Conformational Analysis of C3'-C8 Connected Biflavones

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Two biflavones isolated from *Taxus cuspidata* were identified with ginkgetin (**1**) and sciadopitysin (**2**) which belong to C3'-C8 connected biflavones. The conformation of **2** in the solid state was determined by X-ray analysis and the conformations of **1** and **2** in the liquid state were discussed using nmr techniques. Complete assignments of ^1H and ^{13}C nmr spectra about **1** were made on the basis of COSY, HMQC, HMBC and nOe experiments.

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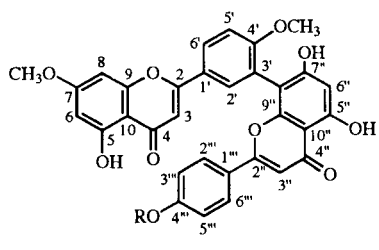
The genus *Taxus is* now very interested in producing the potent anticancer compound, taxol [1-4]. *Taxus cuspidata* belongs to the family Taxane and it grows in several countries in the world, for example, in North America, Japan and China. As a continuation of our work to research bioactive substances, we have examined the constituents of the branches of *Taxus cuspidata* SIEB. and ZUCC., which grows in Jilin province and has been used as a traditional folk medicine in China for antidiabetes, kidney trouble and diuresis, and we isolated two biflavones **1** and **2** together with taxinine, taxinine B, 2'-desacetoxyausrospicatin. Detailed examination of nmr spectra revealed **1** and **2** to be ginkgetin [5] and sciadopitysin [6], respectively. Although complete assignments of nmr on sciadopitysin have been reported [7], there is no report of those on ginkgetin except the report, in which partial assignments have been only made with reference to apigenin and its derivatives [8]. Up to date, structures of biflavones have been generally elucidated by nmr analysis and confirmed by total synthesis. But their structural and conformational studies by X-ray analysis are very rare to our knowledge. Especially, conformational experiments about biflavones belonging to amentflavones which have a linkage between C-3' and C-8 have not yet been reported. Conformational studies of biflavones seem to be very interesting because several biflavones showed restricted rotation and exhibited molecular dissymmetry [9-13], and also recently the absolute configuration of biflavone has been first reported by theoretical calculation of the CD spectrum [14]. Biflavones having molecular dissymmetry will be expected to provide a valuable role to the fields of asymmetric synthesis as a synthon having a dissymmetric field.

This paper deals with, for the first time, the conformation of **2** in the solid state by means of the X-ray method of analysis and also the conformation of **1** and **2** in the liquid state on the basis of nOe measurements by nmr techniques together with the identifications of **1** and **2** with ginkgetin and sciadopitysin and the complete assignments of the ^1H and ^{13}C-nmr spectra of ginkgetin.

Results and Discussion.

Compound **1** was obtained as yellow plates (methanol) and showed mp 238-242°, and possesses the molecular formula $C_{32}H_{22}O_{10}$ according to FAB hrms. The ^1H nmr spectrum (Table 1) shows two hydroxyl signals at δ 13.47 and 13.84 (each, s) shifted to lower magnetic field due to intramolecular hydrogen bonding to the carbonyl groups and also shows two methoxyl groups, twelve olefinic protons which are the three pairs of *ortho* coupling protons, one pair of meta coupling protons, and four isolated protons. The ^{13}C nmr spectrum of **1** exhibited thirty two carbons including two carbonyl signals for flavone at δ 183.0, 183.7, two methoxyl signals and twenty eight olefinic carbons which include twelve tertiary carbons and sixteen quaternary carbons. From the detailed analysis of ^1H-^1H 2D, HMQC, HMBC spectra, **1** was assumed to be ginkgetin which belongs to biflavonoids of amenthoflavone connected between C-3' and C-8. Furthermore, we confirmed that these two methoxyl groups located at C-7 and C-4' on the basis of nOe difference spectra. As shown in Table 2 and Figure 1, nOes were observed between 7-OMe and 8-H, 7-OMe and 6-H, also observed between 4'-OMe and 5'-H respectively. Melting points of **1** and 5, 5", 7", 4"'-tetraacetate of **1** (240-242°, methanol) approximately coincided to the values in the literature

Scheme 1



1 R = H
2 R = CH_3

Table 1

^1H and ^{13}C NMR Data for **1** [a]

position	δc		Correlated H [b]	δH	C coupled [c] with H	H coupled [d] with H
2	164.6	s				
3	105.2	d	H-3	7.01 s	C-2, C-4, C-10, C-1'	
4	183.0	s				
5	162.8	s				
6	98.9	d	H-6	6.49 d (1.5)	C-5, C- 7, C-8, C-10	H-8
7	166.0	s				
8	93.1	d	H-8	6.59 d (1.5)	C-6, C-7, C-9, C-10	H-6
9	158.3	s				
10	106.1	s				
1'	123.5	s				
2'	132.2	d	H-2'	8.42 d (1.5)	C-2, C-4', C-6',C-8"	H-6'
3'	123.5	s				
4'	161.7	s				
5'	112.0	d	H-5'	7.18 d (9.0)	C-1', C-3', C-4'	H-6'
6'	128.7	d	H-6'	7.97 dd (9.0, 1.5)	C-2, C-'2', C-4'	H-2', H-5'
2"	164.6	s				
3"	103.7	d	H-3"	6.87 m	C-2", C-4", C-10", C-1"'	
4"	183.3	s				
5"	162.7	s				
6"	99.9	d	H-6"	6.80 m	C-5", C-7", C-8"', C-10"	
7"	163.6	s				
8"	105.1	s				
9"	155.8	s				
10"	105.2	s				
1"'	122.5	s				
2"'	128.8	d	H-2"'	7.67 brd (8.0)	C-2",C-4"'	H-3"'
3"'	117.0	d	H-3"'	7.04dd (9.0, 1.0)	C-1"',C-4"'	H-2"'
4"'	162.9	s				
5"'	117.0	d	H-5"'	7.04dd (9.0, 1.0)	C-1"',C-4"'	H-6"'
6"'	128.8	d	H-6"'	7.67 brd (8.0)	C-2", C-4"'	H-5"'
7OCH_3	56.0	q		3.59 s	C-7	
4'OCH_3	56.2	q		3.68 s	C-4'	
5OH				13.47 s	C-5, C-6, C-10	
5"OH				13.84 s	C-5", C-6", C-10"	

[a] Chemical shift in pyridine-d_5 (ppm); ^{13}C multiplicities from DEPT experiments; J_{HH} (Hz) in parentheses. [b] HMQC. [c] HMBC. [d] ^1H-^1H COSY.

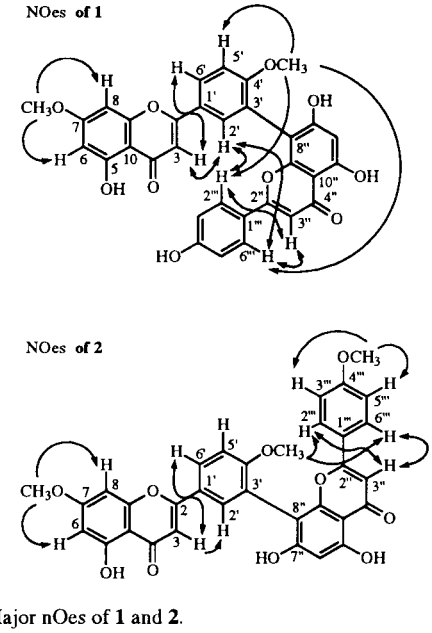


Figure 1. Major nOes of **1** and **2**.

[15, 16], respectively. Complete assignments of ^1H and ^{13}C nmr for **1** are summarized in Table 1.

Compound **2** was obtained as yellow crystals (methanol) and showed mp 295-297°, possessing the molecular formula $C_{33}H_{25}O_{10}$. The ^1H and ^{13}C nmr spectra were similar to compound **1** except possessing three methoxyl groups. From the detailed analysis of ^1H-^1H 2D, HMQC, HMBC, **2** was determined to be sciadopitysin [6]. The nOes between 7-OMe and 8-H, 7-OMe and 6-H, 4'-OMe and 5'-H, 4"'-OMe and 5"'-H, 4"'-OMe and 3"'-H as shown in Table 2 and Figure 1 confirmed the location of the three methoxyl groups in **2**. Our full assignments of ^1H and ^{13}C nmr spectra (in pyridine-d_5) almost coincident to those already reported by Chengbin *et al.* (in DMSO-d_6) [7]. Melting points of **2** and a 7"-0-methyl derivative of **2** (218-220°, methanol) coincided with the values in literature [6].

Next, we examined the stereo structure of **1** and **2** in the liquid state by nOe measurements. Ginkgetin (**1**) and sciadopitysin (**2**) did not show unambiguous optical rotation and also exhibited no Cotton effect in the CD spectra. We

Table 2

NOe Data for **1** and **2** [a]

Irradiated proton	Observed nOes (**1**)	Observed nOes (**2**)
H-3	H-2' (8.7%), H-6' (5.6%)	H-2' (8.5%), H-6' (7.7%)
H-2'	H-3 (5.6%), H-2''',6''' (0.6%)[b]	H-3 (6,.2%)
H-5'	H-6' (6.0%)	H-6'(2.4%)
H-6'	H-3 (5.6%), H-5' (4.1%)	H-5' (5.0%)
H-3''	H-2''',6''' (10.4%)	H-2''' (*), 6''' (*)
H-3''',5'''	H-2''',6''' (5.4%)	H-2''',6''' (*)
H-2''',6'''	H-2' (0.4%)[b], H-3'' (5.2%) H-3''',5''' (4.1%)	H-3'', H-3''',5''' (13.2%)
CH_3O-7	H-6 (3.7%), H-8 (3.0%)	H-6 (1.6%), H-8 (1.7%)
CH_3O-4'	H-5' (2.6%), H-2''',6''' (0.5%) [b]	H-5'(1.1%), H-2''',6''' (0.8%)
CH_3O-4'''		H-3''', 5''' (2.1 %)
OH-5''	H-6'' (1.7%)	
OH-5	H-6 (1.1%)	H-6 (0.7%) *Total 13.2%

[a] Solvent: pyridine-d_6, data for one dimensional nOe. [b] Also appeared in NOESY.

examined one (difference spectra) and two dimensional (NOESY) nOe measurements of **1** and **2** under the same conditions. It is presumed that both **1** and **2** have no restricted rotation about C-2 and C-1' linkage and also about C-2'' and C-1''' linkage because there is no bulky group to have steric repulsion around these two linkages. As shown in Table 2 and Figure 1, nOes of **1** between 3-H and 2'-H, 3-H and 6'-H, 3''-H and 2'''-H, 3''-H and 6'''-H exhibited free rotations about C-2 and C -1', C-2'' and C-1''' linkage respectively. Both one and two dimensional nOe measurements showed nOes between 4'-OMe and 2'''-H, 4'-OMe and 6'''-H, 2'-H and 2'''-H, 2'-H and 6'''-H. Considering the optical inactivity of **1** and nOe data as mentioned above, it is assumed that **1** has no restricted rotation also about the C-3' and C-8'' linkages. In conclusion, these results exhibited no restricted rotation in **1** in liquid state. As shown in Table 2 and Figure 1, nOes of **2** between 3-H and 2'-H, 3-H and 6'-H, 3''-H and 2'''-H, 3''-H and 6'''-H existed in **2**. Though week nOes between 4'-OMe and 2'''-H, 4'-OMe and 6'''-H existed in **2**, but none of nOes between 2'-H and 2'''-H, 2'- H and 6'''-H were found in both one end two dimensional nOe spectra. . These results indicate that restricted rotation exist about C-3' and C-8'' linkage in **2** and this restricted rotation is so weak that sciadopytisin (**2**) easily becomes racemic, because **2** did not clearly show optical rotation. From these results, it was clear that **1** exhibited free rotation about all linkages, alternatively **2** showed partially restricted rotation only about the C-3' and C-8'' linkage and it prefers the conformation as shown in Figure 1 in the liquid state, though the torsional angle about C-3' and C-8'' linkage is not unequivocal. It is assumed that this differentiations caused from the repulsion between the 4'''-OMe and the 7-OMe groups in **2**.

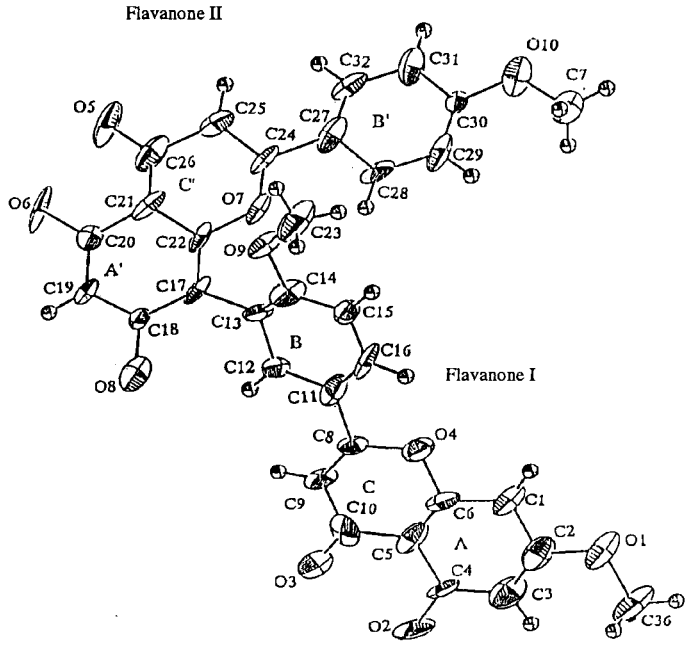


Figure 2. ORTEP view of **2**.

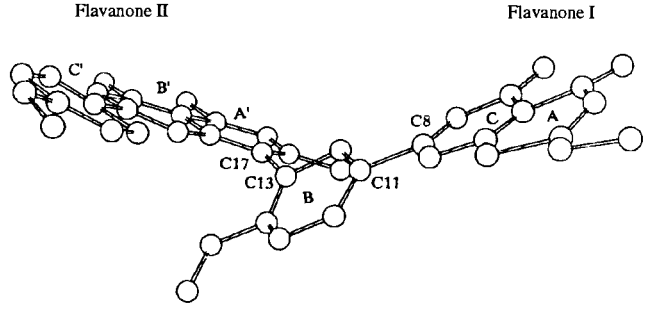


Figure 3. PLUTO view of **2**.

Table 3

Crystallographic Data For Sciadopitysin (**2**)

Crystal dimensions	0.2 x 0.3 x 0.3 mm
Chemical Formula	$C_{33}H_{24}O_{10}$
Formula weight	580.55
Crystal system	triclinic
Space group	$P\bar{1}$ (#2)
Lattice parameters	
a(Å)	9 639(1)
b(Å)	19.374(3)
c(Å)	7.9181(8)
α(°)	94.02(1)
β(°)	110.970(8)
γ(°)	92.23(1)
V(Å^3)	1374.0(3)
Z value	2
D_{Calcd} (g/cm^3)	1.403
μ (CuKoc) (cm^{-1})	6.64
R (R_w)	0.095 (0.074)
No. reflection used	1157

Next, we examined the molecular conformation of sciadopitysin (**2**) in the solid state by the X-ray method. A single crystal of **2** was obtained from acetone by slow evaporation. ORTEP and PLUTO drawings of the molecule are given in Figure 2 and Figure 3. The crystal data are summarized in Table 3. The selected torsional angles are shown in Table 4. Torsional angles of 04-C8-C11-C16, C9-C8-C11-C12 as each -20° and -13° exhibited two planes of ring B and C in Flavone **I** to be torsioned a little about the C-2 and C-1' (C8 and C11 in Figure 2) linkage and O7-C24-C27-C28, C25-C24-C27-C32 as each -1°, -11° also exhibited two planes of ring B' and C' in Flavone **II** to be almost planar as shown in the ORTEP and PLUTO drawings. Torsional angles of C14-C13-C17-C22 and C12-C13-C17-C18 as each 60° and 57° showed two planes of ring B and A' to be torsioned largely about the C-3' and C-8" (C17 and C13 in Figure 2) linkage. It seems that the torsion of the C-3' and C-8" linkage results from the repulsion between 7-OMe and 4"'-OMe, 4'-OMe and 7"-OH groups. Consequently, **2** prefers partially torsioned conformation as shown in Figure 2. In spite of many efforts, we could not obtained a single crystal of ginkgetin (**1**) suitable for X-ray analysis.

Table 4

Selected Torsional Angles of **2**

O4-C8-C11-C12	164°	C9-C8-C11-C12	-13°
O4-C8-C11-C16	-20°	C9-C8-C11-C16	163°
C14-C13-C17-C18	-111°	C12-C13-C17-C18	57°
C14-C13-CI7-C22	60°	C12-C13-C17-C22	-132°
C25-C24-C27-C32	-11°	O7-C24-C27-C32	171°
C25-C24-C27-C28	177°	O7-C24-C27-C28	-1°

Table 5

Positional Parameters and B(eq) of **2**

atom	X	Y	Z	B(eq)
O(1)	-0.539(1)	-0.1176(6)	-0.571(2)	5.7(6)
O(2)	-0.089(1)	-0.2156(5)	-0.192(2)	5.1(6)
O(3)	0.146(1)	-0.1258(6)	-0.053(2)	4.9(6)
O(4)	-0.105(1)	0.0269(5)	-0.327(1)	3.8(5)
O(5)	0.648(1)	0.4304(6)	0.469(1)	5.9(6)
O(6)	0.806(1)	0.3397(6)	0.414(1)	6.3(6)
O(7)	0.282(1)	0.3262(6)	0.090(1)	3.7(5)
O(8)	0.568(1)	0.1472(6)	-0.025(1)	4.7(6)
O(9)	0.257(1)	0.3066(5)	-0.270(1)	3.8(5)
O(10)	-0.322(1)	0.4542(6)	0.025(1)	5.2(6)
C(1)	-0.319(2)	-0.0466(9)	-0.446(2)	4.1(8)
C(2)	-0.386(2)	-0.118(1)	-0.467(2)	4.2(9)
C(3)	-0.313(2)	-0.170(1)	-0.378(2)	4.7(9)
C(4)	-0.160(2)	-0.1626(8)	-0.262(2)	3.4(7)
C(5)	-0.087(2)	-0.094(1)	-0.246(2)	4.0(8)
C(6)	-0.162(3)	-0.0451(8)	-0.336(2)	3.5(8)
C(7)	-0.443(2)	0.419(1)	-0.125(2)	6(2)
C(8)	0.051(2)	0.0355(9)	-0.224(2)	2.6(8)
C(9)	0.129(2)	-0.0102(8)	-0.138(2)	3.6(8)
C(10)	0.073(2)	-0.081 (1)	-0.135(2)	4(1)
C(11)	0.095(2)	0.1094(9)	-0.237(2)	3.7(8)
C(12)	0.235(2)	0.1370(8)	-0.110(2)	2.5(8)
C(13)	0.277(2)	0.2087(9)	-0.117(2)	2.8(8)
C(14)	0.202(2)	0.2395(9)	-0.261(2)	3.8(8)
C(15)	0.061(2)	0.2147(8)	-0.382(2)	2.9(7)
C(16)	0.014(2)	0.149(1)	-0.3671(2)	4.1(8)
C(17)	0.423(2)	0.237(1)	0.0301(2)	3.2(8)
C(18)	0.560(2)	0.2085(8)	0.0591(2)	2.5(7)
C(19)	0.690(2)	0.2422(8)	0.1961(2)	3.3(7)
C(20)	0.684(2)	0.3038(8)	0.2791(2)	2.8(7)
C(21)	0.544(2)	0.3369(9)	0.2584(2)	3.4(7)
C(22)	0.419(2)	0.2974(8)	0.1161(2)	3.0(7)
C(23)	0.179(2)	0.343(1)	-0.4161(2)	6(1)
C(24)	0.270(2)	0.3812(8)	0.1811(2)	3.2(7)
C(25)	0.385(2)	0.4239(8)	0.3121(2)	3.6(8)
C(26)	0.539(2)	0.395(1)	0.3501(2)	4.5(2)
C(27)	0.111(2)	0.4039(9)	0.1351(2)	3.7(8)
C(28)	0.001(2)	0.3605(8)	-0.0051(2)	4.0(8)
C(29)	-0.145(2)	0.377(1)	-0.0441'2)	4.4(8)
C(30)	-0.182(2)	0.4306(8)	0.0481(2)	3.5(7)
C(31)	-0.073(2)	0.4710(9)	0.1891(2)	3.8(8)
C(32)	0.073(2)	0.4539(9)	0.231(2)	4.0(8)
C(33)	-0.613(2)	-0.188(1)	-0.616(2)	6(1)

In conclusion, molecular conformations of ginkgetin (**1**) and sciadopitysin (**2**) in the liquid state, and sciadopitysin in the solid state were elucidated for the first time by nmr and X-ray analysis. It was established that **2** assumes a similar conformation in both the liquid and the solid states as shown in Figures 1 and 2. Complete assignments of the ^{1}H and ^{13}C nmr were first achieved for ginkgetin.

EXPERIMENTAL

General Experimental Procedures.

Melting points were determined on a micro hot-stage apparatus and are uncorrected. Specific rotation were taken on a JASCO DPI-181 polarimeter. The nmr spectra were measured on a Varian XL-400 spectrometer. The hrms were recorded on a JEOL JMX DX-300. The 2D nmr and DEPT experiments were performed using Varian's standard pulse sequences.

Extraction and Isolation.

The branches of *Taxis cuspidata* SIEB. and ZUCC. were collected at the Jiling province in China. Plant material was identified by Professor Y. Gio, Shenyang Pharmaceutical University, and a voucher specimen is deposited in the Herbarium of Shenyang Pharmaceutical University. The air-dried, powdered plant material (3.0 kg) was extracted with boiling ethanol (36 l) for 2 hours to give the ethanol extract (620 g). Water (620 ml) was added to the ethanol extract, which was then extracted with chloroform to give the extract (30 g). The chloroform extract was chromatographed over silica gel and eluted with a mixture of chloroform and methanol. The eluate with (chloroform-methanol = 98:2) afforded sciadopitysin (**2**) (40 mg) and the eluate with (chloroform-methanol = 9:1) afforded ginkgetin (**1**) (10 mg).

X-Ray Data for **2**.

The crystals of **2** used for X-ray analysis was prepared by slow evaporation of a saturated acetone solution of **2** as yellow needles. The diffraction intensities were measured on a Rigaku AFC-5R diffractometer with graphite monochromated CuKα (λ = 1.54178 Å) radiation at 20°. Crystal data and other information are summarized in Table 3. The ω-2θ scan mode with a scan rate of

16°/minute was employed with the ω scan range (1.20 + 0.3 tan θ)°; a total of 4905 reflections with $|F_o|>3\sigma |F_o|$ were collected. The collected reflection intensities were corrected for Lorenz and polarization factors. The structures were solved by patterson and direct methods using the program DIRDIF and MITHRIL, respectively. The non-hydrogen atoms were refined by the full-matrix least-squares method with anisotropic temperature factors. The positions of all hydrogen atoms were calculated. At the final refinement, 1157 reflections, out of 4905 unique reflections with $|F_o|>4\sigma |F_o|$ were used. Final R was 0.074 (R_w = 0.091). Atomic scattering factors were taken from International Tables for X-Ray Crystallography. The final positional and thermal parameters of **2** are listed in Table 5. All calculations were performed using the TEXSAN crystallographic software package of Molecular Structure Corporation. Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

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