Formosadimers A, B, and C from the Bark of *Calocedrus macrolepis* var. *formosana*

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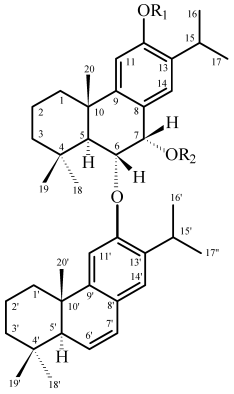
Received April 20, 2005; accepted July 5, 2005

Formosadimers A, B, and C, together with one known compound, sugikurojin B, have been isolated from the bark of *Calocedrus macrolepis* var. *formosana*. Formosadimers A, B, and C, and sugikurojin B are dimers of the abietane-*O*-abietane type. Their structures were elucidated principally based on spectroscopic data.

Key words *Calocedrus macrolepis* var. *formosana*; bark; abietane-*O*-abietane; formosadimer A; formosadimer B; formosadimer C

Calocedrus macrolepis var. *formosana* (=*C. formosana*), commonly called shonan, is an endemic conifer. This plant, a member of the Cupressaceae family, is an economically important tree and indigenous to Taiwan. It is used as an important material in architecture, furniture, and sculpture. The tree grows at elevations ranging from 300 to 2000 m in the central and northern mountains of Taiwan. Previous investigations of the chemical constituents of its wood[1—3] resulted in the isolation of tropolones, monoterpenes, naphthalene-type sesquiterpenes, diterpenoid phenols, lignans, and terpenoid acids. From its leaves,[4—6] researchers have derived a mixture of fatty acids, benzenoids, lignans, sesquiterpenes, diterpenoids, steroids, and pheophorbides. Some of these compounds exhibited cytotoxic activity. In the bark,[7,8] only phenolic diterpenes, such as xanthoperol, ferruginol, and sugiol, have been found. Because only limited findings were reported nearly 30 years ago, we reinvestigated the bark constituents of this plant. In this paper, we report the extraction, isolation, purification, and structure elucidation of three new dimeric compounds, formosadimers A (**1**), B (**2**), and C (**3**), and of the known compound sugikurojin B (**4**).[9] These new structures and known compound are dimers of the abietane-*O*-abietane type.

Formosadimer A (**1**), on the basis of exact mass (HR-EI-MS) at *m*/*z* 598.4385, had the molecular formula $C_{41}H_{58}O_3$, suggesting the presence of 13 degrees of unsaturation. The IR spectrum showed hydroxyl (3459 cm^{-1}) and aromatic (3051, 1605, 1499 cm^{-1}) absorptions. By comparing the ^{1}H- and ^{13}C-NMR data (Tables 1, 2) together with high-resolution mass of **1** with those of sugikurojin B (**4**), compound **1** was found to be an isomeric compound of **4**. The methyl on C-7′ OH of **4** migrated to C-12 OH and formed **1**. A signal at δ_H 3.86 (3H, s) was assigned to the phenolic methyl group instead of phenolic proton. Thus **1** is 6,7-dehydroabieta-8,11,13-trien-12-yl 7α-hydroxy-12-methoxyabieta-8,11,13-trien-6α-yl ether.



1 $R_1 = CH_3$, $R_2 = H$
2 $R_1 = H$, $R_2 = CH_2CH_2OCH_2CH_2CH_2CH_3$
3 $R_1 = Ac$, $R_2 = CH_2CH_2OCH_2CH_2CH_2CH_3$
1" 2" 3" 4" 5" 6"
4 $R_1 = H$, $R_2 = CH_3$

Formosadimer B (**2**) had the molecular formula $C_{46}H_{68}O_4$ based on the exact mass (HR-EI-MS) at *m*/*z* 684.5080. It showed hydroxyl (3364 cm^{-1}) and aromatic (3050, 1604, 1496 cm^{-1}) absorptions in its IR spectrum. Based on the results of ^{1}H- and ^{13}C-NMR data analysis (Tables 1, 2) together with 2D NMR, compound **2** was found to be composed of two dimeric dehydroabietanes and another add-on C_6 ether group chain related to the butoxyethoxyl group. Comparison of the all physical data with those of sugikurojin B (**4**) showed the difference is a side chain of the butoxyethoxyl group linked on C-7 in **2** instead of methoxy group in **4**. Six ^{1}H-NMR signals and six ^{13}C-NMR signals (Tables 1, 2) in addition to their correlated spectroscopy (COSY) and heteronuclear multiple bond correlation spectroscopy (HMBC) (Fig. 1) confirmed the presence of a butoxyethoxy group. The methine proton at δ_H 4.13 was assigned as H-7, which attached to a butoxyethoxy group as revealed from the HMBC correlation (Fig. 1) and nuclear Overhauser en-

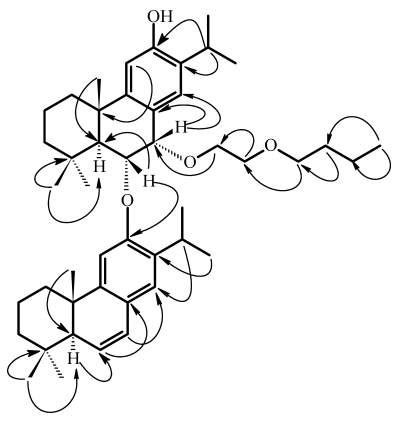


Fig. 1. Major HMBC of **2**

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Table 1. ^{1}H-NMR Spectral Data of Compounds **1**—**3** (500 MHz), δ in ppm, (J in Hz)

No.	**1**[a)]	**2**[b)]	**3**[b)]
1	1.62 m, 2.29 br d (13.9)	1.55 m, 2.11 br d (12.4)	1.55 m, 2.10 br d (12.6)
2	1.68 m, 1.85 m	1.68 m, 1.85 m	1.68 m, 1.85 m
3	1.29 m, 1.49 m	1.27 m, 1.49 m	1.27 m, 1.49 m
5	1.64 d (8.6)	1.61 d (8.5)	1.65 d (8.5)
6	4.91 d (8.6)	4.92 d (8.5)	4.94 d (8.5)
7	4.63 br d (3.1)	4.13 br s	4.17 br s
11	6.89 s	6.70 s	6.92 s
14	7.04 s	6.87 s	6.99 s
15	3.22 sep (7.1)	3.11 sep (6.8)	2.93 sep (6.9)
16	1.10 d (7.1)	1.16 d (6.8)	1.11 d (6.9)
17	1.12 d (7.1)	1.20 d (6.8)	1.15 d (6.9)
18	0.97 s	0.90 s	0.90 s
19	1.18 s	1.16 s	1.15 s
20	1.49 s	1.39 s	1.39 s
1′	1.64 m, 2.34 br d (12.6)	1.61 m, 2.25 br d (12.0)	1.69 m, 2.25 br d (13.2)
2′	1.68 m, 1.79 m	1.71 m, 1.80 m	1.71 m, 1.80 m
3′	1.28 m, 1.53 m	1.29 m, 1.52 m	1.29 m, 1.52 m
5′	2.18 dd (2.2, 3.2)	2.14 dd (2.5, 2.9)	2.14 t (2.9)
6′	5.90 dd (2.2, 9.6)	5.88 dd (2.5, 9.5)	5.88 dd (2.9, 9.5)
7′	6.53 dd (3.2, 9.6)	6.51 dd (2.9, 9.5)	6.49 dd (2.9, 9.5)
11′	7.28 s	7.00 s	6.99 s
14′	6.95 s	6.90 s	6.91 s
15′	3.22 sep (7.0)	3.11 sep (6.8)	3.13 sep (6.8)
16′	1.05 d (7.0)	1.03 d (6.8)	1.04 d (6.8)
17′	1.07 d (7.0)	1.06 d (6.8)	1.05 d (6.8)
18′	0.99 s	0.97 s	0.97 s
19′	1.07 s	1.05 s	1.05 s
20′	1.05 s	1.10 s	1.09 s
C-12 OH		4.78 s	
C-12 C$\underline{H}_3$O	3.86 s		
C-7 OH	4.37 d (3.1)		
C-12 OAc			2.31 s
1″		3.44[c)]	3.43[c)]
2″		3.34[c)]	3.34[c)]
3″		3.33[c)]	3.32[c)]
4″		1.46 m	1.45 m
5″		1.26 m	1.27 m
6″		0.86 t (7.4)	0.85 t (7.4)

a) In CD_3COCD_3; *b*) in $CDCl_3$; *c*) overlapping.

Table 2. ^{13}C-NMR Spectral Data of Compounds **1**—**3** (125 MHz), δ in ppm

No.	**1**[a)]	No.	**1**[a)]	No.	**2**[b)]	**3**[b)]	No.	**2**[b)]	**3**[b)]
1	39.1	5′	51.1	1	39.6	39.5	5′	51.1	51.0
2	18.8	6′	126.7	2	19.1	19.1	6′	127.4	127.4
3	42.9	7′	127.4	3	42.8	42.7	7′	127.3	127.2
4	34.0	8′	125.5	4	34.3	34.3	8′	125.6	125.6
5	56.0	9′	147.0	5	55.9	55.5	9′	147.0	146.9
6	79.4	10′	38.1	6	78.2	78.1	10′	38.2	38.1
7	73.5	11′	106.9	7	80.5	80.6	11′	106.6	106.5
8	128.9	12′	153.5	8	125.0	130.4	12′	153.1	152.9
9	149.2	13′	134.2	9	150.8	150.6	13′	135.0	135.0
10	38.5	14′	124.5	10	38.0	38.1	14′	124.7	124.7
11	105.1	15′	25.3	11	110.4	117.2	15′	25.7	25.5
12	156.7	16′	22.9	12	153.1	148.4	16′	23.1	23.2
13	133.8	17′	22.4	13	130.7	136.7	17′	23.1	23.2
14	127.7	18′	32.1	14	129.5	129.4	18′	32.6	32.6
15	26.1	19′	22.0	15	26.6	27.0	19′	22.6	22.6
16	22.1	20′	19.7	16	22.7	22.9	20′	20.4	20.4
17	22.2	CH_3O–	54.9	17	22.9	23.1	$CH_3\underline{C}OO$–	—	169.7
18	34.9			18	34.9	35.0	$\underline{C}H_3COO$–	—	21.0
19	22.4			19	22.5	22.8	1″	70.0	69.9
20	25.0			20	24.7	24.6	2″	66.4	66.7
1′	36.0			1′	36.2	36.2	3″	71.0	71.0
2′	18.8			2′	19.1	19.2	4″	31.7	31.7
3′	40.9			3′	41.1	41.1	5″	19.2	19.2
4′	32.6			4′	32.9	32.9	6″	13.9	13.9

a) In CD_3COCD_3; *b*) in $CDCl_3$.

chancement-exchange spectroscopy (NOESY) correlation with H-14. Based on the coupling constant of H-7 (br s), H-6 (J=8.5 Hz) and H-5 (J=8.5 Hz) determining H-7 and H-6 are in the equatorial and axial orientation, respectively, and the AB ring is *trans*-fused. Moreover, H-6 also exhibited HMBC correlation with the other dehydroxyferruginol aromatic C-12′ (δ_C 153.1). The second moiety of **2** was identified as a 6,7-dehydroferruginol[10] since the NMR spectral data are similar to those of **1** and **4** (Tables 1, 2). Hence **2** is 7α-butoxyethoxy-12-hydroxyabieta-6α-yl 6,7-dehydroabieta-8,11,13-trien-12-yl ether.

The IR spectrum of formosadimer C (**3**) showed the presence of phenolic acetate (1760 cm^{-1}) and aromatic (3049, 1601, 1494 cm^{-1}) groups. HR-EI-MS at *m/z* 726.5164 established the molecular formula to be $C_{48}H_{70}O_5$. The ^{1}H- and ^{13}C-NMR spectra (Tables 1, 2) of **3** were similar to those of **2**. The only difference was an additional acetyl group at C-12 OH. The signals [δ_H 2.31 (s, 3H); δ_C 21.0 (t), 169.7 (s)] showed one phenolic acetyl. To verify this finding, acetylation of **2** with Ac_2O in pyridine was performed, giving a product that was identical to compound **3**. Thus the structure of formosadimer C was established to be 12-acetoxy-7α-butoxyethoxyabieta-6α-yl 6,7-dehydroabieta-8,11,13-trien-12-yl ether.

Experimental

General Experimental Procedures. General Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 983 G spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance 500 NMR. EI-MS and specific rotation were performed using a JEOL JMS-HX 300 mass spectrometer and JASCO DIP-180 digital polarimeter, respectively. Extracts were chromatographed on silica gel (Merk 70—230 mesh, 230—400 mesh, ASTM) and purified with a semipreparative normal-phase HPLC column (250×10 mm, 7 μm, LiChrosorb Si 60) on an LDC Analytical-III.

Plant Materials The bark of *Calocedrus macrolepis* Kurz var. *formosana* (Florin) Cheng and L. K. Fu was collected in Nan-Tou, Taiwan (1998). The plant was identified by Dr. Shang-Tzen Chang, Department of Forestry. A voucher specimen (No. 223133) has been deposited at the Herbarium of the Department of Botany of National Taiwan University, Taipei, Taiwan.

Extraction and Isolation The dried bark of *C. macrolepis* var. *formosana* (16 kg) was extracted with Me_2CO (140 l) at room temperature (7 d×2). After removal of Me_2CO, H_2O was added to bring the total volume to 1 l. This suspended phase was extracted with EtOAc (1 l×3). Following evaporation, the combined EtOAc layers afforded a black syrup (734 g), which was purified by means of silica gel chromatography and repeated HPLC (normal phase on Lichrosorb Si 60), using the hexane–EtOAc gradient solvent system. Compounds **2** (3.6 mg), **3** (2.4 mg), **1** (4.8 mg), and **4** (2.4 mg) were eluted with 5%, 10%, 20%, and 20% EtOAc in hexane solvent systems, respectively.

6,7-Dehydroabieta-8,11,13-trien-12-yl 7α-Hydroxy-12-methoxyabieta-8,11,13-trien-6α-yl Ether (**1**): Gum; $[\alpha]_D^{23}$ +45.9° (c=0.4, MeOH). UV λ_{max} (MeOH) nm (log ε): 222 (4.34), 280 (4.04). IR (KBr) cm^{-1}: 3459, 3051, 1605, 1499, 1461, 1253. ^{1}H- and ^{13}C-NMR, see Tables 1 and 2. EI-MS (70 eV) (rel. int. %) *m/z*: 598 (M^+, 1), 580 ($M-H_2O^+$, 100), 565 (6), 323 (13), 314 (14), 298 (17), 284 (17), 241 (28), 213 (40); HR-EI-MS *m/z*: 598.4385 (Calcd for $C_{41}H_{58}O_3$, 598.4388).

7α-Butoxyethoxy-12-hydroxyabieta-6α-yl 6,7-Dehydroabieta-8,11,13-trien-12-yl Ether (**2**): Gum; $[\alpha]_D^{25}$ +85.8° (c=0.3, MeOH). UV λ_{max} (MeOH) nm (log ε): 223 (4.40), 283 (4.09). IR (KBr) cm^{-1}: 3364, 3050, 1604, 1496, 1460. EI-MS (70 eV) (rel. int. %) *m/z*: 684 (M^+, 2), 566 (14), 314 (15), 300 (22), 284 (88), 213 (78), 202 (100), 185 (78), 159 (41). ^{1}H- and ^{13}C-NMR, see Tables 1 and 2; HR-EI-MS *m/z*: 684.5080 (Calcd for $C_{46}H_{68}O_4$, 684.5120).

12-Acetoxy-7α-butoxyethoxyabieta-6α-yl 6,7-Dehydroabieta-8,11,13-trien-12-yl Ether (**3**): Gum; $[\alpha]_D^{25}$ +37.0° (c=0.2, MeOH); UV λ_{max} (MeOH) nm (log ε): 221 (4.27), 241 (4.01), 277 (3.76). IR (KBr) cm^{-1}: 3049, 1760, 1601, 1494, 1204; EI-MS (70 eV) (rel. int. %) *m/z*: 726 (M^+, 9), 608 (5), 566 (4), 443 (51), 325 (50), 300 (30), 284 (100), 213 (70), 241 (28), 202 (45). ^{1}H- and ^{13}C-NMR, see Tables 1 and 2; HR-EI-MS *m/z*: 726.5164 (Calcd for $C_{48}H_{70}O_5$, 726.5226).

Acetylation of (2) with Ac_2O–Pyridine Compound **2** (3 mg) was allowed to react with Ac_2O (0.5 ml) and pyridine (0.5 ml) at room temperature overnight. The usual work-up gave compound **3** (3 mg).

Acknowledgments The authors thank the National Science Council of the Republic of China for financial support (NSC 93-2323-B-002-010).

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