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Biflavonoids from the Leaves of *Cephalotaxus koreana* Nakai

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Abstracts – Four biflavonoids were isolated from the leaves of *Cephalotaxus koreana*. Based on spectroscopic data, the compounds were identified as amentoflavone-7',7",4',4"'-tetramethyl ether, sciadopitysin, ginkgetin and bilobetin, respectively.
Key words – *Cephalotaxus koreana*; leaves, biflavonoids, amentoflavone-7',7",4',4"'-tetramethyl ether, sciadopitysin, ginkgetin, bilobetin.

Introduction

Cephalotaxus koreana Nakai is a plant which is indigenous to Korea and distributed in the southern distinct of Korea Peninsula. The leaves of this plant have been used for pierced wounds by insects and the fruits have been used as parasiticide (Yook. C. S., 1993). Several biflavonoids have been isolated from Cephalotaxus species (Khan *et al.*, 1971; Aqil *et al.*, 1976; Ishratullah *et al.*, 1981; Kamil *et al.*, 1982; Ma *et al.*, 1984). However, there have been no reports on constituents of *C. koreana*. As a part of study on this crude drug, we report here the isolation and structural determination of four biflavonoids, amentoflavone-7',7",4',4"'-tetramethyl ether, sciadopitysin, ginkgetin and bilobetin, from the leaves of this medicinal plant.

Materials and Methods

General experimental procedures – Mps was measured using a MEL-TEMP II melting point apparatus. Optical rotations were determined on a JASCO DIP-1000 KUY polarimeter ($l = 0.5$). UV was measured using a HITACHI UV spectrophotometer. IR spectra were obtained with a Perkin-Elmer 283B IR spectrophotometer. FABMS spectra were obtained in a 3-nitrobenzylalcohol matrix in the positive ion mode using VG70-VSEQ and VG analytical UK. NMR spectra were measured in DMSO-d_6 on Bruker AMX500 spectrometer and solvent signals used as internal standards. Column chromatography was carried out with silica gel 60 (70-230 mesh, Merck). TLC was performed on precoated silica gel $60F_{254}$ (Merck).

Plant material – The leaves of *C. koreana* were collected at Mt. Nae-Jang and Mt. Back-Yang in Korea in July 1995. A voucher specimen is deposited at the Herbarium of College of Pharmacy, Kyung Hee University in Korea.

Extraction and isolation – The dried leaves (2.6 kg) of *C. koreana* were cut into small pieces and extracted with 70% MeOH in reflux. The filtrate was evaporated *in vacuo* to give an extract (about 400 g), a portion of which (30 g) was chromatographed on silica gel and eluted with $CHCl_3$-MeOH by gradient. A mixture of crude crystals were obtained from Fr. 68-74 and further separated by silica gel column chromatography by eluting with $CHCl_3$-MeOH (9:1) to yield three compounds which were recrystallized from MeOH to give **1** (10 mg), **2** (14 mg), **3** (30 mg). A crude crystal was obtained from Fr. 83-90 and recrystallized from MeOH to give **4** (22 mg).

Compound 1 (Amentoflavone-7,7",4',4"'-tetramethyl ether): A pale yellow amorphous powder, mp 292-294°C, UV λ_{max}^{MeOH}nm: 270, 323; +NaOMe: 285, 365sh; +$AlCl_3$: 279, 301sh, 344, 385sh; +$AlCl_3$/HCl: 279, 302sh, 340, 384sh; +NaOAc: 267, 327; +NaOAc/H_3BO_3: 270, 327; FABMS: *m/z* 595 $[M+H]^+$; ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

Compound 2 (Sciadopitysin): A pale yellow amorphous powder, mp 295-297°C, IR $\nu_{max}^{KBr}cm^{-1}$: 3400 (OH), 1660 (conjugated C=O), 1605, 1580,

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1510 (aromatic C=C); UV λ_{max}^{MeOH}nm: 271, 323; +NaOMe: 284, 364; +$AlCl_3$: 260sh, 280, 302sh, 344, 383sh; +$AlCl_3$/HCl: 259sh, 281, 301sh, 340, 383sh; +NaOAc: 270, 283, 316sh, 339; +NaOAc/H_3BO_3: 270, 326; FABMS: *m/z* 581 $[M+H]^+$; ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

Compound 3 (Ginkgetin): A pale yellow amorphous powder, mp 338-340°C, IR $\nu_{max}^{KBr}cm^{-1}$: 3400 (OH), 1664 (conjugated C=O), 1610, 1585, 1502, 1446 (aromatic C=C); UV λ_{max}^{MeOH}nm: 271, 328; +NaOMe: 282, 328, 392; +$AlCl_3$: 280, 301sh, 349, 387sh; +$AlCl_3$/HCl: 280, 300sh, 343, 382sh; +NaOAc: 273, 281sh, 322sh, 340, 395sh; +NaOAc/H_3BO_3: 272, 330; FABMS: *m/z* 567 $[M+H]^+$; ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

Compound 4 (Bilobetin): A pale yellow amorphous powder, mp 365-367°C, IR $\lambda_{max}^{KBr}cm^{-1}$: 3400 (OH), 1660 (conjugated C=O), 1610, 1580, 1504, 1446 (aromatic C=C); UV λ_{max}^{MeOH}nm: 270, 329; +NaOMe: 279, 389; +$AlCl_3$: 260sh, 280, 301sh, 347, 388sh; +$AlCl_3$/HCl: 260sh, 280, 301sh, 344, 388sh; +NaOAc: 276, 303sh, 373; +NaOAc/H_3BO_3: 271, 331; FABMS: *m/z* 553 $[M+H]^+$; ^{1}H- and ^{13}C-NMR: see Tables 1 and 2.

Results and Discussion

The dried leaves were extracted with 70% MeOH. The extract were chromatographed on silica gel with $CHCl_3$-MeOH. A mixture of crude crystals was separated by silica gel column chromatography to afford compounds **1-4**. Compounds **1-4** were obtained as pale yellow amorphous powders and were positive to Mg-HCl reagent and $FeCl_3$ reagent, indicating that these compounds are phenolic compounds. The IR spectra of **1-4** showed absorption bands due to phenolic hydroxyl groups, conjugated C=O groups and aromatic C=C groups. The MeOH solutions of **1-4** showed UV absorption maxima at 270-271 nm (band II) and 323-329 nm (band I). Furthermore, the TLC chromatographic spots of **1-4** showed deep purple fluorescences under UV lights, and turned into yellow fluorescences by spraying with 5% alcoholic $AlCl_3$ solution, indicating that they are biflavonoids.

Compound **1** exhibited molecular ion peak due to $[M+1]^+$ at *m/z* 595 ($C_{34}H_{26}O_{10}$ + H) in positive FAB-MS spectrum. The ^{1}H-NMR spectrum of **1** displayed signals due to four methoxyl groups at δ 3.76 (s), 3.80 (s), 3.84 (s) and 3.87 (s), three *meta*-coupled doublets of each one proton at δ 6.37 (*J*=2.2 Hz, H-6), 6.78 (*J*=2.2 Hz, H-8) and 8.09 (*J*=2.6 Hz, H-2'), two *ortho*-coupled doublets of each two protons at δ 6.93 (*J*=8.7 Hz, H-3''' and H-5''') and 7.61 (*J*=8.9 Hz, H-2''' and H-6'''), an *ortho*-coupled doublet of one proton at δ 7.38 (*J*=8.9 Hz, H-5'), a doublet of doublets of one proton at δ 8.24 (*J*=8.8, 2.6 Hz, H-6') and three singlets of each one proton at δ 6.69 (H-6''), 6.99 (H-3'') and 7.01 (H-3) as listed in Table 1. However signals of H-3' and H-8'' were not observed. These results suggested that compound **1** is C-3'-C-8'' linked biflavonoids (Shin and Kim, 1991). The ^{13}C-NMR spectrum of **1** showed thirty-four carbon signals including four methoxy carbon signals at δ 55.5, 56.0, 56.1 and 56.5 as listed in

Table 1. ^{1}H NMR (500 MHz) spectral data of compounds **1 - 4** in DMSO-d_6 (δ values in ppm)†

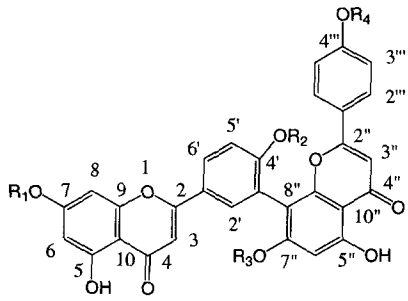
No. of H	1	2	3	4
3	7.01 s	6.88 s	6.88 s	6.91 s
6	6.37 d (2.2)	6.36 d (2.2)	6.36 d (2.2)	6.19 d (2.0)
8	6.78 d (2.2)	6.79 d (2.2)	6.78 s	6.48 d (2.0)
2'	8.09 d (2.6)	8.09 d (2.4)	8.09 d (2.3)	8.06 d (2.3)
5'	7.38 d (8.9)	7.37 d (8.9)	7.36 d (8.9)	7.35 d (8.8)
6'	8.24 dd (8.8, 2.6)	8.22 dd (8.8, 2.6)	8.22 dd (8.7, 2.3)	8.18 dd (8.8, 2.3)
3''	6.99 s	6.98 s	6.99 s	6.78 s
6''	6.69 s	6.41 s	6.38 s	6.39 s
2''', 6'''	7.61 d (8.9)	7.60 d (8.9)	7.49 d (8.8)	7.50 d (8.8)
3''', 5'''	6.93 d (8.7)	6.93 d (8.9)	6.72 d (8.8)	6.71 d (8.8)
-OMe	3.76 s	3.76 s	3.79 s	3.78 s
	3.80 s	3.80 s	3.88 s	---
	3.84 s	3.83 s	---	---
	3.87 s	---	---	---

†J values (in Hz) in Parentheses.

Table 2. ^{13}C NMR (125 MHz) spectral data of compound 1-4 in DMSO-d_6 (δ values in ppm)

No. of C	1	2	3	4
2	162.6	163.0	163.1	163.3
3	103.2	103.6	103.7	103.5
4	182.0	181.9	181.9	181.7
5	161.5	161.1	161.1	161.4
6	98.1	98.1	98.7	98.6
7	165.2	165.2	165.2	163.5
8	92.8	92.7	92.7	94.1
9	157.3	157.3	157.4	157.4
10	104.8	104.7	104.4	103.7
1'	122.5	122.4	122.4	122.4
2'	128.5	128.3	128.2	128.1
3'	121.3	121.7	121.8	121.6
4'	161.1	160.6	160.6	160.6
5'	111.9	111.7	111.7	111.7
6'	130.8	130.9	130.8	130.9
2"	163.5	163.6	163.4	164.2
3"	103.2	103.2	102.5	102.5
4"	182.3	182.0	181.9	181.7
5"	160.5	160.5	160.4	160.4
6"	95.6	98.7	98.7	98.9
7"	161.1	162.6	162.1	161.8
8"	103.9	103.7	103.8	103.6
9"	153.3	154.3	154.3	154.3
10"	104.1	103.6	103.7	103.5
1'"	122.7	122.8	121.2	121.2
2'"	127.9	127.8	127.9	128.0
3'"	114.5	114.5	115.8	115.8
4'"	162.3	162.2	161.0	161.0
5'"	114.5	114.5	115.8	115.8
6'"	127.9	127.8	127.9	128.0
-OMe	55.5	55.5	55.9	55.8
	56.0	55.9	56.3	---
	56.1	56.0	---	---
	56.5	---	---	---

Table 2. The comparison of ^{13}C-NMR spectral data of compound **1** with those of the known biflavonoid, amentoflavone (Chari *et al.*, 1977; Markham *et al.*, 1987; Joly *et al.*, 1980; Shin and Kim, 1991), revealed substituent effect of 4'-*O*-methylation (about 4 ppm upfield shift of C-5' and 2 ppm downfield shift of C-1'), 4'"-*O*-methylation (about 1.5 ppm upfield shift of C-3'" and C-5'" and 2 ppm downfield shift of C-1'"), 7-*O*-methylation (about 1.5 ppm upfield shift of C-8) and 7"-*O*-methylation (about 3.5 ppm upfield shift of C-6") (Chari *et al.*, 1977; Markham *et al.*, 1987). All of chemical shifts of **1** were well agreed to those of amentoflavone-7,7",4',4'"-tetramethyl ether (Okuyama *et al.*, 1979; Joly *et al.*, 1980; Ishratullah *et al.*, 1981; Ma *et al.*, 1984; Markham *et al.*, 1987). Based on the above data, **1** was identified to be as amentoflavone-7,7",4',4'"-tetramethyl ether.



Amentoflavone-7,7",4',4'"-tetramethyl ether: R_1, R_2, R_3, R_4 = CH_3
Sciadopitysin : R_1, R_2, R_4 = CH_3 ; R_3= H
Ginkgetin: R_1, R_2 = CH_3 ; R_3, R_4 = H
Bilobetin : R_1, R_3, R_4 = H ; R_2 = CH_3

Fig. 1. Chemical Structure of Biflavonoid from the leaves of C. koreana.

Compound **2** exhibited molecular ion peak due to $[M+1]^+$ at *m/z* 581 ($C_{33}H_{24}O_{10}$ + H) in positive FAB-MS spectrum. The ^{13}C-NMR spectrum of **2** showed thirty-three carbon signals. The ^{13}C- and ^1H-NMR spectral data of **2** were similar to those of **1**. However, one methoxy group at δ 56.5 in ^{13}C-NMR and 3.87 (s) in ^1H-NMR spectra of **1** was not observed in those of **2**. The difference of ^{13}C-NMR spectral data of compound **2** with those of amentoflavone, revealed substituent effect of 4'-*O*-methylation, 4'"-*O*-methylation and 7-*O*-methylation. However substituent effect of 7"-*O*-methylation was not observed. From the spectral data, it is obvious that one methoxy group at C-7" of **1** is substituted to one hydoxyl group in **2**. All chemical shifts of **2** are well agreed to those of sciadopitysin (Khan *et al.*, 1971; Okuyama *et al.*, 1979; Ma *et al.*, 1984; Kang *et al.*, 1990). Based on the above data, **2** was identified to be as sciadopitysin.

Compound **3** exhibited molecular ion peak due to $[M+1]^+$ at *m/z* 567 ($C_{32}H_{22}O_{10}$+H) in positive FAB-MS spectrum and ^{13}C-NMR spectrum of **3** showed thirty-two carbon signals. The ^{13}C- and ^1H-NMR spectral data of **3** were similar to those of **2**. However, one methoxy group at δ 56.0 in ^{13}C-NMR and δ 3.83 (s) in ^1H-NMR spectra of **2** was not observed in those of **3**. The difference of ^{13}C-NMR spectral data of compound **3** with those of amentoflavone, revealed substituent effect of 4'-*O*-methylation and 7-*O*-methylation. However substituent effect of 4'"-*O*-methylation and 7"-*O*-methylation was not

observed. From the spectral data, it is obvious that one methoxy group at C-4''' of **2** is substituted to one hydoxyl group in **3**. All chemical shifts of **3** were well accorded with those of ginkgetin (Khan *et al.*, 1971; Ishratullah *et al.*, 1981; Markham *et al.*, 1987; Kang *et al.*, 1990). Based on the above data, **3** was identified to be as ginkgetin.

Compound **4** showed molecular ion peak due to $[M+1]^+$ at *m/z* 553 ($C_{31}H_{20}O_{10}$+H) in positive FAB-MS spectrum. ^{13}C-NMR spectrum of **4** exhibited thirty-one carbon signals. The ^{13}C- and ^{1}H-NMR spectral data of **4** were similar to those of **3**. However, one methoxy group at δ 56.3 in ^{13}C-NMR and δ 3.88 (s) in ^{1}H-NMR spectra of **3** was not observed in those of **4**. When we compared ^{13}C-NMR spectral data of compound **4** with those of amentoflavone, only substituent effect of 4'-*O*-methylation was observed. From the spectral data, we could presumed that one methoxy group at C-7 of **3** was substituted to one hydoxyl group in **4**. All chemical shifts of **4** were well agreed to those of bilobetin (Joly *et al.*, 1980; Markham *et al.*, 1987; Kang *et al.*, 1990). Based on the above data, **4** was identified to be as bilobetin.

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