Three Abietane Diterpenes and Two Diterpenes Incorporated Sesquiterpenes from the Bark of *Cryptomeria japonica*

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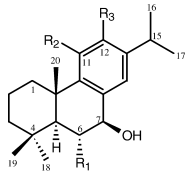
Three new abietane diterpenes, sugikurojins D (1), E (2), and F (3), and two new abietanes which incorporate cadinane, sugikurojins G (4) and H (5) were isolated from the bark of *Cryptomeria japonica*. These structures were elucidated primarily by extensive NMR experiments. The structure of sugikurojin D (1) was deduced to be 6α-acetoxy-7β,11-dihydroxy-12-methoxy-8,11,13-abietatriene. Sugikurojin E (2) was deduced to be 6α-acetoxy-7β,12-dihydroxy-8,11,13-abietatriene. Sugikurojin F (3) was 7α-methoxy-8,13-abietadien-11,12-dione. Sugikurojins G (4) and H (5) had a unique skeleton incorporating an α-cadinol or a 1α-hydroxy-T-cadinol in ferruginol, respectively. Also obtained in this investigation were the known diterpenes (6—14). An antibacterial activity of ten among these against *Staphylococcus aureus* and *Escherichia coli* was inactive at the (MIC: 125 μg/ml) level. Meanwhile, in the cytotoxic activity against HL-60, compounds 4, 8, and 11 showed moderate (IC_{50}: 4, 35.4; 8, 28.0; 11, 52.4 μM) though weak (IC_{50}: 4, 100; 8, 80.8; 11, 100 μM) activity against HCT-15.

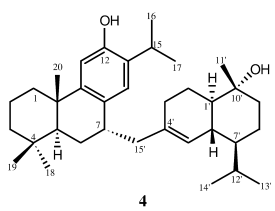
Key words *Cryptomeria japonica*; bark; diterpenoid; sesquiterpenoid; antibacterial activity; cytotoxicity

The Japanese cedar, *Cryptomeria japonica* D. DON. (Taxodiaceae), is a widely distributed conifer called *sugi* in Japanese. This wood is the most popular building material for Japanese housing.

Our previous chemical studies of the black heartwood of *C. japonica* D. DON. reported the isolation and structure determination of 42 compounds containing 7 new compounds and antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.[1,2] However, very little is known of the chemical constituents of the bark of *C. japonica*.[3] Further investigation of the bark of this plant material led us to isolate three new abietane diterpenes, sugikurojins D (**1**), E (**2**), and F (**3**), and two new abietanes incorporating cadinane, sugikurojins G (**4**) and H (**5**), along with 12-hydroxy-6,7-secoabieta-8,11,13-triene-6,7-dial (**6**),[4] ferruginol (**7**),[5] 7β-methoxydeoxocryptojaponol (**8**),[6] sugikurojin B (**9**),[1] 6,12,15-trihydroxy-5,8,11,13-abietatetraene-7-one (**10**),[7] 5,6-dehydrosugiol (**11**),[8] trilobine (**12**),[9] 6,7-dehydroferruginol (**13**),[10] and 11,14-dihydroxy-8,11,13-abietatriene-7-one (**14**).[11] This paper deals with the elucidation of the structures of compounds **1**—**5** and the antibacterial activity of the isolated compounds against *S. aureus* and *E. coli*, together with cytotoxic activity against HL-60 and HCT-15 cell lines.



	R_1	R_2	R_3		R_1	R_2
1	OAc	OH	OMe	**3**	OMe	H
1a	OH	OH	OMe	**3a**	H	OH
2	OAc	H	OH			
2a	OH	H	OH			



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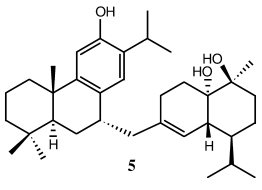

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The air-dried bark of *C. japonica* was milled and exhaustively extracted with acetone at room temperature for 6 weeks. The acetone extract was fractionated in seven fractions by column chromatography (silica gel), followed by repeated separation of four of the portions with chromatography over silica gel and reversed-phase silica gel furnished sugikurojins D (**1**)—H (**5**) along with nine known compounds (**6**—**14**).

Sugikurojin D (**1**), $[\alpha]_D^{25}$ +45.5° was obtained as a colorless solid and was considered to have the molecular formula of $C_{23}H_{34}O_5$ based on the positive HR-FAB-MS of the molecular ion at *m/z* 413.2288 $[M+Na]^+$, indicating **1** to have seven equivalents of unsaturation. The IR spectrum of **1** showed absorption bands at 3400 (OH), 1730 and 1235 (acetoxyl), and 1610 and 1500 cm^{-1} (aromatic). The presence of the latter was supported by the UV data (λ_{max} 210, 282 nm). The 23 carbon signals observed in the ^{13}C-NMR spectrum (Table 1) and distortionless enhancement by polarization transfer (DEPT) experiment showed the presence of an acetyl group at δ 172.1 (s) and 21.8 (q); three double bonds at δ 145.8 (s) 144.1 (s), 139.0 (s), 133.7 (s), 131.6 (s), and 116.1 (d), suggested that **1** should contain three rings. The ^{1}H-NMR spectrum (Table 1) of **1** showed three tertiary methyl signals at δ 0.99, 1.15, and 1.49, an isopropyl group at δ 1.21, 1.24 (each d, J=7.0 Hz), and 3.19 (sep, J=7.0 Hz), an acetyl group at δ 2.19 (s), ABX type signals at δ 1.73 (d, J=12.0 Hz), 5.42 (dd, J=12.0, 8.5 Hz), and 4.61 (br d, J=8.5 Hz), and one aromatic proton at δ 7.03 (s). These data suggest that **1** was a derivative of salviviridinol (**1a**)[12] because of the similarity of its ^{1}H-NMR spectral data to that of **1a**, except for an extra acetyl group. The gross structure of **1** was determined by analysis of the NMR data including heteronuclear multiquantum coherence (HMQC), heteronuclear multiple bond connectivity (HMBC), and rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments, and by referring to the data for **1a**. The HMBC spectrum of **1** showed long-range couplings from the proton at δ 5.42 (H-6) to acetyl group (δ 172.1) and the methoxy proton at δ 3.76 to C-12, indicating that **1** had an acetoxyl group at C-6 and a methoxyl group at C-12. The stereochemistry of **1** was de-

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Table 1. NMR Data on Compounds **1**—**5** in $CDCl_3$

	1		**2**		**3**		**4**		**5**	
C No.	^{13}C d (mult.)	^{1}H d (mult. *J* in Hz)	^{13}C d (mult.)	^{1}H d (mult. *J* in Hz)	^{13}C d (mult.)	^{1}H d (mult. *J* in Hz)	^{13}C d (mult.)	^{1}H d (mult. *J* in Hz)	^{13}C d (mult.)	^{1}H d (mult. *J* in Hz)
1	36.3 (t)	H-α 1.32 (ddd, 13.0, 13.0, 4.0) H-β 3.15 (ddd, 13.0,4.0, 4.0)	38.8 (t)	H-α 1.49 (ddd, 13.0, 13.0, 4.0) H-β 2.15 (br d, 13.0)	35.8 (t)	H-α 1.16 (ddd, 13.0, 13.0, 4.0) H-β 2.63 (br d, 13.0)	38.7 (t)	H-α 1.36 (ddd, 12.5, 12.5, 3.5) H-β 2.36 (br d, 12.5)	38.2 (t)	H-α 1.36 (m) H-β 2.16 (br d, 12.5)
2	18.8 (t)	H-α 1.51 (m) H-β 1.68 (m)	18.6 (t)	H-α 1.60 (m) H-β 1.70 (ddddd, 13.0, 13.0, 13.0, 4.0, 4.0)	18.9 (t)	H-α 1.53 (m) H-β 1.72 (ddddd, 13.0, 13.0, 13.0, 4.0, 4.0)	19.4 (t)	H-α 1.60 (m) H-β 1.72 (ddddd, 12.5, 12.5, 12.5, 3.5, 3.5)	19.4 (t)	H-α 1.60 (m) H-β 1.73 (ddddd, 12.5, 12.5, 12.5, 3.5, 3.5)
3	42.9 (t)	H-α 1.28 (ddd, 13.0, 13.0, 4.0) H-β 1.45 (ddd, 13.0, 4.0, 4.0)	43.3 (t)	H-α 1.27 (ddd, 13.0, 13.0, 4.0) H-β 1.47 (br d, 13.0)	41.0 (t)	H-α 1.24 (ddd, 13.0, 13.0, 4.0) H-β 1.46 (br d, 13.0)	41.7 (t)	H-α 1.22 (m) H-β 1.48 (br d, 12.5)	41.7 (t)	H-α 1.22 (m) H-β 1.48 (br d, 12.5)
4	33.2 (s)		33.4 (s)		33.1 (s)		33.4 (s)		33.3 (s)	
5	52.7 (d)	1.73 (d, 12.0)	52.2 (d)	1.74 (d, 12.0)	45.5 (d)	1.53 (dd, 13.0, 1.5)	44.7 (d)	1.40 (dd, 12.5, 1.5)	44.8 (d)	1.40 (dd, 12.5, 1.5)
6	75.9 (d)	5.42 (dd, 12.0, 8.5)				H-α 2.04 (br d, 13.0)	21.6 (t)	H-α 1.70 (br d, 12.5)	21.4 (t)	1.69 (2H, m)
			79.0 (d)	5.40 (dd, 12.0, 5.5)	22.1 (t)	H-β 1.39 (ddd, 13.0, 13.0, 4.0)		H-β 1.14 (m)		
7	76.6 (d)	4.61 (br d, 8.5)	76.7 (d)	4.68 (br d, 5.5)	70.9 (d)	4.31 (dd, 4.0, 2.0)	35.3 (d)	2.92 (br q, 7.5)	35.4 (d)	2.96 (br q, 7.5)
8	133.7 (s)		133.1 (s)		139.6 (s)		131.6 (s)		131.4 (s)	
9	131.6 (s)		148.0 (s)		151.9 (s)		148.6 (s)		148.7 (s)	
10	42.5 (s)		39.4 (s)		39.1 (s)		37.9 (s)		37.9 (s)	
11	145.8 (s)		117.1 (d)	6.83 (s)	188.2 (s)		110.7 (d)	6.61 (s)	110.8 (d)	6.61 (s)
12	144.1 (s)		148.0 (s)		187.1 (s)		150.8 (s)		150.9 (s)	
13	139.0 (s)		138.2 (s)		153.6 (s)		131.5 (s)		131.6 (s)	
14	116.1 (d)	7.03 (s)	127.4 (d)	7.36 (s)	131.7 (d)	6.35 (d, 1,0)	129.1 (d)	6.88 (s)	127.1 (d)	6.88 (s)
15	26.6 (d)	3.19 (sept, 7.0)	27.3 (d)	2.94 (sept, 7.0)	26.3 (d)	3.02 (dsept, 7.0, 1.0)	27.0 (d)	3.21 (sept, 7.0)	27.0 (d)	3.12 (sept, 7.0)
16	23.7 (q)	1.24 (d, 7.0)	22.9 (q)	1.21 (d, 7.0)	21.4 (q)	1.27 (d, 7.0)	22.7 (q)	1.25 (d, 7.0)	22.7 (q)	1.25 (d, 7.0)
17	23.6 (q)	1.21 (d, 7.0)	22.8 (q)	1.19 (d, 7.0)	21.3 (q)	1.12 (d, 7.0)	22.5 (q)	1.23 (d, 7.0)	22.5 (q)	1.23 (d, 7.0)
18	36.0 (q)	1.15 (s)	35.7 (q)	1.07 (s)	33.1 (q)	0.95 (s)	33.8 (q)	0.92 (s)	33.9 (q)	0.92 (s)
19	22.7 (q)	0.99 (s)	22.1 (q)	1.01 (s)	21.9 (q)	0.92 (s)	21.5 (q)	0.89 (s)	21.4 (q)	0.89 (s)
20	21.1 (q)	1.49 (s)	25.8 (q)	1.26 (s)	18.5 (q)	1.24 (s)	25.0 (q)	1.14 (s)	25.0 (q)	1.14 (s)
O-Me	61.7 (q)	3.76 (s)			57.3 (q)	3.46 (s)				
O-Ac	172.1 (s)	2.19 (s)	172.7 (s)	2.32 (s)						
	21.8 (q)		20.9 (q)							
1′							50.1 (d)	1.30 (m)	73.2 (s)	
2′							22.9 (t)	H-a 2.10 (m) H-b 1.30 (m)	27.3 (t)	H-a 1.94 (ddd, 12.5, 5.0, 5.0) H-b 1.84 (ddd, 12.5, 12.5, 5.0)
3′							28.2 (t)	H-a 2.03 (m) H-b 2.26 (br d, 12.5)	24.2 (t)	H-a 2.14 (m) H-b 2.34 (br d, 12.5)
4′							137.1 (s)		137.3 (s)	
5′							125.0 (d)	5.55 (br s)	122.7 (d)	5.49 (br s)
6′							39.8 (d)	1.80 (m)	40.2 (d)	1.40 (dd, 12.5, 1.5)
7′							46.6 (d)	1.07 (dddd, 12.5, 12.3, 3.5, 3.5)	39.6 (d)	1.42 (dddd, 12.5, 12.3, 3.5, 3.5)
8′							21.9 (t)	1.60—1.66 (2H, m)	19.1 (t)	1.60—1.66 (2H, m)
9′							42.1 (t)	H-a 1.46 (ddd, 12.5, 12.5, 3.5) H-b 1.83 (ddd, 12.5, 3.5, 3.5)	35.5 (t)	H-a 2.00 (ddd, 12.5, 12.5, 3.5) H-b 1.51 (ddd, 12.5, 3.5, 3.5)
10′							72.6 (s)		73.0 (s)	
11′							20.8 (q)	1.15 (s)	24.2 (q)	1.29 (s)
12′							26.0 (q)	2.10 (m)	25.9 (d)	2.04 (m)
13′							15.0 (q)	0.74 (d, 7.0)	15.0 (q)	0.76 (d, 7.0)
14′							21.6 (q)	0.91 (d, 7.0)	21.5 (q)	0.93 (d, 7.0)
15′							46.2 (t)	2.20 (2H, d, 7.5)	46.5 (t)	2.24 (2H, d, 7.5)

duced by ROESY experiments (see Experimental) and coupling constants. The α-acetoxyl group at C-6 and the β-OH group at C-7 could be assigned from the observed coupling constants (dd, J=12.0, 8.5 Hz) for H-6 at δ 5.42 and for H-7 (br d, J=8.5 Hz) at δ 4.61. Thus **1** was shown to be 6α-acetoxy-7β,11-dihydroxy-12-methoxy-8,11,13-abietatriene and designated sugikurojin D.

Sugikurojin E (**2**), $[\alpha]_D^{25}$ +30.0° a colorless solid, had the molecular formula $C_{22}H_{32}O_4$ based on HR-FAB-MS, *i.e.*, 30 mass units less than that of **1**. The ^{1}H-NMR spectrum (Table 1) of **2** exhibited signals typical of a derivative of ferruginol: an isopropyl group attached to a phenyl group; two para aromatic protons and a typical Hβ-1 proton; an acetyl group; as well as three singlet methyl groups (Table 1). The same ABX type signals as observed in **1** were also obtained at δ 1.74 (d, J=12.0 Hz), 5.40 (dd, J=12.0, 5.5 Hz), and 4.68 (br t, J=5.5 Hz) due to H-5, H-6, and H-7, respectively. In the ^{1}H-NMR spectrum of **2**, the Hβ-1 signal was shifted upfield by 1.00 ppm (δ 3.15 in **2**→2.15 in **1**) compared with that of **1** owing to the disappearance of the anisotropic effect of the hydroxyl group at C-11. These data suggest that **2** was a derivative of 6α,7β-dihydroxyferruginol (**2a**)[13] except for an extra acetyl group. The α-acetyl group at C-6 could be assigned from the observed long-range coupling from H-6 proton to acetyl group and the coupling constant for H-6 at 5.40 (dd, J=12.0, 5.5 Hz). Thus the structure of **2** was determined to be 6α-acetoxy-7β,12-dihydroxy-8,11,13-abietatriene and designated sugikurojin E.

Sugikurojin F (**3**), $[\alpha]_D^{25}$ −43.9° was obtained as a colorless solid. The molecular formula $C_{21}H_{30}O_3$, which was determined based on HR-FAB-MS, suggested the presence of seven degrees of unsaturation. The IR spectrum of **3** exhibited carbonyl stretching vibration at 1675 and 1655 cm^{-1} reminiscent of an *ortho*-quinone moiety. Further, the presence of two carbonyl groups at δ 188.2 (s) 187.1 (s) and two double bonds at δ 153.6 (s), 151.9 (s), 139.6 (s), and 131.7 (d) in the ^{13}C-NMR spectrum (Table 1) and the UV maximum at 256 (log ε 4.23) indicated that **3** should include an *ortho*-quinone moiety.[14] The presence of carbonyl group at C-11 was deduced from the anisotropy effect of carbonyl group to Hα-1, compared with that of **2**. Accordingly, the only phenyl proton at δ 6.35, with a corresponding carbon signal at δ 131.7 was assigned to H-14, which was coupled with H-15 at δ 3.02 due to allyl coupling (J=1.0 Hz). The ^{1}H-NMR spectrum (Table 1) of **3** contained three methyl singlets at δ 0.92, 0.95, and 1.24; an isopropyl attached to an aromatic ring [1.12 (d, J=7.0 Hz), 1.27 (d, J=7.0 Hz), 3.02 (dsep, J=7.0, 1.0 Hz)], and a carbinol proton at δ 4.31 (dd, J=4.0, 2.0 Hz), together with one methoxyl group at δ 3.46 (s). These data suggest that **3** was a derivative of 6-deoxysalviphlomone [7β-hydrxy-8,13-abietadiene-11,12-dione (**3a**)][14] except for a methoxyl group at C-7. The methoxyl group at C-7 could be assigned from the observed long-range coupling from H-7 to methoxyl group (δ 57.3). The stereochemistry of the 7α-OCH$_3$ group of **3** was proven by the sharp (dd, J=4.0, 2.0 Hz) of the H-7 at δ 4.31 in the ^{1}H-NMR spectrum. Thus the structure of **3** was determined to be 7α-methoxy-8,13-abietadiene-11,12-dione and designated sugikurojin F.

Sugikurojin G (**4**), $[\alpha]_D^{25}$ −5.2° was obtained as a colorless solid. The FAB-MS of **4** gave pseudomolecular ions at *m*/*z* 529 $[M+Na]^+$ and 545 $[M+K]^+$ corresponding to a molecular formula of $C_{35}H_{54}O_2$, which was confirmed by negative HR-FAB-MS, suggesting the presence of nine degrees of unsaturation. The EI-MS displayed fragment ions at *m*/*z* 368 (30%), 313 (11%), 285 (26%), 236 (18%), and 221 (12%). The HR-EI-MS exhibited fragment ions at *m*/*z* 285 (observed 285.2244; calcd 285.2218 for $C_{20}H_{29}O$) and 221 (observed 221.1936; calcd 221.1915 for $C_{15}H_{25}O$), indicating that compound **4** is composed of a diterpene constituent-1 [$C_{20}H_{29}O$] and a sesquiterpene constituent-2 [$C_{15}H_{25}O$] (Fig. 1). The absorption bands at 3400, 1605, 1585, 1255 1085, and 890 cm^{-1} in the IR spectrum and the λ_{max} at 278 and 206 nm in the UV spectra suggested the presence of a hydroxyl group and an aromatic ring. The ^{13}C-NMR spectrum indicated the presence of four double bonds [δ 150.8 (s, phenolic), 148.6 (s), 137.1 (s), 131.6 (s), 131.5 (d), 129.1 (d), 125.0 (d), 110.7 (d)] and an oxygenated carbon [δ 72.6 (s)]. Its ^{1}H-NMR spectrum had signals for four tertiary methyl groups (δ 0.89, 0.92, 1.14, 1.15), two isopropyl groups [δ 0.74, 0.91, 1.23, 1.25 (each d, J=7.0 Hz)], an olefinic proton (δ 5.55, br s), and two aromatic protons [δ 6.61, 6.88 (each s)]. Those data suggested that the diterpene constituent-1 was ferruginol. Therefore, the constituent-2 was a bicyclic sesquiterpene having a tri-substituted double bond (δ 132.1, 125.0) an isopropyl group, and a tertiary methyl group accompanied by a hydroxyl group, suggesting it to be a cadinane-type sesquiterpene like α-cadinol,[15] T-cadinol,[16] cubenol,[16] or epi-cubenol.[17,18]

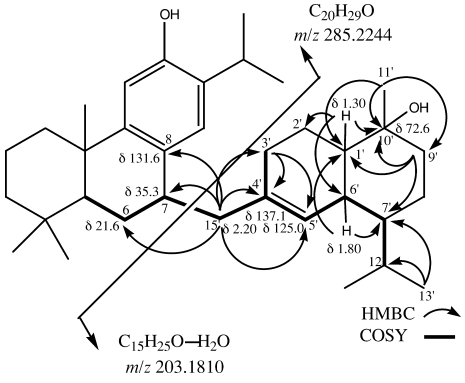

Fig. 1. Some Key HMBC and HR-EI-MS for Compound **4**

The gross structure of **4** was determined by analysis of NMR data including HMBC and ROESY experiments, and by referring to the NMR data for ferruginol, α-cadinol, or T-cadinol. An HMBC experiment (Fig. 1) revealed long-range couplings from H$_2$-15′ at δ_C 46.2 to C-6, -7, -8, -3′, -4′ and -5′, establishing the connectivity between ferruginol and α-cadinol. The stereochemistry of C-7 was determined based on ROESY experiments (Fig. 2) and coupling constants. The α-methylene function at C-7 could be assigned from the NOEs between H$_2$-15′/H-5 and Hα-6, and the large J_{H6-H7} coupling constant (J=7.5 Hz). Thus the structure of sugikurojin G was established as **4**.

Sugikurojin H (**5**), $[\alpha]_D^{25}$ −6.1° $C_{35}H_{54}O_3$, an amorphous solid, exhibited a deprotonated molecular ion $[M-H]^-$ at *m*/*z*

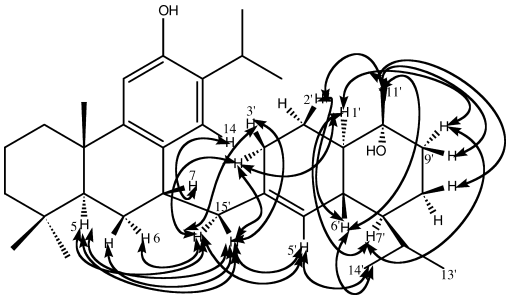

Fig. 2. Some Key NOESY Correlations for Compound **4**

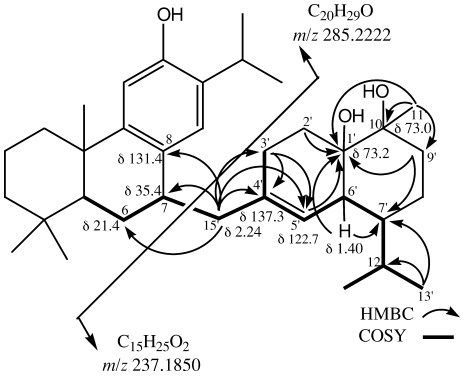

Fig. 3. Some Key HMBC and HR-EI-MS for Compound **5**

521, 16 mass units higher than that of **4**. The EI-MS displayed fragment ions at *m/z* 368 (30%), 313 (11%), 285 (26%), 239 (15%), 237 (10%), and 211 (12%). The HR-EI-MS exhibited fragment ions at *m/z* 285 (observed 285.2222; calcd 285.2219 for $C_{20}H_{29}O$) and 237 (observed 237.1850; calcd 237.1855 for $C_{15}H_{25}O_2$), indicating that compound **5** is composed of a diterpene constituent-1 ($C_{20}H_{29}O$) and a sesquiterpene constituent-2 ($C_{15}H_{25}O_2$) (Fig. 3). Comparison of the ^{13}C-NMR spectra of **5** with that of **4**, by referring to those of α-cadinol, T-cadinol, cubenol, or epi-cubenol, showed that **5** also had the same skeleton as **4**, incorporating a ferruginol and a hydroxy-α-cadinol or -T-cadinol. Namely, NMR data due to the sesquiterpene moiety of **5** indicated the presence of two secondary methyl groups at δ 0.76 and 0.93 (each 3H, d, J=7.0 Hz), one tertiary methyl group at δ 1.29 (3H, s), a trisubstituted double bond at δ 137.3 (s) and 122.7 (d) [δ_H 5.49 (br s)], and two oxygenated quaternary carbons at δ 73.2 (s) and 73.0 (s), indicating that **5** should contain two tertiary hydroxyl groups, one of which connects to the same carbon as the methyl group. The other hydroxyl group could connect to C-1′ due to the disappearance of the H-1′ proton signal and the deshielded C-1′ carbon signal (δ 73.2), by comparison of NMR data with that of **4**. The HMBC experiments (Fig. 3) of **5** showed couplings from H-2′ to C-1′; H-3′ to C-4′ and -5′; H-5′ to C-1′; H-6′ to C-1′ and -7′; H-9′ to C-1′ and -7′; H_3-11′ to C-1′, -9′, and -10′; H_3-13′ (H_3-14′) to C-7′ and -12′. Thus, the sesquiterpene moiety of **5** was determined as 4-isopropyl-1,6-dimethyl-2,3,4,4a,7,8-hexahydro-1*H*-naphthalene-1,8a-diol. The relative stereochemistry of the sesquiterpene moiety was deduced by ROESY experiments (Fig. 4). The NOE between H-6′/H-2′β, -5′, -8′β, -12′, -13′, and -14′, and H-7′α/H-9′α established a half chair confomation for ring A and a chair for B, a trans-junction between rings A and B, and α-axial for the hydroxy group at C-1′. Also, the NOE between H_3-11′/H_2-2β′, H-6′ and -9β′, and H_3-13′ (14′)/H-6′, and H_2-8′ showed that the methyl group at C-10′ and the isopropyl group at C-7′ could be β-axial and β-equatorial, respectively. Thus, the sesquiterpene moiety of **5** was determined as 1α-hydroxy-T-cadinol. The connectivity between the ferruginol moiety and the 1α-hydroxy-T-cadinol moiety was C-7 in ferruginol and C-15′ in 1α-hydroxy-T-cadinol, deduced by HMBC of H-15′ to C-6, -7, and -8. Thus the structure of sugikurojin G was established as **5**.

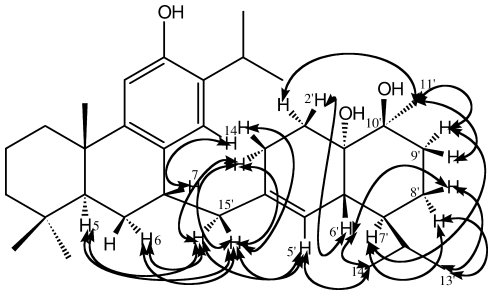

Fig. 4. Some Key NOESY Correlations for Compound **5**

In antibacterial activity against the gram-positive bacteria *S. aureus* and gram-negative bacteria *E. coli*, compounds **1**—**5**, **8**, **10**—**12** and **14** were all found to be inactive at the (MIC: 125 μg/ml) level. Meanwhile, in the cytotoxic activity against HL-60, compounds **4**, **8**, and **11** were moderate (IC_{50}: **4** 35.4; **8** 28.0; **11** 52.4 μM) though weak (IC_{50}: **4** 100; **8** 80.8; **11** 100 μM) activity against HCT-15.

Experimental

General Experimental Procedures Optical rotations were recorded on a JASCO DIP-140 digital polarimeter. IR spectra were measured on a JASCO FT/IR-5300 instrument. UV spectra were recorded with a Shimadzu UV-6000 spectrophotometer. NMR spectra were recorded on a Varian UNITY 600 spectrometer in $CDCl_3$ solution using tetramethylsilane (TMS) as an internal standard. NMR experiments included ^{1}H–^{1}H COSY, HMQC, HMBC, and ROESY. Coupling constants (J values) are given in Hertz (Hz). Mass spectra were taken on a JEOL JMS-700 MS station. Kieselgel 60 (230—400 mesh, Merck) was used for column chromatography, and silica gel 60F-254 (Merck) for TLC. HPLC was carried out on a JASCO-PU 1580 instrument using a COSMOSIL C18 P-MS (4.6×150 mm, 20×250 mm) column.

Plant Material The air-dried bark of black heartwood of *C. japonica* trees, aged 70 to 80 years from Kaifu, Tokushima, was collected in October 2003. A voucher specimen (3002) is deposited in the Herbarium of the Department of Pharmacognosy, Tokushima Bunri University, Tokushima, Japan.

Extraction and Isolation The powder bark (2.0 kg) of *C. japonica* was exhaustively extracted with acetone at room temperature for 4 weeks. The acetone extract was evaporated under vacuum to yield a brown residue (70 g), which was subjected to silica gel column chromatography with hexane–acetone (30 : 1→0 : 10) to afford fractions 1—7. Fraction 2 (6.6 g) was passed through silica gel with hexane–acetone (5 : 1→1 : 3) and purified by preparative HPLC (80% MeOH, flow rate 8 ml/min), to afford sugikurojin F (**5**, 15 mg), 12-hydroxy-6,7-secoabieta-8,11,13-triene-6,7-dial (**6**, 15 mg), ferruginol (**7**, 20 mg), 7β-methoxydeoxocryptojaponol (**8**, 93 mg), sugikurojin B (**9**, 12 mg). Fraction 3 (6.7 g) was purified by preparative HPLC (75% MeOH, flow rate 8 ml/min) to afford 11,14-dihydroxy-8,11,13-abietatriene-7-one (**14**, 12 mg). Fraction 5 (3.5 g) was passed through silica gel with hexanes–EtOAc (1 : 1) and purified by preparative HPLC (70% MeOH, flow rate 8 ml/min) to afford sugikurojins D (**1**, 18 mg), F (**3**, 19 mg), G (**4**, 17 mg), 6,12,15-trihydroxy-5,8,11,13-abietatetraene-7-one (**10**, 7 mg), 5,6-dehydrosugiol (**11**, 104 mg), trilobine (**12**, 7 mg), and 6,7-dehydroferruginol (**13**, 14 mg). Fraction 6 (1.2 g) was purified through silica gel with [hexane–EtOAc (1 : 1→2 : 8) to afford sugikurojin E (**2**, 8 mg).

Sugikurojin D (**1**): A colorless solid; $[\alpha]_D^{25}$ +45.5° (c=1.2, $CHCl_3$); FT-IR (dry film) 3400, 1730, 1605, 1585, 1235, 1040, 890 cm^{-1}; UV (MeOH) λ_{max}

nm (log ε) 210 (4.65), 282 (3.62); ^{1}H- and ^{13}C-NMR, see Table 1; HMBC (H/C) 6/5, 6/7, 6/10, 6/acetyl, 7/9, 14/7, 14/12, 14/13, 15/12, 15/13, 15/14, 15/16, 15/17, 16/13, 16/15, 17/13, 17/15, 18/3, 18/4, 18/5, 19/3, 19/4, 19/5, 20/1, 20/5, 20/9, 20/10, MeO/12. Selected ROESY data, 1α/3α, 1α/5α, 3α/5α, 5α/18, 5α/7α, 6β/20, 7α/14, 14/15, 14/16, 14/17, 16/17, 18/19, 19/20; EI-MS: 390 (5), 373 (27), 331 (42), 330 (74), 313 (100), 248 (19); negative FAB-MS *m/z*: 389 $(M-H)^-$; positive FAB-MS *m/z*: 413 $(M+Na)^+$, 429 $(M+K)^+$; HR-FAB-MS *m/z*: 413.2288 (Calcd for $C_{23}H_{34}O_5+Na$, 413.2304).

Sugikurojin E (**2**): A colorless solid; $[\alpha]_D^{25}$ +30.0° (*c*=1.2, $CHCl_3$); FT-IR (dry film) 3400, 1735, 1600, 1590, 1235, 1035, 880 cm^{-1}; UV (MeOH) λ_{max} nm (log ε) 210 (4.66), 280 (3.65); ^{1}H- and ^{13}C-NMR, see Table 1; HMBC (H/C) 6/5, 6/7, 6/10, 6/acetyl, 7/9, 11/10, 11/13, 14/7, 14/12, 14/13, 15/12, 15/13, 15/14, 15/16, 15/17, 16/13, 16/15, 17/13, 17/15, 18/3, 18/4, 18/5, 19/3, 19/4, 19/5, 20/1, 20/5, 20/9, 20/10, MeO/12. Selected ROESY data, 1α/3α, 1α/5α, 1β/11, 3α/5α, 5α/18, 5α/7α, 6β/20, 7α/14, 14/15, 14/16, 14/17, 16/17, 18/19, 19/20; EI-MS: 360 (2), 342 (100), 300 (50), 285 (27), 260, (53), 218 (52); HR-EI-MS *m/z*: 360.2304 (Calcd for $C_{22}H_{32}O_4$, 360.2307).

Sugikurojin F (**3**): A colorless solid; $[\alpha]_D^{25}$ −43.9° (*c*=0.4, $CHCl_3$); FT-IR (dry film) 3400, 1675, 1655, 1590, 1040, 880 cm^{-1}; UV (MeOH) λ_{max} nm (log ε) 256 (4.23); ^{1}H- and ^{13}C-NMR, see Table 1; HMBC (H/C) 5/7, 6/7, 6/10, 7/9, 7/OMe, 14/9, 14/12, 14/15, 15/12, 15/13, 15/14, 15/16, 15/17, 16/13, 16/15, 17/13, 17/15, 18/3, 18/4, 18/5, 19/3, 19/4, 19/5, 20/1, 20/5, 20/9, 20/10. Selected ROESY data, 1α/3α, 1α/5α, 3α/5α, 5α/18, 5α/7α, 6β/20, 14/15, 14/16, 14/17, 16/17, 18/19, 19/20; EI-MS: 330 (2), 342 (100), 300 (50), 285 (27), 260, (53), 218 (52); HR-EI-MS *m/z*: 330.4600 (Calcd for $C_{22}H_{32}O_4$, 330.4611).

Sugikurojin G (**4**): A colorless solid; $[\alpha]_D^{25}$ −5.2° (*c*=1.5, $CHCl_3$); FT-IR (dry film) 3400, 1600, 1590, 1040, 880 cm^{-1}; UV (MeOH) λ_{max} nm (log ε) 206 (5.23), 278 (4.48); ^{1}H- and ^{13}C-NMR, see Table 1; HMBC, see Fig. 1; Selected ROESY, see Fig. 2; EI-MS: 360 (2), 342 (100), 300 (50), 285 (27), 260, (53), 218 (52); negative FAB-MS *m/z*: 505 $(M-H)^-$; positive FAB-MS *m/z*: 529 $(M+Na)^+$, 545 $(M+K)^+$; HR-FAB-MS *m/z*: 505.4059 (Calcd for $C_{35}H_{54}O_2-H$, 505.4045), 529.3991 (Calcd for $C_{35}H_{54}O_2+Na$, 529.4021).

Sugikurojin H (**5**): A colorless solid; $[\alpha]_D^{25}$ −6.1° (*c*=0.2, $CHCl_3$); FT-IR (dry film) 3400, 1600, 1595, 1045, 890 cm^{-1}; UV (MeOH) λ_{max} nm (log ε) 210 (4.99), 280 (4.62); ^{1}H- and ^{13}C-NMR, see Table 1; HMBC, see Fig. 3; Selected ROESY, see Fig. 4; EI-MS: 522 (2), 368 (30), 313 (11), 285 (26), 239 (15), 237 (10), 236 (18), 211 (12); negative FAB-MS *m/z*: 521 $(M-H)^-$; HR-FAB-MS *m/z*: 521.3995 (Calcd for $C_{35}H_{54}O_3-H$, 521.3995).

Antibacterial Activity Testing The MICs against bacteria were measured by a broth dilution method. A nutrient broth [Bacto beef extract 0.3% (w/v), Bacto peptone 0.5% (w/v), Difco Lab., Detroit. MI, U.S.A.] was used for the antibacterial test. Two milliliters of the broth containing 2 mg of each tested compound was diluted stepwise with the broth. The bacterium was preincubated in L-broth (Bacto tryptone 1% (w/v), yeast extract 0.5% (w/v), NaCl 0.5% (w/v), pH 7.2) for 18 h at 37 °C. A 1 ml aliquot of the culture was inoculated into 500 ml of nutrient broth, then 2 ml portions of the inoculated broth were pipetted into sterilized test tubes each containing 2 ml of diluted quality assurance control (QAC). The mixture was incubated at 37 °C for 24 h, and the MICs were determined by visual inspection.

Cytotoxicity Assay The MTT Cell Growth Assay Kit (Chemicon International Inc., Temecula, CA, U.S.A.) was used in this assay. Cells were maintained in the RPMI-1640 medium (Sigma, St. Louis, MO, U.S.A.) supplemented with 10% fetal bovine serum (ICN Biomedicals Inc., Solon, OH, U.S.A.) in a humidified atmosphere of 5% CO_2 at 37 °C throughout the study. Cells (90 μl) at a density of 5×10^5 cells/ml in the exponential growth phase were plated in 96-well flat-bottomed microplates with various drug concentrations (10 μl). After 24 h (HL-60) or 48 h (HCT-15), 10 μl of 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was added to each well. After a further 4 h of incubation, 100 μl of 2-propanol with 0.04 N HCl solution was added to each well, and the formazen crystals in each well were dissolved by stirring with a pipet. The optical density measurements were made using a microplate reader (BIO-RAD Co., Ltd., Tokyo, Japan) at 570 nm.

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