Bioactive Constituents of Chinese Natural Medicines. V.¹⁾ Radical Scavenging Effect of Moutan Cortex. (1): Absolute Stereostructures of Two Monoterpenes, Paeonisuffrone and Paeonisuffral

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The methanolic extract and the ethyl acetate-soluble and methanol-eluted fractions from Chinese Moutan Cortex, the roots of *Paeonia suffruticosa* ANDREWS, were found to exhibit scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radical and superoxide anion radical generated by the xanthine-xanthine oxidase system. Two monoterpenes called paeonisuffrone and paeonisuffral were isolated from the ethyl acetate-soluble fraction. Their absolute stereostructures were elucidated on the basis of chemical and physicochemical evidence, which included the application of a modified Mosher's method and lipase catalyzed debenzoylation reaction.

Key words *Paeonia suffruticosa*; Moutan Cortex; paeonisuffrone; paeonisuffral; monoterpene; radical scavenging effect

The root cortex of *Paeonia suffruticosa* ANDREWS (Paeoniaceae) has been used as a Chinese herbal medicine, Moutan Cortex (牡丹皮 in Japanese). Moutan Cortex is one of the most important herbal medicines known and is used as an analgesic, a sedative, an antiinflammatory agent, and a remedy for female diseases in Chinese traditional medicine. Particularly, this herbal medicine is prescribed in various Chinese preparations used for treatment of "Oketsu" syndrome, which is presumed to be caused by blood stagnation. Extensive chemical and pharmacological studies were made on the constituents of Moutan Cortex to determine its bioactive principles, and the presence of paeonol (10) and its glycosides, and monoterpene glycosides, paeoniflorin (6), oxypaeoniflorin, benzoyl-paeoniflorin (7), and benzoyl-oxypaeoniflorin (8), has hitherto been reported.²⁾

Active oxygen species and free radicals react with biomolecular constituents (e.g. lipids, protein, and DNA) to cause certain clinical diseases, such as atherosclerosis, inflammation, diabetes, and cancer³⁾ which are regarded as Oketsu syndrome.

During the course of our chemical studies on the bioactive constituents of Chinese herbal medicine,^{1,4)} the methanolic extract of Chinese Moutan Cortex was found to show scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and superoxide anion radical (O_2^-) generated by the xanthine-xanthine oxidase system.

The radical scavenging effect was concentrated in the ethyl acetate-soluble fraction from the methanolic extract. Two then new monoterpenes, paeonisuffrone (1) and paeonisuffral (3), were isolated from the ethyl acetate-soluble fraction. In this report, we describe a full account of the structural elucidation of 1 and 3.⁵⁾

Chinese Moutan Cortex was extracted with methanol under reflux. The methanolic extract was found to show DPPH radical scavenging effect and inhibitory effect on the formazan formation from nitroblue tetrazolium (NBT) in the xanthine-xanthine oxidase system, while the extract showed little inhibition for xanthine oxidase activity. These results indicated that the extract scavenged O_2^- as well as DPPH radical. The methanolic extract was then partitioned into an ethyl acetate and water mixture to give an ethyl acetate-soluble fraction and an aqueous phase. The aqueous phase was

subjected to XAD-2 column chromatography to give a water-eluted fraction and a methanol-eluted fraction. As is apparent from Table 1, the ethyl acetate-soluble and methanol-eluted fractions showed scavenging effects on both DPPH radical and O_2^- . Particularly, DPPH radical scavenging effect of the ethyl acetate-soluble fraction was more potent than that of positive control, α -tocopherol. The ethyl acetate-soluble fraction was separated by repeated silica gel column chromatography to give 1 (0.0003%), 3 (0.0014%), and 3-O-methylpaeonisuffral (4, 0.0003%) together with paeoniflorigenone (5,⁶⁾ 0.0110%), benzoyl-paeoniflorin (7,⁷⁾ 0.10%), benzoyl-oxypaeoniflorin (8,⁸⁾ 0.05%), paeonol (10,^{2,9)} 1.3%), resacetophenone (11,^{2,9)} 0.0030%), acetovanillone (12,^{2,9)} 0.0010%), acetoisovanillone (13,^{2,9)} 0.0030%), 2,5-dihydroxy-4-methylacetophenone (14,^{2,9)} 0.0020%), 2,5-dihydroxy-4-methoxyacetophenone (15,^{2,9)} 0.0010%).

Compound 1 was isolated as a white powder with negative optical rotation ($[\alpha]_D^{20}$ -16.8°). The IR spectrum of 1 showed absorption bands ascribable to hydroxyl and ketone functions at 3426 and 1725 cm^{-1} . The molecular formula $C_{10}H_{14}O_4$ of 1 was determined from the quasimolecular ion peaks observed in the positive- and negative-ion FAB-MS and by high-resolution MS analysis. Namely, quasimolecular ion peaks were observed at m/z 237 ($M+K$)⁺ and m/z 221 ($M+Na$)⁺ in the positive-ion FAB-MS of 1, while its nega-

Table 1. DPPH radical and O_2^- Scavenging Activities of the Methanolic Extract and its Fractions

	DPPH radical SC ₅₀	O_2^-	
		Formozan formation IC ₅₀	Xanthine oxidase IC ₅₀
MeOH ext.	5.3 $\mu g/ml$	12 $\mu g/ml$	>50 $\mu g/ml$ (27%) ^{a)}
AcOEt-soluble fraction	2.5 $\mu g/ml$	5.5 $\mu g/ml$	>25 $\mu g/ml$ (40%) ^{b)}
MeOH-eluted fraction	10 $\mu g/ml$	11 $\mu g/ml$	>50 $\mu g/ml$ (15%) ^{c)}
H ₂ O-eluted fraction	17 $\mu g/ml$	14 $\mu g/ml$	>50 $\mu g/ml$ (14%) ^{c)}
α -Tocopherol	11 μM	—	—
Gallic acid	3.9 μM	1.8 μM	>10 μM (-7%) ^{a)}
(+)-Catechin	6.0 μM	5.3 μM	>10 μM (-2%) ^{a)}

Values in parentheses represent the inhibition (%) at a) 10 μM , b) 25 $\mu g/ml$, and c) 50 $\mu g/ml$.

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Chart 1

tive-ion FAB-MS showed a quasimolecular ion peak at m/z 197 ($\text{M}-\text{H}$)⁻. The ^1H -NMR (CD_3OD) and ^{13}C -NMR (CD_3OD , Table 2) spectra of **1**, which were assigned with the aid of various NMR analytical methods,¹⁰ showed the presence of a tertiary methyl [δ 1.31 (s, 10- H_3)], two methylenes [δ 2.31 (d, $J=18$ Hz), 2.91 (dd, $J=1, 18$ Hz) (3- H_2); δ 2.21 (d, $J=11$ Hz), 2.47 (ddd, $J=1, 7, 11$ Hz) (7- H_2)], two methylenes bearing an oxygen function [δ 3.57, 3.88 (ABq, $J=10$ Hz, 9- H_2); δ 3.84, 3.89 (ABq, $J=12$ Hz, 8- H_2)], and a methine [δ 2.86 (d, $J=7$ Hz, 5- H)] together with a ketone carbonyl carbon [δ_{C} 212.9 (4-C)], two quaternary carbons bearing an oxygen function [δ_{C} 82.2, 87.6 (1, 2-C)], and another quaternary carbon [δ_{C} 63.0, (6-C)].

Acetylation of **1** with acetic anhydride (Ac_2O) in pyridine furnished the monoacetate (**1a**), which showed signals assignable to a tertiary methyl [δ 1.42 (s, 10- H_3)], an acetyl [δ 2.10 (s)], two methylenes [δ 2.21 (d, $J=11$ Hz), 2.50 (dd, $J=7, 11$ Hz) (7- H_2); 2.54, 2.78 (ABq, $J=18$ Hz, 3- H_2)], a methine [δ 2.84 (d, $J=7$ Hz, 5- H)], a methylene bearing an oxygen function [δ 3.70, 3.92 (ABq, $J=11$ Hz, 9- H_2)], and a

methylene bearing an acetoxyl group [δ 4.38, 4.42 (ABq, $J=12$ Hz, 8- H_2)] in the ^1H -NMR (CDCl_3) spectrum. On the other hand, acetylation of **1** with Ac_2O in pyridine in the presence of dimethylaminopyridine (DMAP) yielded the diacetate (**1b**), which showed no absorption bands ascribable to a hydroxyl function in the IR spectrum. Comparison of the ^{13}C -NMR data (Table 2) of **1** with those for **1a** and **1b** revealed acylation shifts around the 1 and 8-positions in **1**. The connectivities of the quaternary carbons (C-1, 2, 6) were clarified by heteronuclear multiple bond correlation (HMBC) experiment with **1a**. Thus, long-range correlations were observed between the following carbons and protons of **1a**: 1-C and 3- H_2 , 5- H , 8- H_2 ; 2-C and 3- H_2 , 10- H_3 ; 6-C and 7- H_2 , 8- H_2 (Fig. 1).

Reduction of **1** with sodium borohydride (NaBH_4) in methanol was found to stereoselectively give the 4 β -hydroxyl derivative (**2**), which was subjected to acetylation with Ac_2O , pyridine, and DMAP to afford the triacetate (**2a**). The ^1H - ^1H correlation spectroscopy (H-H COSY) experiment on **2** and **2a** indicated the presence of a partial structure (from 3-C to

7-C). In the HMBC experiment, long-range correlations were observed between the following carbons and protons of **2** (1-C and 3-H₂, 5-H, 8-H₂; 2-C and 3-H₂, 10-H₃; 6-C and 4-H, 7-H₂, 8-H₂). Comparison of the ¹H-NMR data for **2** and **2a** with those for **1**, **1a**, and **1b** showed the presence of a partial structure from the 3-position to the 7-position. Furthermore, the nuclear Overhauser enhancement and exchange spectroscopy (NOESY) experiment on **2a** showed NOE correlations between the signals of the following proton pairs (3β-H and 10-H₃; 3α-H and 5-H; 4-H and 5-H; 7β-H and 8-H₂) (Fig. 1). On the basis of the above-mentioned evidence and comparison of the NMR data for **1** and **2** with those for paeoniflorin (**6**) and albiflorin (**9**),⁷⁾ the relative stereostructures of **1** and **2** were determined to be as shown.

Table 2. ¹³C-NMR Data for **1**, **1a**, **1b**, **2**, **3a**, **3b**, and **4**

	1 ^{a)}	1 ^{b)}	1a ^{a)}	1b ^{a)}	2 ^{b)}	3a ^{b)}	3b ^{b)}	4 ^{b)}
C-1	81.7	82.2	81.4	85.6	82.6	79.7	79.7	79.7
C-2	86.1	87.6	86.1	86.0	90.5	45.2	48.1	43.9
C-3	48.6	49.8	48.5	48.9	45.4	107.6	213.0	110.9
C-4	209.9	212.9	209.1	208.2	70.4	45.6	47.9	43.9
C-5	48.5	49.5	48.6	51.0	40.1	32.0	35.7	32.0
C-6	60.4	63.0	59.8	59.0	59.1	102.7	97.3	102.9
C-7	31.2	31.7	31.2	31.9	30.8	44.0	47.3	40.5
C-8	61.2	62.7	69.8	70.5	64.1	67.2	61.2	68.3
C-9	68.8	71.7	63.0	63.1	70.3	101.1	101.0	101.1
C-10	18.9	19.2	19.0	19.8	20.4	22.6	21.9	22.7
COCH ₃			171.7	171.0				
				169.7				
COCH ₃			21.0	20.9 ^{c)}				
				21.1 ^{c)}				
OCH ₃								49.3

The spectra were measured a) in CDCl₃, or b) in CD₃OD, and c) assignments may be interchangeable.

$\Delta\delta$ values in Hz (= $\delta_S - \delta_R$, measured in 270 MHz)

Chart 2

The absolute configuration of **1** has been determined by application of a modified Mosher's method.¹¹⁾ That is, **1** was treated with *t*-butyldimethylsilyl chloride (TBDMS-Cl) and imidazole in dimethylformamide (DMF) to furnish the 8-TBDMS derivative, which was subjected to the stereoselective reduction to provide the alcohol (**2b**). The alcohol (**2b**) was treated with (*R*)- or (*S*)-2-methoxy-2-trifluoromethylphenylacetic (MTPA) acid in the presence of dicyclohexylcarbodiimide (DCC) and DMAP in dichloromethane (CH₂Cl₂) to give the (*R*)-MTPA ester (**2c**) and (*S*)-MTPA ester (**2d**), respectively. As shown in Chart 2, the signals due to protons on the 3 and 10-carbons in the (*R*)-MTPA ester (**2c**) appeared at higher fields than those of the (*S*)-MTPA ester (**2d**) [$\Delta\delta$: positive], whereas the signals due to protons attached to the 5 and 7-carbons of **2c** were observed at lower fields as compared to those of **2d** [$\Delta\delta$: negative]. Consequently, the absolute configuration at the 4-position in **2** has been elucidated to be *R*. On the basis of the above evidence, the absolute structure of a pinane-type monoterpene, **1**, has been characterized as shown.

Compound **3** was also obtained as a white powder with positive optical rotation ($[\alpha]_D^{20} -39.7^\circ$). The IR spectrum of **3** showed absorption bands ascribable to hydroxyl and ketone functions at 3400, 1719, and 1071 cm⁻¹. The positive-ion FAB-MS of **3** showed a quasimolecular ion peak at *m/z* 215 (M+H)⁺ and high-resolution MS analysis revealed the molecular formula of **3** to be C₁₀H₁₄O₅. However, the ¹H-NMR (CD₃OD) and ¹³C-NMR (Table 2) spectra of **3** showed two pairing signals in *ca.* 3:1 ratio. The detailed assignments of the major peaks in the ¹H- and ¹³C-NMR spectra showed the presence of a tertiary methyl [δ 1.14 (s, 10-H₃)], two methylenes [δ 1.77 (d, *J*=14 Hz), 2.01 (dd, *J*=1, 14 Hz) (2-H₂); 1.85 (dd, *J*=1, 13 Hz), 2.13 (dd, *J*=4, 13 Hz) (5-H₂)], a methylene bearing an oxygen function [δ 3.64 (d, *J*=8 Hz), 3.96 (dd, *J*=5, 8 Hz) (8-H₂)], two methines [δ 2.33 (m, 7-H), 2.46 (m, 4-H)], an acetal methine [δ 5.19 (d, *J*=3 Hz, 9-H)], and two ketal carbons (3, 6-C), whereas the minor signals were found to be superimposable on those for **5**, except for some signals due to the benzoyl group of the latter. Treatment of **3** with silica gel (Merck, 60–230 mesh) in methanol yielded **4**, which showed no absorption due to a ketone function in its IR spectrum. The ¹H- and ¹³C-NMR data of **4** were very similar to the major signals in those of **3**, except for the signals due to the methoxyl group. Finally, debenzoylation of **5** with lipase from *Candida cylindracea* furnished **3**, which was converted to **4** by methanol treatment. On the basis of those findings, **3** was a mixture of the 3-ketal form (**3a**) and

3-keto form (**3b**) and the absolute stereostructure of a menthane-type monoterpene, **3**, was clarified as shown.

Experimental

The instruments used for obtaining physical data and the experimental conditions for chromatography were the same as described in our previous paper.¹⁾

Extraction and Isolation Moutan Cortex (10.0 kg, cultivated in Anhwei, China and purchased from Koshiro Co. Ltd., Osaka) were finely minced and extracted with MeOH under reflux. Evaporation of the solvent under reduced pressure gave the MeOH extract (1.98 kg). The MeOH extract (1.97 kg) was partitioned in a AcOEt and H₂O (1:1) mixture, and removal of the solvent under reduced pressure from the AcOEt- and H₂O-soluble fractions yielded 400 g and 1.55 kg of residues, respectively. The H₂O-soluble portion (960 g) was separated by XAD-2 (Organo Co., Ltd., 2 kg, H₂O→MeOH) column chromatography to give a H₂O-eluted fraction (781 g) and a MeOH-eluted fraction (179 g). The AcOEt-soluble fraction (200 g) was subjected to repeated ordinary-phase silica gel column chromatography (4 kg, i) *n*-hexane–AcOEt, ii) CHCl₃–MeOH–H₂O) to give paeonisuffrone (**1**, 0.0003%), paeonisuffral (**3**, 0.0014%), 3-*O*-methylpaeonisuffral (**4**, 0.0003%), paeoniflorigenone (**5**, 0.0110%), benzoyl-paeoniflorin (**7**, 0.10%), benzoyl-oxypaeoniflorin (**8**, 0.05%), paeonol (**10**, 1.3%), resacetophenone (**11**, 0.0030%), acetovanillone (**12**, 0.0010%), acetoisovanillone (**13**, 0.0030%), 2,5-dihydroxy-4-methylacetophenone (**14**, 0.0020%), and 2,5-dihydroxy-4-methoxyacetophenone (**15**, 0.0010%). Five known compounds (**7**, **8**, **10**, **11**, **12**) were identified by comparison of TLC behavior, ¹H- and ¹³C-NMR spectra with those of authentic samples,⁸⁾ while other known compounds (**5**, **13**, **14**, **15**) were identified by comparison of their physical data with reported values.^{6,9)}

Paeonisuffrone (1): A white powder, [α]_D²⁰ –16.8° (*c*=1.45, MeOH). High-resolution FAB-MS: Calcd for C₁₀H₁₄O₄Na (M+Na)⁺: 221.0790. Found: 221.0802. IR (KBr): 3426, 1725, 1705, 1597, 1541, 1408 cm⁻¹. ¹H-NMR (CD₃OD) δ : 1.31 (3H, s, 10-H₃), 2.21 (1H, d, *J*=11 Hz), 2.47 (1H, ddd, *J*=1, 7, 11 Hz) (7-H₂), 2.31 (1H, d, *J*=18 Hz), 2.91 (1H, dd, *J*=1, 18 Hz) (3-H₂), 2.86 (1H, d, *J*=7 Hz, 5-H), 3.57, 3.88 (2H, ABq, *J*=10 Hz, 9-H₂), 3.84, 3.89 (2H, ABq, *J*=12 Hz, 8-H₂); (CDCl₃) δ : 1.40 (3H, s, 10-H₃), 2.19 (1H, d, *J*=11 Hz), 2.55 (1H, dd, *J*=7, 11 Hz) (7-H₂), 2.50, 2.75 (2H, ABq, *J*=18 Hz, 3-H₂), 2.66 (1H, d, *J*=7 Hz, 5-H), 3.62, 4.09 (2H, ABq, *J*=10 Hz, 9-H₂), 3.82, 4.07 (2H, ABq, *J*=12 Hz, 8-H₂). ¹³C-NMR (CDCl₃ and CD₃OD) δ : given in Table 2. Positive-ion FAB-MS *m/z*: 237 (M+K)⁺, 221 (M+Na)⁺. Negative-ion FAB-MS *m/z*: 197 (M-H)⁻.

Paeonisuffral (3): A white powder, [α]_D²⁰ +39.7° (*c*=0.6, MeOH). High-resolution FAB-MS: Calcd for C₁₀H₁₄O₅ (M+H)⁺: 215.0920. Found: 215.0898. IR (KBr): 3400, 2936, 1719, 1356, 1071 cm⁻¹. ¹H-NMR (CD₃OD) δ : the major signals assignable to the ketal form (**3a**): 1.14 (s, 10-H₃), 1.77 (d, *J*=14 Hz), 2.01 (dd, *J*=1, 14 Hz) (2-H₂), 1.85 (dd, *J*=1, 13 Hz), 2.13 (dd, *J*=4, 13 Hz) (5-H₂), 2.33 (m, 7-H), 2.46 (m, 4-H), 3.64 (d, *J*=8 Hz), 3.96 (dd, *J*=5, 8 Hz) (8-H₂), 5.19 (d, *J*=3 Hz, 9-H); the minor signals assignable to the ketone form (**3b**): 1.22 (s, 10-H₃), 2.01 (m, 7-H), 2.18 (dd, *J*=2, 13 Hz), 2.28 (dd, *J*=3, 13 Hz) (5-H₂), 2.48 (d, *J*=17 Hz), 2.72 (d, *J*=17 Hz) (2-H₂), 2.71 (m, 4-H), 3.29 (dd, *J*=9, 11 Hz), 3.47 (dd, *J*=6, 11 Hz) (8-H₂), 5.37 (brs, 9-H). ¹³C-NMR (CD₃OD) δ : given in Table 2. Positive-ion FAB-MS *m/z*: 215 (M+H)⁺.

3-*O*-Methylpaeonisuffral (4): A white powder, [α]_D²⁰ +21.1° (*c*=0.4, MeOH). High-resolution FAB-MS: Calcd for C₁₁H₁₆O₅Na (M+Na)⁺: 251.0895. Found: 251.0908. IR (KBr): 3419, 2936, 1356, 1073 cm⁻¹. ¹H-NMR (CD₃OD) δ : 1.15 (3H, s, 10-H₃), 1.76, 2.61 (1H each, both br d, *J*=*ca.* 14 Hz, 2-H₂), 1.76 (1H, d, *J*=13 Hz), 2.07 (1H, dd, *J*=5, 13 Hz) (5-H₂), 2.34 (1H, m, 7-H), 2.70 (1H, m, 4-H), 3.25 (3H, s, OMe), 3.71 (1H, d, *J*=9 Hz), 3.92 (1H, dd, *J*=4, 9 Hz) (8-H₂), 5.21 (1H, d, *J*=3 Hz, 9-H). ¹³C-NMR (CD₃OD) δ : given in Table 2.

Acetylation of 1 with Ac₂O in Pyridine A solution of **1** (20 mg) in pyridine (0.43 ml) was treated with Ac₂O (0.14 ml) and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with 1% aqueous HCl, saturated aqueous NaHCO₃, and brine, then dried over MgSO₄ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by silica gel column chromatography (0.5 g, *n*-hexane–AcOEt=1:3) to give **1a** (1 mg).

1a: A white powder, [α]_D²⁰ –48.9° (*c*=0.55, EtOH). High-resolution FAB-MS: Calcd for C₁₂H₁₆O₅Na (M+Na)⁺: 263.0895. Found: 263.0876. IR (KBr): 3566, 2975, 1732, 1240, 1042 cm⁻¹. ¹H-NMR (CDCl₃) δ : 1.42 (3H,

s, 10-H₃), 2.10 (3H, s, OAc), 2.21 (1H, d, *J*=11 Hz), 2.50 (1H, dd, *J*=7, 11 Hz) (7-H₂), 2.54, 2.78 (2H, ABq, *J*=18 Hz, 3-H₂), 2.84 (1H, d, *J*=7 Hz, 5-H), 3.70, 3.92 (2H, ABq, *J*=11 Hz, 9-H₂), 4.38, 4.42 (2H, ABq, *J*=12 Hz, 8-H₂). ¹³C-NMR (CDCl₃) δ : given in Table 2. Positive-ion FAB-MS *m/z*: 263 (M+Na)⁺.

Acetylation of 1 with Ac₂O in Pyridine and DMAP A solution of **1** (20 mg) in pyridine (0.28 ml) was treated with Ac₂O (0.10 ml) and DMAP (2 mg) and the mixture was stirred at 40 °C for 12 h. The reaction mixture was poured into ice-water and the whole mixture was extracted with AcOEt. The AcOEt was treated in the usual manner to give a residue, which was purified by silica gel column chromatography (1 g, *n*-hexane–AcOEt) to provide **1b** (19 mg).

1b: A white powder, [α]_D²⁰ –9.0° (*c*=0.5, EtOH). High-resolution FAB-MS: Calcd for C₁₄H₁₈O₆Na (M+Na)⁺: 305.1001. Found: 305.1026. IR (KBr): 2930, 1790, 1744, 1372, 1242, 1134, 1046, 1022 cm⁻¹. ¹H-NMR (CDCl₃) δ : 1.36 (3H, s, 10-H₃), 2.09, 2.12 (3H each, both s, OAc×2), 2.54, 2.92 (2H, ABq, *J*=17 Hz, 3-H₂), 2.76 (2H, s, 7-H₂), 3.00 (1H, m, 5-H), 3.72, 3.92 (2H, ABq, *J*=16 Hz, 9-H₂), 4.36, 4.40 (2H, ABq, *J*=12 Hz, 8-H₂). ¹³C-NMR (CDCl₃) δ : given in Table 2. Positive-ion FAB-MS *m/z*: 305 (M+Na)⁺.

Reduction of 1 with NaBH₄ A solution of **1** (13 mg) in EtOH (1.0 ml) was treated with NaBH₄ (6 mg) and the mixture was stirred at room temperature for 1.5 h. Excess NaBH₄ was quenched with Dowex 50W×8 (H⁺ form) and filtered. Removal of the solvent from the filtrate yielded **2** (8 mg).

2: A white powder, [α]_D²⁰ –6.2° (*c*=0.33, EtOH). High-resolution FAB-MS: Calcd for C₁₀H₁₆O₄Na (M+Na)⁺: 223.0946. Found: 223.0927. IR (KBr): 3400, 2930 cm⁻¹. ¹H-NMR (CD₃OD) δ : 1.22 (3H, s, 10-H₃), 1.54 (1H, d, *J*=10 Hz), 2.03 (1H, dd, *J*=8, 10 Hz) (7-H₂), 1.78 (1H, d, *J*=16 Hz), 2.29 (1H, dd, *J*=8, 16 Hz) (3-H₂), 2.42 (1H, dd, *J*=4, 8 Hz, 5-H), 3.68, 4.62 (2H, ABq, *J*=8 Hz, 9-H₂), 3.80, 3.83 (2H, ABq, *J*=12 Hz, 8-H₂), 4.11 (1H, dd, *J*=4, 8 Hz, 4-H). ¹³C-NMR (CD₃OD) δ : given in Table 2. Positive-ion FAB-MS *m/z*: 223 (M+Na)⁺.

Acetylation of 2 with Ac₂O in Pyridine and DMAP A solution of **2** (3 mg) in pyridine (0.28 ml) was treated with Ac₂O (0.14 ml) and DMAP (1 mg) and the mixture was stirred at 40 °C for 12 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was treated in the usual manner to give a residue, which was purified by silica gel column (0.5 g, *n*-hexane–AcOEt) to furnish **2a** (4.8 mg).

2a: A white powder, [α]_D²⁰ –1.5° (*c*=0.3, EtOH). IR (KBr): 2924, 1740, 1250. ¹H-NMR (CDCl₃) δ : 1.27 (3H, s, 10-H₃), 1.98 (1H, d, *J*=16 Hz, 3- α -H), 2.06, 2.07, 2.09 (3H each, all s, OAc×3), 2.23 (1H, d, *J*=12 Hz, 7- α -H), 2.43 (1H, dd, *J*=8, 12 Hz, 7- β -H), 2.51 (1H, dd, *J*=8, 16 Hz, 3- β -H), 2.67 (1H, dd, *J*=4, 8 Hz, 5-H), 3.83, 4.44 (1H each, both d, *J*=9 Hz, 9-H₂), 4.27, 4.39 (2H, ABq, *J*=12 Hz, 8-H₂), 5.12 (1H, dd, *J*=4, 8 Hz, 4-H).

Preparation of 4 from 3 A solution of **3** (10 mg) in MeOH (2 ml) was treated with silica gel (0.5 g, Merck, 60–230 mesh) and the mixture was stirred at room temperature for 12 h. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (1 g, CHCl₃–MeOH=8:1) to give **4** (8 mg), which was identified by comparison of TLC behavior and IR, ¹H-NMR, and ¹³C-NMR spectra with those of natural authentic **4**.

Debenzoylation of 5 Followed by Methanol Treatment A solution of **5** (23.7 mg) in a mixture of phosphate buffer (pH 7.0, 0.5 ml) and acetone (0.5 ml) was treated with 20 mg lipase (from *Candida cylindracea*, Type VII, Sigma) and the mixture was stirred at 37 °C for 10 h. The reaction mixture was poured into MeOH and filtered. The filtrate was evaporated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (1 g, CHCl₃–MeOH=8:1) to give **3** (15.2 mg), which was identical by comparison of TLC behavior and ¹H-NMR spectrum with those of natural authentic **3**.

DPPH Radical Scavenging Activity DPPH radical scavenging activities of the MeOH extract and fractions were investigated according to the method of Uchiyama *et al.*¹²⁾ Briefly, a solution of the extract in acetate buffer (pH 5.5, 2.0 ml) and EtOH (2.0 ml) was treated with 2×10⁻⁴ M DPPH EtOH solution (1.0 ml) and the mixture was incubated at room temperature for 30 min. Reduction of the DPPH radical was measured at 517 nm. Measurements were performed in duplicate, and the concentration required for a 50% reduction (50% scavenging concentration, SC₅₀) of 40 μ M DPPH radical was determined graphically. Results are shown in Table 1.

O₂⁻ Scavenging Activity The improved assay method for superoxide dismutase described by Imanari *et al.* was used.¹³⁾ Briefly, a reaction mixture containing 100 μ M xanthine, 100 μ M EDTA, 25 μ M NBT, 0.005% bovine

serum albumin, and *ca.* 1.8 mU/ml xanthine oxidase in 33.3 mM sodium carbonate buffer (pH 10.2) was incubated with or without each test sample for 20 min at 25 °C (total volume: 3.0 ml). After incubation, the solution was mixed with 0.1 ml of 6 mM CuCl₂ to stop the reaction. The formazan formation was monitored at 560 nm. In addition, inhibitory effect of test samples on xanthine oxidase activity was examined to clarify whether the inhibition of the formazan formation was due to inhibition of xanthine oxidase. The reaction mixture without NBT was incubated in similar conditions described above and 0.1 ml of 2 N HCl was added to stop the reaction. Uric acid formation was monitored at 290 nm. IC₅₀ values for the formazan formation and xanthine oxidase activity were determined.

References and Notes

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