A Novel Cytotoxic C-Methylated Biflavone from the Stem of *Cephalotaxus wilsoniana*

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Bioassay-directed fractionation of an ethanolic extract of *Cephalotaxus wilsoniana* has resulted in the isolation of a novel C-methylated biflavone, taiwanhomoflavone-A (1). Its structure was elucidated on the basis of spectroscopic analysis. Taiwanhomoflavone-A is cytotoxic with ED₅₀ values of 3.4, 1.0, 2.0 and 2.5 µg/ml, respectively, against KB epidermoid carcinoma of nasopharynx, COLO-205 colon carcinoma, Hepa-3B hepatoma, and Hela cervix tumor cells.

Key words *Cephalotaxus wilsoniana*; cytotoxicity; taiwanhomoflavone-A; 6-C-methyl-7, 4'-O-dimethylamentoflavone

Several antitumor alkaloids^{1–6)} have been reported from *Cephalotaxus* spp. *Cephalotaxus wilsoniana* HAYATA (Cephalotaxaceae) is an evergreen tree distributed over the middle mountain of Taiwan. During a search for antitumor agents from the terrestrial plants of Taiwan,^{8–11)} we found a crude extract from *C. wilsoniana* which possesses inhibitory effect against KB, Hepa-3B, and Hela cancer cell lines. Bioassay-guided fractionation of an EtOH extract led to the isolation of a novel C-methylated biflavone (1) in addition to the known compounds, harringtonolide (=hainanolide), *epi*-wilsonine, sugiol, isopimaric acid and kayaflavone. In this paper we describe the structural elucidation of 1 using H–H correlation spectroscopy (COSY), ¹³C–¹H heteronuclear multiple quantum coherence (HMQC) and ¹³C–¹H heteronuclear multiple bonds coherence (HMBC) experiments.

The molecular formula of taiwanhomoflavone (1) (C₃₃H₂₄O₁₀) was indicated by a molecular ion (*m/z* 579 [M–H]⁺) in the FAB-MS spectra. The IR spectrum suggested that 1 contained hydroxyl, conjugated carbonyl and aromatic functions.

Observing the ¹H-NMR spectrum of 1, an AMX coupling system with signals at δ 8.49 (H-2'), 8.08 (H-6'), and 7.29 (H-5') revealed a 1,3, 4-trisubstituted benzene ring. The signals at δ_H 7.76 (H-2'', 6'', d, *J*=8.5 Hz) and δ_H 7.15 (H-3'', 5'', d, *J*=8.5 Hz), together with the signals of δ_C 128.56 (C-2'', 6''), and 116.81 (3'', 5'') in the ¹³C-NMR spectrum indicated another aromatic moiety in an A₂B₂ coupling system. In addition, four aromatic protons in each singlet, two aromatic methoxys in singlet, and a unique methyl group in sin-

glet were observed in the ¹H-NMR spectrum. This evidence revealed that 1 has a biflavone skeleton with a C-methyl group and excluded the possibility of linkage between the two flavone moieties at C-2'', -6'', -3'' and -5'' in the B-ring.

In the HMBC spectrum, the correlations between C-8'' and H-6'' and H-2' revealed the connective positions to be at C-3'

Table 1. ¹H- and ¹³C-NMR Data^{a)} (Pyridine-*d*₅) for Compound 1

Carbon	¹³ C (ppm)	¹ H (ppm)	¹³ C– ¹ H connectivities ^{b)}
2	164.14 s	—	H-3
3	104.76 d	7.12 (s)	—
4	182.73 s	—	H-3
4a	105.54 s	—	H-3
5	158.83 s	—	6-Me
6	108.67 s	—	H-8, 6-Me
7	163.64 s	—	H-8, OMe
8	90.31 d	6.71 (s)	—
8a	156.31 s	—	H-8
1'	123.25 s	—	H-5'
2'	131.87 d	8.49 (d, 2.0)	H-6'
3'	104.93 s	—	H-5'
4'	161.44 s	—	H-2', 6', OMe
5'	111.82 d	7.29 (d, 8.5)	—
6'	128.40 d	8.08 (dd, 2.0, 8.5)	H-2'
2''	164.36 s	—	H-3''
3''	103.44 d	6.95 (s)	—
4''	183.02 s	—	H-3''
4'a	104.85 s	—	H-3'', 6''
5''	162.40 s	—	H-3'', 6''
6''	99.31 d	6.93 (s)	—
7''	163.64 s	—	—
8''	104.76 s	—	H-2', 6''
8'a	155.53 s	—	—
1'''	122.18 s	—	H-3''', 5'''
2'''	128.56 d	7.76 (d, 8.5)	H-6'''
3'''	116.81 d	7.15 (d, 8.5)	H-5'''
4'''	162.67 s	—	H-3''', 5''', 2'', 6''
5'''	116.81 d	7.15 (d, 8.5)	H-3'''
6'''	128.56 d	7.76 (d, 8.5)	H-2'''
6-Me	7.60 q	2.22 (s)	— ^{c)}
7-O-Me	56.02 q	3.74 (s)	— ^{c)}
4'-O-Me	55.98 q	3.77 (s)	— ^{c)}

a) All assignments (¹³C; 75.5 MHz, multiplicity; ¹H; 300 MHz) are based on one dimensional (1D) and two dimensional (2D) NMR experiments, including COSY 90, HETCOR, and HMBC spectra. b) HMBC corresponded to two or three bonds connectivities. c) These assignments were explained in the text.

and C-8" between the flavones. Generally, the carbonyl carbon signal (C-4 or -4") observed near δ_C 183 for biflavones such as ginkgetin¹²⁾ would permit the assignment of a peri-OH at C-5 or -5". The signal for C-8 at δ_C 90.3 in **1** was consistent with the carbon bearing a methoxyl group at the C-7 position; otherwise, a chemical shift for C-8 would appear around δ_C 94.0 suggesting a hydroxyl group at C-7.¹²⁾ After detailed examination of the HMBC spectrum of **1**, a methyl group was assigned at C-6 due to the correlation between the methyl group and C-5 and C-6, respectively. Together with the above evidence, the structure of **1** was deduced to be an analogue of 6-*C*-methyl-7-*O*-methylamentoflavone (**2**)¹³⁾ except for a methoxyl group in **1** and a hydroxyl group in **2**. The remaining methoxyl group in **1** was assigned at C-4' due to the HMBC spectrum. Then, the structure of **1** was confirmed unambiguously, and was tentatively named taiwanhomoflavone-A.

Furthermore, bioassays showed that taiwanhomoflavone-A (**1**) exhibited cytotoxic effects against four cancer cell lines: KB, COLO-205, Hepa-3B and Hela with ED₅₀ of 3.45, 1.06, 2.03 and 2.53 μ g/ml, respectively. To our knowledge, this is the first report that 6-*C*-methyl biflavone as compound **1** has cytotoxic activity.

Experimental

General Experimental Procedures ¹H- and ¹³C-NMR spectra were recorded at 300.13 and 75.46 MHz, respectively, on a Bruker 300 AC spectrometer. The spectra of heteronuclear correlation, HMBC was established by the coupling of 8 Hz. Electron impact (EI)-MS and FAB-MS were performed on a JEOL SX-102A instrument. Si gel (Merck 70—230 mesh) was used for column chromatograph, and precoated Si gel (Merck 60F-254) plates were used for TLC. HPLC was accomplished on an SPD-6AV liquid chromatography using a preparative C₁₈ column. Melting points were determined on a Fisher-Johns apparatus and are uncorrected.

Plant Material The stems of *Cephalotaxus wilsoniana* were collected in September 1995 on Taipei, Taiwan. A voucher specimen is deposited at the National Research Institute of Chinese Medicine, Shih-Pai, Taipei, Taiwan, R.O.C.

Extraction and Isolation The dried stems of *C. wilsoniana* (6.3 kg) were extracted exhaustively with ethanol. An EtOH extract (200 g) of dried

stems of *Cephalotaxus wilsoniana* was extracted successively with *n*-hexane and CHCl₃. The CHCl₃ extract was chromatographed by column chromatography over Si gel and eluted with *n*-hexane-EtOAc and EtOAc to give 8 fractions. The bioactive fr. 5 (*n*-hexane : EtOAc = 2 : 1) was further separated by HPLC (5C₁₈, 250×10 mm) with MeOH-H₂O (9 : 1) to furnish taiwanhomoflavone-A (**1**) (11 mg).

Taiwanhomoflavone-A (1**):** Pale yellow crystal, mp 245—248 °C; IR $\nu_{\max}$ (KBr) 3400 (OH), 1660 (conjugated CO), 1620 (aromatic rings) cm⁻¹; ¹H- and ¹³C-NMR, see Table 1; FAB-MS *m/z* 579 [M-H]⁺; HR-EI-MS *m/z* 580.1379 [M]⁺ (Calcd for C₃₃H₂₄O₁₀, 580.1369).

Cytotoxicity Assay An *in vitro* cytotoxicity assay was performed as previously described.¹¹⁾

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