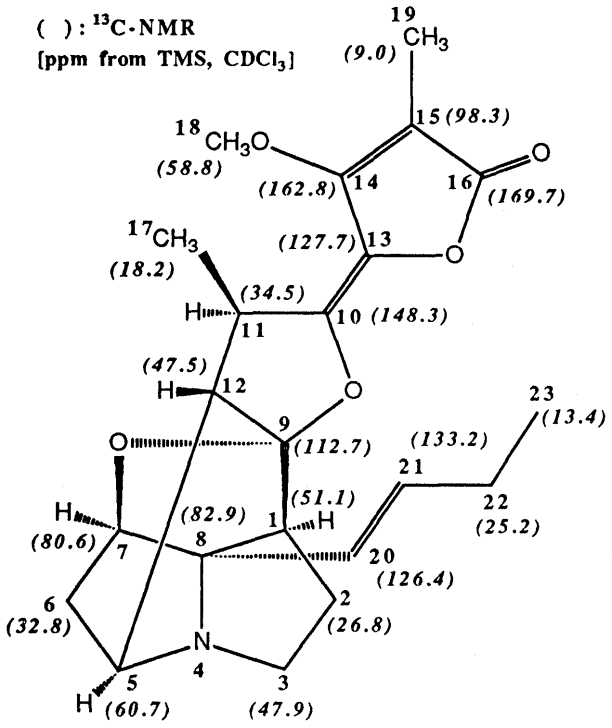
A new cage-type alkaloid, asparagamine A (**1**), was isolated from the roots of *Asparagus racemosus* Willd. (Liliaceae). The relative stereostructure was elucidated by spectroscopic, chemical and single crystal X-ray analyses as a novel polycyclic pyrrolizidine derivative.

KEYWORDS alkaloid; asparagamine A; *Asparagus racemosus*; Liliaceae; polycyclic pyrrolizidine; cage compound

In our previous papers, we have reported the isolation and characterization of several new biologically active products, such as camelliaside A-C,[1,2] L-methionine S(*S*)-sulfoximine[3] and entadamide A-C,[4,5] from Asian tropical medicinal plants and crude drugs. As a series of this program, the present paper deals with the isolation and the structural elucidation of a new alkaloid (**1**) named asparagamine A from the roots of a Thai medicinal plant, *Asparagus racemosus* Willd. (Liliaceae). *A. racemosus* is a woody small plant growing in Southeast Asia, India, Australia and Africa, and the root has been used as a stimulant, restorative and demulcent in India and Thailand.[6]

From 75% EtOH extracts of the dried roots of *A. racemosus*, **1** was isolated as a major compound (0.13 % / dry wt.), positive to Dragendorff's and I_2-platinate reagents, by chromatographic (silica gel) separation. Compound **1** is a colorless prism, mp 180°C (ether), $[\alpha]_D$ + 202.5° (MeOH), and its molecular formula $C_{22}H_{27}NO_5$ [observed *m/z* 385.1895 $(M)^+$, calcd. *m/z* 385.1889] was confirmed by a high-resolution electron impact mass spectrometry (HR-EI-MS)[7]. The IR (ν_{max} 1750, 1620, 1015 cm^{-1} [KBr]) and UV (λ_{max} 294.4 nm [MeOH]) spectra indicated the presence of an α, β-unsaturated lactone structure in the molecule.

The ^{1}H-NMR spectrum ($CDCl_3$)[8] of **1** exhibited signals for three methyl [δ 0.93 (t), 1.32 (d) and 2.01 (s)] groups, one methoxyl [δ 4.08 (s)] group and a set of olefinic hydrogens [δ 5.44 (d) and 5.71 (dt)]. The ^{13}C-NMR spectrum ($CDCl_3$) of **1** showed signals of 22 carbons including one carbonyl (δ 169.7), one ketal carbon (δ 112.7), and three sets of olefinic carbons (δ 126.4/133.2, 148.3/127.7 and 162.8/98.3) with a substituted pyrrolizidine ring (Fig. 1). Further two-dimensional (2D)-NMR analysis of **1** and its *N*-oxide (**2**)[9], obtained by an usual *N*-oxidation with *m*-chloroperbenzoic acid, by changing the solvent to benzene-d_6, allowed us to deduce a pyrrolizidine skeleton with an additional substituent at C-8 and a fused-ring structure for **1** as shown in Fig. 1. The ^{1}H detected heteronuclear multiple bond connectivity (HMBC) spectra (*J*=8 Hz) of **1** and **2** corroborated the proposed structure (Fig. 1).



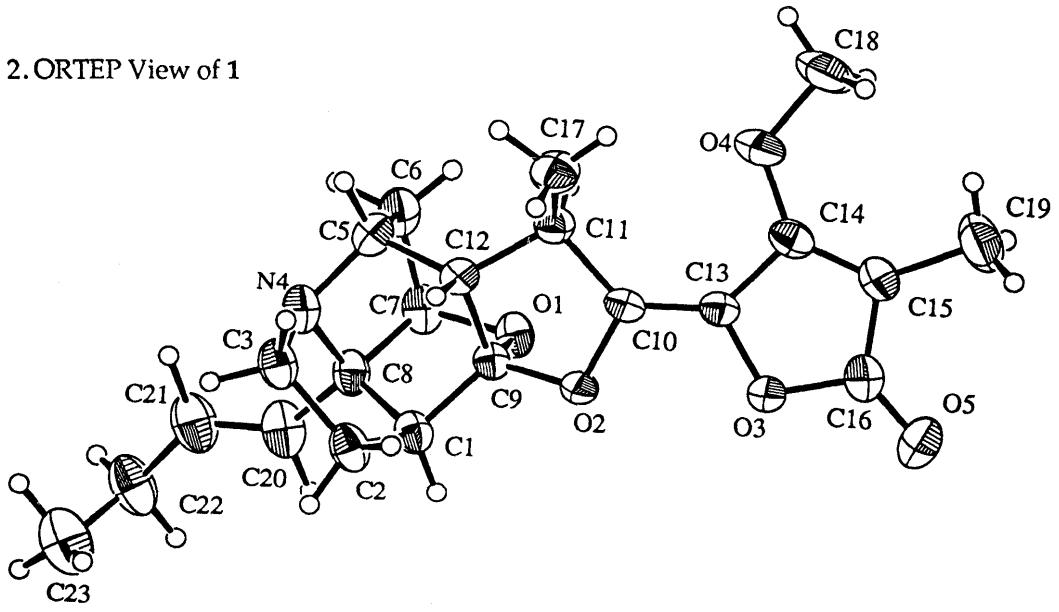
HMBC Data for **1**

Position	^{13}C	HMBC (^{1}H)
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

Fig. 1. Structure and Significant Two and Three Bond Correlation Revealed by HMBC Spectra of **1**

In order to establish the stereochemical configurations of each chiral center in the polycyclic rings of **1**, a single crystal X-ray diffraction analysis[10] was undertaken on a crystal obtained from ether. The molecular structure and relative stereochemistry of **1** was unequivocally established as shown in Fig. 2.

Fig. 2. ORTEP View of **1**



Numerous macrocyclic types of pyrrolizidine alkaloid have been isolated from several pyrrolizidine-bearing plants.[13] To our knowledge, asparagamine A (**1**) is the first example that has a complicated polycyclic structure fused with a pyrrolizidine ring. Its cage-type structure seems to be extremely unique and interesting from the biosynthetic and pharmacological points of view. In fact, asparagamine A in dose form 10 to 100 μg/ml showed anti-tumor activity on several tumor models *in vitro*

in a dose dependent manner. On the other hand, we are trying to discover other pharmacological activities in other models. The results will be reported in a subsequent paper.

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7) EI-MS of **1** *m/z* (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), 55 (7.0).
8) ^{1}H-NMR (500MHz, $CDCl_3$) δ 5.71 (1H, dt, *J*= 15.5, 6.4 Hz, H-21), 5.44 (1H, d, *J*= 15.5 Hz, H-20), 4.14 (1H, brs, H-7), 4.08 (3H, s, H-18), 3.45 (1H, brs, H-5), 3.04 (1H, m, H-11), 3.03 (1H, m, H-3), 2.93 (1H, m, H-3), 2.79 (1H, d, *J*= 5.9Hz, H-1), 2.01 (3H, s, H-19), 2.01 (2H, m, H-22), 1.89 (1H, d, *J*= 12.2 Hz, H-6), 1.82 (1H, m, H-2), 1.77 (1H, m, H-12), 1.74 (1H, m, H-6), 1.73 (1H, m, H-2), 1.32 (3H, d, *J*= 6.4 Hz, H-17), 0.93 (3H, t, *J*= 7.5 Hz, H-23).
9) colorless syrup, $[\alpha]_D$ + 146.5° (MeOH), HR-FAB-MS *m/z* 402.1919 $[M+H]^+$ (Calcd. for $C_{22}H_{28}NO_6$, 402.1917).
10) Crystal data : $C_{22}H_{27}NO_5$, orthorhombic, space group $P2_12_12_1$, a=9.841 (1), b=27.067 (1), c=7.768 (1)Å, V=2069.8 (4) Å^3, Z=4, Dc=1.237 g cm^{-3}, μ(CuKa)=7.14cm^{-1}, F(000)=824.000, R=0.052, 1287 uniqe reflections. The structure was solved by the direct method[11] and expanded using Fourier techniques.[12]
11) by SHELXS86: G. M. Sheldrick, "*Crystallographic Computing 3*", ed. by G. M. Sheldrick, C. Kruger and R. Goddard, Oxford University Press, 1985, pp.175.
12) by DIRDIF92: P. T. Beurkens, G. Admiraal, G. Beurkens, W. P. Bosmann, S. Garcia-Granda, R. O. Gould, J. M. M. Smits, C. Smykalla, "*The DIRDIF program system, Technical Report of the Crystallography Laboratory*", University of Nijmegen, The Netherlands, 1992.
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