Three New Sesquiterpenes from the Black Heartwood of *Cryptomeria japonica*

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Three new sesquiterpenes, (4*S*)-2,6,10-bisaboratrien-4-ol-1-one (1), 1,8-epoxy-1(6),2,4,7,10-bisaborapentaen-4-ol (2), and 1-methoxy-4-cadinene (3) have been isolated from the black heartwood of *Cryptomeria japonica*. Compounds 1 and 2 were designated sugikurojinols A and B, respectively, and the structures of compounds 1—3 were established by extensive NMR experiments. Compounds 1 and 2 were also examined for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, and for termiticidal activity against *Coptotermes formosanus* SHIRAKI.

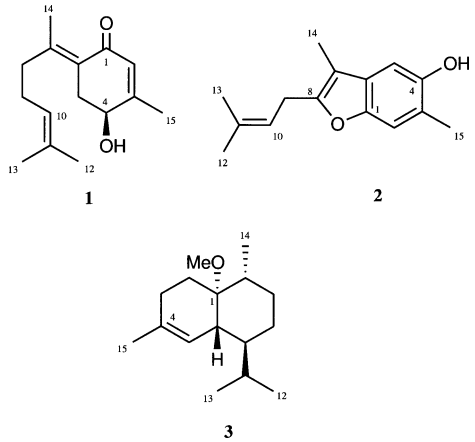
Key words *Cryptomeria japonica*; Taxodiaceae; black heartwood; sesquiterpenoid; antibacterial activity; termiticidal activity

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. Although many cedar cores are reddish-brown (called *akajin* in Japanese), about 20% are dark brown, as a result of genetic factors, physical damage, and infection of fungus, *etc.*, are called *kurojin* in Japanese. *Akajin* are known to have a greater resistance against *Coptotermes formosanus* SHIRAKI. Chemical research on the terpene components, termiticidal activity, and antibacterial activity of cedar cores has been limited to the reddish-brown ones.[1—7)]

In a previous paper,[8)] we reported the structures of an abietane sugikurojin A (**4**) and two abietane dimers sugikurojins B (**5**) and C (**6**), together with 36 known compounds from the black heartwood of *C. japonica* D. DON. Further investigation of this plant material led us to isolate the three new sesquiterpenoids **1**—**3**. This paper deals with the elucidation of the structures of compounds **1**—**3** isolated from the black heartwood of *C. japonica* D. DON., and the antibacterial activity of these compounds against *Saphylococcus aureus* and *Escherichia coli*, together with termiticidal activity against *C. formosanus* SHIRAKI.

A shaving chip (3.92 kg) of the black heartwood was exhaustively extracted with MeOH at room temperature for 4 weeks. The MeOH extract was partitioned between H_2O and EtOAc. Repeated separation of the EtOAc-soluble portion with chromatography over silica gel and reverse-phase silica gel furnished two novel bisabolanes sugikurojinols A (**1**) and B (**2**), and a cadinane 1-methoxy-4-cadinene (**3**).

Compound (**1**), $[\alpha]_D^{25}$ −75.2° was obtained as a colorless oil and was considered to have the molecular formula of $C_{15}H_{22}O_2$ based on the high-resolution electron ion mass spectrum (HR-EI-MS) of the molecular ion at *m*/*z* 234.1608. The IR spectrum of **1** showed absorption bands at 3395, 1655, and 1600 cm^{-1} ascribable to hydroxyl and conjugated carbonyl groups, respectively. The presence of the latter was supported by the UV data (λ_{max} 240, 283 nm), indicating that **1** had a cross-conjugated enone system.[9)] The 15 carbon signals observed in the ^{13}C-NMR spectrum and distortionless enhancement by polarization transfer (DEPT) experiment revealed the presence of a carbonyl group 190.9 (s); three double bonds at δ 159.3 (s) 149.6 (s), 132.7 (s), 129.6 (d), 125.9 (s), and 123.2 (d); and an oxygenated carbon at δ 69.5 (d), which requires that **2** should contain one ring. The ^{1}H-NMR spectrum of **1** showed four methyl signals at δ 2.11 (br s), 2.03 (d, J=0.8 Hz), 1.69 (d, J=8 Hz), and 1.61 (d, J=0.8 Hz), two olefinic protons at δ 5.89 (br s) and 5.13 (t sep, J=7.0, 0.8 Hz), and one oxygenated methine proton at δ 4.25 (br s). These data suggest that **1** was a bisabolane-type sesquiterpene like cryptomerione,[10)] 7(14),10-bisaboladien-1-ol-4-one,[11)] and 2,7(14),10-bisabolatrien-1-ol-4-one.[12)] The ^{1}H–^{1}H shift correlation spectroscopy (COSY) spectrum of **1** revealed only one spin system (H-2—H-15). 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 2,7(14),10-bisabolatrien-1-ol-4-one.[12)] Thus **1** was shown to be 2,6,10-bisaboratrien-4-ol-1-one. To determine the absolute stereochemistry at C-4, the generalized Mosher's method was applied.[13)] Thus the epimeric esters of **1** with (*S*)- and (*R*)-methoxytrifluoromethylphenyl acetic acid (MTPA esters) were prepared and analyzed using ^{1}H-NMR. The resonance of the H-2 methine and H_3-15 methyl groups had higher δ values in the *S*-ester than *R*-ester ($\delta_S-\delta_R=\Delta\delta>0$), and the signals of H_2-5, H_2-8, H_2-9, and H_3-14



1 **2** **3**

Chart 1

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Table 1. NMR Data on Compounds **1** and **2** in $CDCl_3$

C No.	Compound **1**			Compound **2**		
	^{13}C δ (mult.)	^{1}H δ (mult. J in Hz)	HMBC (from C to H)	^{13}C δ (mult.)	^{1}H δ (mult. J in Hz)	HMBC (from C to H)
1	190.9 (s)		H-5	148.8 (s)		H-2, H-5
2	129.6 (d)	5.89 (br s)	H-15	111.2 (d)	7.25 (br s)	H-15
3	159.3 (s)		H-5, H-4, H-15	120.0 (s)		H-2, H-5, H-15
4	69.5 (d)	4.25 (br s)	H-2, H-5, H-15	149.5 (s)		H-5, H-15
5	37.2 (t)	2.75 (ddq, 14.0, 7.3, 1.2) 2.96 (ddq, 14.0, 5.0, 0.8)		103.6 (d)	6.76 (br s)	
6	125.9 (s)		H-2, H-5, H-8, H-14	129.1 (s)		H-1, H-14
7	149.6 (s)		H-5, H-8, H-14	108.8 (s)		H-5, H-9, H-14
8	36.4 (t)	2.23 (m)	H-9, H-14	153.2 (s)		H-9, H-10, H-14
9	26.6 (t)	2.13 (m)	H-8, H-10	25.6 (t)	3.39 (br d, 7.0)	H-10
10	123.2 (d)	5.13 (t sep, 7.0, 0.8)	H-8, H-9, H-12, H-13	119.5 (d)	5.31 (t sep, 7.0, 1.4)	H-9, H-12, H-13
11	132.7 (s)		H-9, H-12, H-13	133.6 (s)		H-9, H-12, H-13
12	25.7 (q)	1.69 (d, 0.8)	H-10, H-13	25.6 (q)	1.73 (d, 1.4)	H-10, H-13
13	17.6 (q)	1.61 (d, 0.8)	H-10, H-12	17.8 (q)	1.73 (d, 1.4)	H-10, H-12
14	21.1 (q)	2.11 (br s)	H-8	7.9 (q)	2.08 (s)	
15	20.1 (q)	2.03 (d, 0.8)	H-2	16.5 (q)	2.31 (s)	H-2

showed $\Delta\delta<0$. It was concluded that the C-4 center had the *S* configuration. Hence **1** was shown to be (4*S*)-2,6,10-bisaboratrien-4-ol-1-one and designated sugikurojin A.

Compound (**2**), a colorless oil, had the molecular formula $C_{15}H_{18}O_2$ based on HR-EI-MS, with absorption bands at 3385, 1655, 1620, 1510, 1185, and 855 cm^{-1} due to hydroxyl, aromatic ring, and olefinic functions in the IR spectrum, while its UV spectrum showed absorption maxima at 306, 265, and 216 nm due to an aromatic ring. The ^{13}C-NMR spectrum indicated the presence of five double bonds at δ 153.2 (s), 149.5 (s), 148.8 (s), 133.6 (s), 129.1 (s), 120.0 (s), 119.5 (d), 111.2 (d), 108.8 (s), and 103.6 (d), which requires that **2** should contain two rings. The ^{1}H-NMR spectrum contained four methyl signals at δ 2.31 (s), 2.08 (s), 1.73 (d, J=1.4 Hz), and 1.73 (d, J=1.4 Hz), and three olefinic protons at δ 7.25 (br s), 6.76 (br s), and 5.31 (m). These data suggested that **2** is a bicyclic sesquiterpene with a benzofurane ring. The ^{1}H–^{1}H COSY and HMQC experiments (see Table 1) implied the partial structures a [CH=C(CH_3)=CH: from C-2 to C-5 with 15] and b [$(CH_3)_2$C=CHCH_2C=C(CH_3): from 7 to 14]. The HMBC experiment revealed long-range couplings from H-2 to C-1, -3, -4, -6, and -15, from H-5 to C-1, -3, -4, and -7, from H-9 to C-7, -8, -10, and -11, from H-10 to C-8, -11, -12, and -13, and from H-14 to C-6, -7 and -8. Thus the structure of **2** was determined to be 1,8-epoxy-1(6),2,4,7,10-bisaborapenta-en-4-ol and designated sugikurojin B.

Compound (**3**), $[\alpha]_D^{25}$ −65.2° was obtained as a colorless oil. The molecular formula $C_{16}H_{28}O$, which was determined based on HR-EI-MS, suggested the presence of three degrees of unsaturation. The ^{13}C-NMR spectrum indicated the presence of one double bond at δ 133.9 (s) and 119.8 (d); and two oxygenated carbons at δ 74.9 (s) and 52.3 (q), indicating that **3** should contain two rings. The ^{1}H-NMR spectrum contained four methyl signals at δ 1.71 (d, J=1.0 Hz), 1.07 (d, J=7.0 Hz), 0.90 (d, J=7.0 Hz), and 0.72 (d, J=7.0 Hz), one olefinic proton at δ 5.44 (br s), and one methoxy group at δ 3.37 (s). These data suggest that **3** is a cadinane-type sesquiterpene like T-cadinol,[14] cubenol,[14] and epicubenol.[15,16] Comparisons of the ^{13}C-NMR spectrum of **3** with cubenol[17] isolated together with **3** indicated that **3** should be the methyl ether of cubenol. Furthermore, the HMBC spectrum of **3** showed coupling from OCH_3 to C-1 and NOEs were detected between OCH_3/H-14α, OCH_3/H-9α, H-2β/H-6β, H-2β/H-10β, and H-6β/H-10β. Accordingly, the structure of **3** was established to be 1-methoxy-4-cadinene.

The termiticidal effect of sugikurojinols A and B was weaker than that of hinokitiol against *C. formosanus*. At a dose of 5 mg sugikurojinols A and B and hinokitiol killed three, four, and 10 of 33 termites over 14 d in the termite test A,[18] respectively. The antimicrobial activity of sugikurojinol B (MIC=15.6 μg/ml) was as strong as that of hinokitiol (MIC=15.6 μg/ml) against *S. aureus*. However, sugikurojinol A showed no activity (MIC>250 μg/ml), and sugikurojinols A and B (MIC>250 μg/ml) showed no activity against *E. coli*.

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). HR-EI-MS were measured on a JEOL JMS-PX303 mass spectrometer. 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 COSUMOSIL C18 P-MS (4.6×150 mm and 20×250 mm) column.

Plant Material The black heartwood of *C. japonica* trees, aged 70 to 80 years from Kaifu, Tokushima, was collected in October 2000. A voucher specimen (3001) is deposited in the Herbarium of the Department of Pharmacognosy, Tokushima Bunri University, Tokushima, Japan.

Extraction and Isolation The chips (3.92 kg) of *C. japonica* heartwood were exhaustively extracted with MeOH at room temperature for 4 weeks. The methanol extract was evaporated under vacuum to yield a brown residue (296 g), which was partitioned between H_2O and EtOAc. Repeated separation of the aliquot (120 g) of the EtOAc-soluble portion (240 g) with chromatography over normal-phase silica gel (using increasing concentrations of diisopropyl ether in hexane as eluent) and reverse-phase HPLC (70—90% MeOH) furnished sugikurojinols A (**1**, 17 mg) and B (**2**, 35 mg), and 1-methoxy-4-cadinene (**3**, 2.5 mg).

Sugikurojinol A (**1**): A colorless oil; $[\alpha]_D^{25}$ −75.2° (c=0.59, $CHCl_3$); FT-IR (dry film) 3395, 1655, 1600, 1220, 1040, 880 cm^{-1}; UV (MeOH) λ_{max} nm (log ε) 202 (4.18), 240 (4.00), 283 (3.85); CD $\Delta\varepsilon$ −2.26 (300 nm),

−1.67 (271 nm), +1.02 (222 nm); ^{1}H-, ^{13}C-NMR, and HMBC, see Table 1; selected NOESY data, 2/15, 4/5a, 4/5b, 4/15, 5a/8a, 5a/ 8b, 8a/10, 8b/10, 9a/10, 9a/14, 9b/10, 9b/14. 10/12. EI-MS *m/z* 234 (M^+, 40), 191 (100), 166 (30), 123 (22), 98 (20), 93 (21), 69 (67), 41 (43); HR-EI-MS *m/z* 234.1608 (Calcd for $C_{15}H_{22}O_2$, 234.1620).

Sugikurojinol B (**2**): A colorless oil; FT-IR (dry film) 3385, 1655, 1620, 1510, 1460, 1435, 1285, 1185, 885, 855 cm^{-1}; UV (MeOH) λ_{max} (log ε) nm 216 (3.07), 265 (3.19), 306 (3.06); ^{1}H-, ^{13}C-NMR, and HMBC, see Table 1; selected NOESY data, 2/15, 5/14, 9a/10, 9a/14, 9b/10, 9b/14, 10/12. EI-MS *m/z* 230 (M^+, 80), 215 (100), 175 (17), 162 (18); HR-EI-MS *m/z* 2230.1313 (Calcd for $C_{15}H_{18}O_2$, 230.1307).

1-Methoxy-4-cadinene (**3**): A colorless oil; $[\alpha]_D^{25}$ −65.2° (*c*=0.11, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 5.44 (1H, br s, H-5), 3.37 (3H, s, OCH_3), 2.48 (1H, ddd, *J*=14.0, 6.5, 1.5 Hz, H-2α), 2.11 (1H, m, H-11), 2.04 (1H, ddd, *J*=17.8, 12.0, 6.5 Hz, H-3α) 1.90 (1H, ddd, *J*=17.8, 6.5, 1.5 Hz, H-3β), 1.81 (1H, d, *J*=9.5 Hz, H-6), 1.71 (3H, d, *J*=1.0 Hz, Me-15), 1.66 (1H, dddd, *J*=13.0, 4.5, 4.5, 4.5 Hz, H-8β), 1.65 (1H, m, H-7), 1.56 (1H, dddd, *J*=13.0, 13.0, 13.0, 4.5 Hz, H-9β), 1.51 (1H, dddd, *J*=13.0, 4.5, 4.5, 4.5 Hz, H-9α), 1.35 (1H, ddt, *J*=13.0, 4.5, 7.0 Hz, H-10), 1.32 (ddd, *J*=14.0, 12.0, 6.5 Hz, H-2β), 1.07 (3H, d, *J*=7.0 Hz, Me-14), 1.04 (dddd, *J*=13.0, 13.0, 13.0, 4.5 Hz, H-8α), 0.90 (3H, d, *J*=7.0 Hz, Me-12), 0.72 (3H, d, *J*=7.0 Hz, Me-13). ^{13}C-NMR ($CDCl_3$) δ 133.9 (s, C-4), 119.8 (d, C-5), 74.9 (s, C-1), 52.3 (q, OCH_3), 47.7 (d, C-6), 42.1 (d, C-10), 39.1 (d, C-7), 30.2 (t, C-9), 27.3 (t, C-3), 27.0 (t, C-2), 26.0 (d, C-11), 24.5 (t, C-8), 23.9 (q, C-15), 21.3 (q, C-12), 17.3 (q, C-14), 14.6 (q, C-13). HMBC (H/C) 2/1, 2/3, 2/4, 2/6, 3/1, 3/4, 3/5, 5/1, 5/3, 5/4, 5/6, 5/7, 5/15, 6/4, 7/1, 7/12, 7/13, 8/7, 8/10, 9/8, 12/7, 12/11, 12/13, 13/7, 13/11, 13/12, 14/1, 14/9, 14/10, 15/3, 15/4, 15/5, OMe/1. Selected NOESY data, 2α/14, 3α/15, 3α/OMe, 3β/15, 5/6, 5/11, 5/12, 5/13, 5/15, 6β/8β, 6β/10β, 6β/13, 8β/10β, 8β/12, 9α/14, 9α/OMe, 9β/14, 9β/10β, 10β/14, 14/OMe. EI-MS *m/z* 236 (M^+, 6), 204 (92), 193 (88), 161 (100), 119 (57), 85 (43); HR-EI-MS *m/z* 236.2127 (Calcd for $C_{16}H_{28}O$, 236.2140).

Determination of the Absolute Configuration of 1 Using Modified Mosher's Method Samples of **1** (3.5 mg) in dry pyridine (50 μl) were treated with (+)-*S* or (−)-*R*-methoxy-trifluoromethylphenylacetyl chloride (15 μl). After 30 min, the reaction was completed based on TLC. The mixture was added to 10 ml of 0.1 M HCl and extracted with 3×10 ml of EtOAc, the organic phase was washed with 10 ml of 5% aqueous $NaHCO_3$ and saline, dried ($MgSO_4$), and evaporated, and the residue was analyzed using ^{1}H-NMR in CDC1$_3$. The signals of the H-2 and H_3-15 protons in the *S* ester appeared at δ 5.982 and 1.891, and the corresponding signals in the *R* ester appeared at δ 5.963 and 1.742 ($\Delta\delta$>0). The signals of the H-5a, H-5b, H_2-8, H_2-9, and H_3-14 protons appeared at δ 3.091, 2.670, 2.482, 2.175, and 2.103 in the *S* ester and at δ 3.153, 2.819, 2.497, 2.237, and 2.1301 in the *R* ester ($\Delta\delta$<0).

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