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Polygonapholine, an Alkaloid with a Novel Skeleton, Isolated from *Polygonatum alte-lobatum*

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Abstract: A novel alkaloid, polygonapholine, is isolated from the rhizome of *Polygonatum alte-lobatum* and determined by spectroscopic methods.

The rhizome of *Polygonatum alte-lobatum* Hayata, a Formosan endemic plant, has been used as a tonic drug in Taiwan. The literature showed that various steroidal saponins and flavonoids have been reported from several *Polygonatum* species.[1,2] Recently we have isolated and characterized two new homologous series of 1,4-benzoquinones, named polygonaquinones A and B, respectively, a novel homoisoflavanone, a new gentrogen glycoside and thirteen known compounds from the rhizome of *P. alte-lobatum*.[3] Continuing our studies on Formosan medicinal plants, we further investigated the MeOH extract of the rhizome of this plant. The MeOH extract was chromatographed over silical gel. Elution with EtOAc-MeOH(4:1) yielded polygonapholine(**1**).

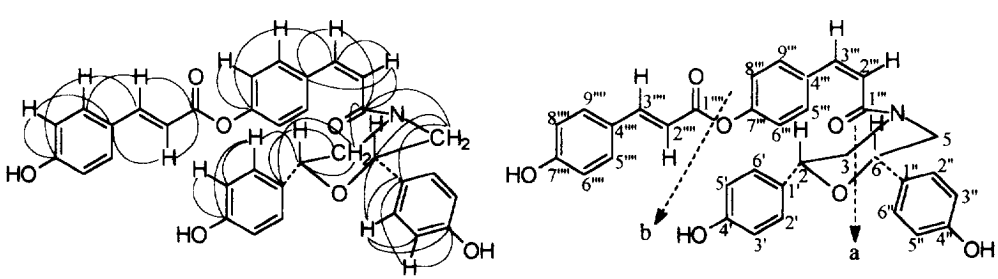

FIGURE 1-Structure of **1**. ^{1}H-^{13}C long-range correlations observed in its HMBC spectrum of **1**.

Compound **1**, a pale yellow powder, showed $[\alpha]_D^{25}$ -76° (c=0.5, MeOH). It gives a positive test with Dragendorff's reagent and possesses the molecular formular $C_{34}H_{29}NO_7$ as determined from negative D/CI mass spectra ($[M-H]^-$ at m/z 562) and from ^{1}H and ^{13}C counting in NMR spectra. IR absorptions were indicative of hydroxyl group (3450cm^{-1}), conjugated C=O (1680 and 1640cm^{-1}) and aromatic ring (1560cm^{-1}).

The present of a base peak [M-a-b+H] (Fig. **1**) at m/z 281 and characteristic peaks at m/z 176, 165, 154(base peak), 136, and 120 in its negative D/CI and positive FAB mass spectra, respectively suggest that **1** possesses a N-*p*-coumaroyltyramine moiety.[4] The ^{13}C NMR spectrum of **1** had signals of all 34 arbon atoms in the molecule,among which 2 cinnamoyl carbonyls, 24 aromatic carbons, four olefinic (2 -CH=CH-) carbons and 4 aliphatic (2>CHO-,2 -CH_2 N<) carbons[5] (Table **1**). Analysis of COSY 90 and HMQC spectra for established the connectivities of five ^1H-^1H and ^1H-^{13}C spin systems corresponding to a *p*-hydroxy-trans-cinnamoyl, a *p*-oxygenated cis-cinnamoyl[6], two *p*-hydroxyphenyl and a 2,4,6-trisubstituted morpholine moieties. The HMBC spectra of **1** showed connections between these moieties. The HMBC of C-1' to H-2 and H-3 and C-2 to H-2' and H-6' confirmed that the C-1' of *p*-hydroxyphenyl moiety was linked to the C-2 of the morpholine moiety. The HMBC of C-1" to H-5 and H-6 and C-6 to H-2" and H-6" also confirmed that the C-2" of the other *p*-hydroxyphenyl moiety was linked to the C-6 of the morpholine moiety. In addition to the above connectivity between the two *p*-hydroxyphenyl and morpholine moieties, the HMBC of C-1"' to H-3 and H-5 confirmed the connectivity between C-1"' and nitrogen atom. The above evidence and the presence of a N-*p*-coumaroyltyramine moiety in the positive FAB and negative D/CI mass spectra, confirmed that the C-1"" was linked by a oxygen bridge to C-7"'. Based on the above evidence, the structure of polygonapholine(**1**) was established as **1** (Fig. **1**).

The ^1H and ^{13}C NMR spectra of **1** (Table **1**) were assigned by COSY, HMQC, HMBC and comparison with those of N-*p*-coumaroyltyramine.[4,7] The ^{13}C NMR spectra also supported the characterization of **1**. This structure was also supported by positive FAB and negative D/CI Mass spectra.

Table 1. ^1H and ^{13}CNMR Spectra of **1**.(400 MHz, TMS-CD_3OD).

C	δ_C	δ_H	(m)	J	C	δ_C	δ_H	(m)	J	C	δ_C	δ_H	(m)	J
2	73.5	β4.69	dd	8.0,4.8	2"	128.5	7.19	d	8.8	7"'	162.5			
3	47.9	3.40	m		3"	116.4	6.79	d	8.8	8"'	117.4	6.74	d	8.8
5	47.9	3.40	m		4"	158.2				9"'	132.3	7.37	d	8.8
6	73.3	β4.73	dd	8.0,4.8	5"	116.4	6.79	d	8.8	1""	169.8			
1'	134.4				6"	128.5	7.19	d	8.8	2""	117.5	6.41	d	15.6
2'	128.4	7.22	d	8.8	1"'	170.5				3""	142.3	7.45	d	15.6
3'	116.4	6.77	d	8.8	2"'	120.8	5.79	d	12.8	4""	126.6			
4'	158.2				3"'	138.7	6.60	d	12.8	5""	130.6	7.36	d	8.8
5'	116.4	6.77	d	8.8	4"'	127.4				6""	116.8	6.72	d	8.8
6'	128.4	7.22	d	8.8	5"'	132.3	7.37	d	8.8	7""	160.2			
1"	134.5				6"'	117.4	6.74	d	8.8	8""	116.8	6.72	d	8.8
										9""	130.6	7.36	d	8.8

* All assignments were confirmed by HMQC,HMBC and NOESY spectra data.Coupling constants (J in Hz) are given in parentheses .

The relative configuration of **1** was determined by the phase-sensitive NOESY spectrum. The NOE correlations are illustrated by arrows in Fig. **2**. To clarify the conformation of **1** (Fig. **2**), a computer-assisted 3D structure (Fig. **2**) was obtained using the molecular modelling program INSIGHT II [8] modelling system,

using where possible, units from within the fragment library. Geometry optimization was performed using DISCOVER utilizing the CVFF (Consistent Valence Force Field) force field calculations for energy minimization. The results were visualized using INSIGHT II running on a Silicon Graphics IRIS (SGI) INDIGO XS24-4000. The conformational search suggested the stable conformation as shown in Fig. 2. It indicates that the H-2 and H-6, and the two *p*-hydroxyphenyl groups are β -axial and α -equatorial, respectively, with respect to morpholine ring. The stereochemistry of H-2 and H-6 was also supported by their coupling constants as indicated in Table 1.[9]

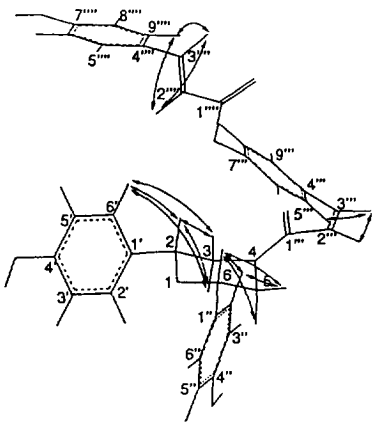

FIGURE 2-Stereoview of **1** generated from computer modeling. The bold lines show the NOE relationships.

EXPERIMENTAL

General.-^{1}H , ^{13}C and bidimensional spectra were recorded on Bruker 400 MHz FT-NMR spectrometer, UV spectra were taken on Hitachi Model 260-30 spectrometer,$[\alpha]_D$ on a Jasco model DIP-370 Digital Polarimeter and mass spectra were run on a Jeol JMS-SX 102 mass spectrometer.

Isolation of Products.-*Polygonatum alte-lobatum* Hayata (20kg) was collected at Pin-Tung Hsien, Taiwan, R.O.C.,during October 1995. A voucher specimen deposited in the authors' laboratory. The fresh rhizomes were chipped and extacted with MeOH at room temp. in a closed container several times. The extract was chromatographed on silica gel column. Elution with EtOAc-MeOH(4:1) yielded **1**.

Polygonapholine (1).-(20mg) was isolated as pale yellow powder, mp 238-240°C ; $[\alpha]_D^{25}$ -76° (MeOH; c 0.5); UVλmax(MeOH)nm: 420(sh), 330(sh), 306, 225; IR ν max(KBr)cm^{-1}:3450, 1680, 1640, 1600, 1560, 1240;^{1}H NMR (400MHz, CD_3OD): see Table **1**; ^{13}C NMR (400MHz,CD_3OD): see Table **1**; FABMS (positive) (rel. int.)m/z:(no molecular ion peak) 329(10), 307(7), 289(5), 176(98),165(12),155(28),154(100), 147(17), 138(41), 137(69), 136(91), 120(21), 107(39), 91(34), 89(41), 77(48); D/CI(negative)(rel. int.)m/z : 562[M-1]$^-$

(5), 487(5), 444(1), 427(2), 416(2), 400(4), 386(1), 371(5), 359(3), 341(43), 327(15), 313(17), 299(3), 281(100); 280(49), 195(2), 179(3), 153(2), 135(1), 119(5).

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4. N-*p*-coumaroyltyramine obtained from the rhizome of this same plant. Its EIMS m/z (rel. int.) :284(M+1)$^+$(9), 273(1), 257(1), 239(1), 226(1), 208(2), 190(4), 176(5), 167(12), 155(28),154(100), 149(49), 137(86),136(99),120(26), 107(47).^{13}C NMR(CD_3OD): δ 35.8, 42.5, 116.3(2C), 116.7(2C), 118.5, 127.8, 130.5 (2C),130.7(2C), 131.3, 141.8, 156.9, 160.5, 169.3.
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