Bioactive Constituents of Chinese Natural Medicines. VI.[1] Moutan Cortex. (2): Structures and Radical Scavenging Effects of Suffruticosides A, B, C, D, and E and Galloyl-oxypaeoniflorin

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Five paeonol glycosides, suffruticosides A, B, C, D, and E, and a monoterpene glucoside, galloyl-oxypaeoniflorin, were isolated from the glycosidic fraction of Chinese Moutan Cortex, the root cortex of *Paeonia suffruticosa* ANDREWS, together with paeonolide, apiopaeonoside, galloyl-paeoniflorin, oxypaeoniflorin, and paeoniflorin. The structures of five suffruticosides and galloyl-oxypaeoniflorin were elucidated on the basis of chemical and physicochemical evidence. Suffruticosides A, B, C, and D, galloyl-oxypaeoniflorin, and galloyl-paeoniflorin exhibited more potent radical scavenging effects than α-tocopherol.

Key words *Paeonia suffruticosa*; suffruticoside; galloyl-oxypaeoniflorin; radical scavenging effect; Moutan Cortex

In the course of characterization studies on bioactive constituents of Chinese herbal medicines,[1,2] we have found that the methanolic extract of Chinese Moutan Cortex showed a potent scavenging effect on 1,1-diphenylpicryl-2-hydrazyl (DPPH) radical, and this radical scavenging effect was concentrated on the ethyl acetate-soluble fraction and the methanol-eluted fraction of XAD-2 column chromatography of the water-soluble portion from the methanolic extract. In a previous paper, we reported the isolation and structure elucidation of two monoterpenes, paeonisuffrone and paeonisuffral, from the ethyl acetate-soluble fraction.[1] As a continuation of our studies on Moutan Cortex,[1,3] we isolated five paeonol glycosides called suffruticosides A (**1**), B (**2**), C (**4**), D (**5**), and E (**7**) and galloyl-oxypaeoniflorin (**8**) from the methanol-eluted portion. This paper offers a full account of the structure elucidation of these suffruticosides and **8**. In addition, we describe the DPPH radical scavenging effects of constituents from the methanol-eluted portion.[4]

Moutan Cortex was extracted with methanol under reflux. Since the methanolic extract was found to exhibit the DPPH radical scavenging effect, it was partitioned in an ethyl acetate and water mixture to give an ethyl acetate and a water-soluble portion.[1] The water-soluble portion was subjected to Amberlite XAD-2 column chromatography to give a water-eluted and a methanol-eluted fraction. The methanol-eluted fraction showed the DPPH radical scavenging activity (SC_{50}=10 μg/ml),[1] so this fraction was subjected to ordinary silica gel column chromatography to give suffruticosides A (**1**, 0.0004%), B (**2**, 0.0003%), C (**4**, 0.0002%), D (**5**, 0.0004%), and E (**7**, 0.0005%) and galloyl-oxypaeoniflorin (**8**, 0.0010%), together with apiopaeonoside[5] (**3**, 0.0002%), paeonolide[6] (**6**, 0.0002%), paeonoside[7] (**13**, 0.0001%), galloyl-paeoniflorin[8] (**9**, 0.0005%), oxypaeoniflorin[9] (**10**, 0.26%), and paeoniflorin[9] (**11**, 0.52%).

Structures of Suffruticosides A (1) and B (2) Suffruticoside A (**1**) was isolated as a white powder. The positive-ion FAB-MS of **1** showed quasimolecular ion peaks at *m/z* 651 $(M+K)^+$, 635 $(M+Na)^+$, and 613 $(M+H)^+$. The molecular formula $C_{27}H_{32}O_{16}$ of **1** was determined from the quasimolecular ion peak $(M+Na)^+$ and by high-resolution MS analysis. The IR spectrum of **1** showed absorption bands at 3450, 1703, 1651, 1605, and 1070 cm^{-1} ascribable to hydroxyl, ester, and aromatic ring, while absorption maxima were observed at 219 nm (ε 30300) and 272 nm (ε 16200) in the UV spectrum. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned using various NMR analytical methods,[10] showed a paeonol moiety [δ 3.01 (s, 1-Ac), 3.84 (s, 4-OCH_3), 6.67 (dd, J=2.0, 8.8 Hz, 5-H), 7.40 (d, J=2.0 Hz, 3-H), 8.12 (d, J=8.8 Hz, 6-H)], and a galloyl moiety [δ 7.87 (s, 2‴, 6-H)] together with apiofuranosyl and glucopyranosyl moieties. Treatment of **1** with 3% sodium methoxide in methanol furnished apiopaeonoside (**3**)[5] and methyl gallate. Comparison of the ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) data for **1** with those for **3** revealed an acylation shift around the 5″-position of **1** [δ 4.78, 4.84 (ABq, J=10.0 Hz, 5″-H_2)]. Consequently, the structure of suffruticoside A was determined to be paeonol 2-*O*-5″-*O*-galloyl-β-D-apiofuranosyl (1″-6′)-β-D-glucopyranoside (**1**).

Suffruticoside B (**2**), also isolated as a white powder, liberated **3** and methyl gallate by alkaline treatment. The IR and UV spectra of **2** were found to be similar to those of **1**. The molecular formula $C_{27}H_{32}O_{16}$, which was the same as that of **1**, was confirmed from the quasimolecular ion peaks at *m/z* 635 $(M+Na)^+$ and 613 $(M+H)^+$ in the positive-ion FAB-MS and by high-resolution MS measurement. The ^{1}H-NMR and ^{13}C-NMR (Table 1) spectra[10] of **2** indicated the presence of an apiopaeonoside moiety and galloyl group. By detailed comparison of the ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR data for **2** with those for **1** and **3**, an acylation shift was observed around the 4′-position of **2** [δ 5.77 (dd, J=9.5, 9.5, 4′-H)]. On the basis of the above evidence, the structure of suffruticoside B was characterized as paeonol 2-*O*-β-D-apiofuranosyl (1″-6′)-4′-*O*-galloyl-β-D-glucopyranoside (**2**).

Structures of Suffruticosides C (4), D (5), and E (7) Suffruticosides C (**4**) and D (**5**) were each obtained as a white powder. The IR spectra of **4** and **5** were very similar and showed absorption bands due to hydroxyl, ester, and aromatic ring. The UV spectra of **4** and **5** were superimposable on those of **1** and **2** and their absorption maxima suggested the paeonol glycoside structure having a galloyl group. Suffruticosides C (**4**) and D (**5**) were found to have the same molecular formula $C_{27}H_{32}C_{16}$ as **1** and **2**, which was determined from their positive-ion FAB-MS and by high-resolution analysis. Treatment of **4** and **5** with sodium methoxide in

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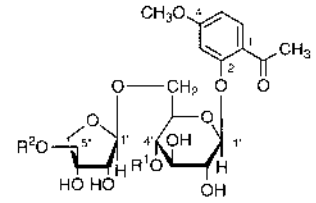
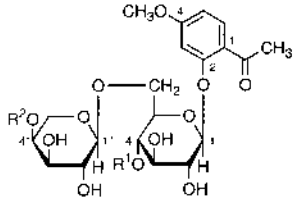
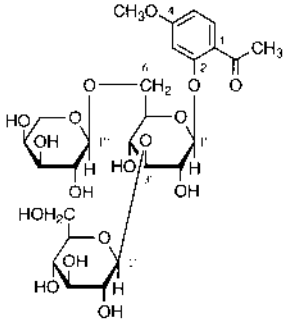


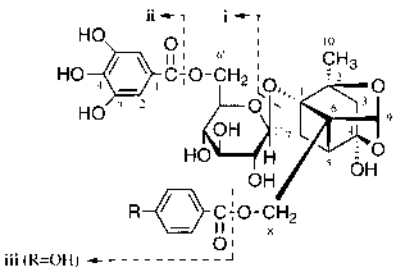
suffruticoside A (**1**) : R^1=H, R^2=galloyl
suffruticoside B (**2**) : R^1=galloyl, R^2=H
apiopaeonoside (**3**) : R^1=R^2=H



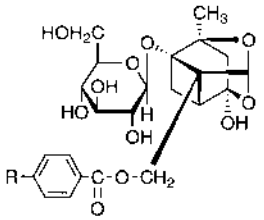
suffruticoside C (**4**) : R^1=H, R^2=galloyl
suffruticoside D (**5**) : R^1=galloyl, R^2=H
paeonolide (**6**) : R^1=R^2=H



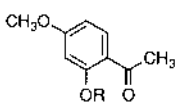
suffruticoside E (**7**)



galloyl-oxypaeoniflorin (**8**) : R=OH
galloyl-paeoniflorin (**9**) : R=H



oxypaeoniflorin (**10**) : R=OH
paeoniflorin (**11**) : R=H



paeonol (**12**) : R=H
paeonoside (**13**) : R=β-D-glucopyranosyl

Chart 1

methanol furnished **6**[6] and methyl gallate. Comparison of the ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra for **4** or **5** with those for **6** revealed an acylation shift at the 4″-position of **4** or at the 4′-position of **5**. This evidence led us to clarify the structures of suffruticosides C and D to be paeonol 2-*O*-4″-*O*-galloyl-α-L-arabinopyranosyl (1″-6′)-β-D-glucopyranoside (**4**) and paeonol 2-*O*-α-L-arabinopyranosyl (1″-6′)-4′-*O*-galloyl-β-D-glucopyranoside (**5**), respectively.

The IR and UV spectra of **7** were similar to those of **3** and **6** and suggestive of the paeonol glycoside structure. Hydrolysis with 5% aqueous sulfuric acid in 1,4-dioxane (1 : 1, v/v) liberated D-glucose and L-arabinose, which were identified by GLC analysis of their thiazolidine derivatives.[11] In the positive-ion and negative-ion FAB-MS of **7**, quasimolecular ion peaks were observed at *m/z* 645 $(M+Na)^+$ and *m/z* 621 $(M-H)^-$, respectively, and high-resolution MS analysis revealed the molecular formula of **7** as $C_{26}H_{38}O_{17}$. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **7** showed signals due to an α-L-arabinopyranosyl [δ 4.81 (d, J=8.0 Hz, 1‴-H)] and two β-D-glucopyranosyl [δ 5.32 (d, J=7.9 Hz, 1″-H), 5.60 (d, J=7.3 Hz, 1′-H)] parts together with a paeonol moiety. The trisaccharide structure of **7** was determined by ^{1}H-NMR nuclear Overhauser and exchange spectroscopy (NOESY) and heteronuclear multiple bond connectivity (HMBC) experiments. Thus, the NOE correlations were observed between the 3-proton and the 1′-proton, between the 1″-proton and the 3′-proton, and between the 1‴-proton and the 6′-proton, whereas long-range correlations were observed between the 1′-proton and the 2-carbon, between the 1″-proton and the 3′-carbon, and between the 1‴-proton and the 6′-carbon. Consequently, the structure of suffruticoside E was determined to be paeonol 2-*O*-[β-D-glucopyranosyl (1″-3′)] [α-L-arabinopyranosyl (1‴-6′)] β-D-glucopyranoside (**7**).

Structure of Galloyl-oxypaeoniflorin (8) Galloyl-oxypaeoniflorin (**8**), obtained as a white powder, showed absorption bands assignable to hydroxyl, ester, and aromatic ring in the IR spectrum. The UV spectrum of **8** showed absorption maxima at 215 nm (ε 17000) and 267 nm (ε 9550). The positive-ion SIMS of **8** showed a quasimolecular ion peak at *m/z* 649 $(M+H)^+$ in addition to fragment ion peaks at *m/z* 315 (i), 153 (ii), and 121 (iii), which were derived by cleavage of the galloyl-glucosyl, galloyl, and *p*-hydroxybenzoyl moieties. The ^{1}H-NMR spectrum of **8** showed the presence of a galloyl and a *p*-hydroxybenzoyl groups. Comparison of the ^{13}C-NMR (Table 2) for **8** with those for **10**,[3a] **9**,[8] and benzoyl-

Table 1. ^{13}C-NMR Data for **1**, **2**, **4**, **5**, **7**, and **8** (75 MHz, Pyridine-d_5, δ_C)

		1	**2**	**4**	**5**	**7**			**8**
Paeonol moiety	C-1	121.9	122.1	122.0	121.9	121.8	Monoterpene moiety	C-1	88.8
	C-2	160.0	159.9	159.9	159.8	159.7		C-2	85.9
	C-3	102.4	102.3	102.0	102.2	101.6		C-3	44.6
	C-4	164.8	164.9	164.8	164.8	164.8		C-4	105.9
	C-5	108.0	108.4	108.4	108.9	108.8		C-5	43.6
	C-6	132.1	132.2	132.1	132.1	135.6		C-6	71.5
	Me	32.3	32.4	32.3	32.3	32.3		C-7	22.6
	C=O	197.2	197.0	197.2	197.0	197.1		C-8	60.6
	OMe	55.6	55.7	55.6	55.8	55.8		C-9	101.5
β-D-Gluco-	C-1′	102.4	102.4	102.5	101.7	101.8		C-10	19.7
pyranosyl	C-2′	74.6	74.6[a]	74.5	74.1	73.3	β-D-Gluco-	C-1′	101.0
moiety	C-3′	78.4[a]	76.0	78.6	75.9[a]	88.3	pyranosyl	C-2′	74.7
	C-4′	71.5	72.3	71.0	72.3	69.2	moiety	C-3′	78.0
	C-5′	77.2	74.9[a]	77.5	75.3[a]	77.0		C-4′	71.2
	C-6′	69.1	68.5	70.4	69.4	69.7		C-5′	75.0
α-L-Arabino-	C-1″	110.6	111.2	106.0	105.6	105.9		C-6′	64.4
pyranosyl	C-2″	78.3	77.7	72.0[a]	71.9	72.2	*p*-Hydroxy-	C-1	121.1[a]
or β-D-apio-	C-3″	78.8[a]	80.2	72.6[a]	74.5	75.3	benzoyl	C-2	132.2
furanosyl	C-4″	74.6	75.1	64.8	69.1	69.2	moiety	C-3	115.9
moiety	C-5″	67.4	65.4	72.8	66.7	66.8		C-4	163.4
	C-1	120.8	120.7	121.2	120.7			C=O	166.5[b]
Galloyl	C-2	110.2	110.5	110.3	110.5		Galloyl	C-1	121.0[a]
moiety	C-3	147.5	147.6	147.4	147.5		moiety	C-2	110.1
	C-4	141.0	141.2	140.8	141.2			C-3	147.4
	C=O	167.0	166.7	167.0	166.9			C-4	140.9
β-D-Gluco-	C-1‴					105.5		C=O	167.0[b]
pyranosyl	C-2‴					74.3			
moiety	C-3‴					78.1			
	C-4‴					71.4			
	C-5‴					78.6			
	C-6‴					62.3			

a, *b*) Assignments may be interchangeable within the same column.

Table 2. Radical Scavenging Effect on DPPH Radical for Constituents from Moutan Cortex

Compounds	SC_{50} (μM)
Suffruticoside A (**1**)	6.2
Suffruticoside B (**2**)	6.6
Apiopaeonoside (**3**)	No effect
Suffruticoside C (**4**)	6.6
Suffruticoside D (**5**)	5.1
Paeonolide (**6**)	No effect
Suffruticoside E (**7**)	No effect
Galloyl-oxypaeoniflorin (**8**)	5.7
Galloyl-paeoniflorin (**9**)	5.4
Oxypaeoniflorin (**10**)	No effect
Paeoniflorin (**11**)	No effect
α-Tocopherol	9.8
Gallic acid	3.9

Concentration required for 50% reduction of 40 μM DPPH radical solution. Measurement in acetic acid buffer (pH 5.5). No effect: $SC_{50}>100$ μM.

oxypaeoniflorin[3a] led us to presume that the galloyl group in **8** is attached to the 6′-position of the glucose moiety. Finally, partial alkaline hydrolysis of **8** with 1% aqueous potassium hydroxide yielded **10**, and thus the structure of **8** was determined.

Radical Scavenging Effect on DPPH Radical The methanol-eluted fraction of the water-soluble portion from Chinese Moutan Cortex showed a scavenging effect on DPPH radical ($SC_{50}=10$ μg/ml).[1] We then examined the radical scavenging effects of seven paeonol glycosides (**1**—**7**) and four monoterpene glucosides (**8**—**11**) from the methanol-eluted fraction of Chinese Moutan Cortex. As shown in Table 2, four paeonol glycosides (**1**, **2**, **4**, **5**) and two monoterpene glucosides (**8**, **9**) showed more potent radical scavenging effect than α-tocopherol. Since the paeonol glycosides (**3**, **6**), **10**, and **11**, which lacked a galloyl group, showed no effect, the galloyl group was confirmed to be essential for the radical scavenging effect.

Experimental

The instruments used for obtaining physical data and experimental conditions for chromatography were the same as we described previously.[2]

Isolation of Paeonol Glycosides and Monoterpene Glucosides The MeOH extract (1.98 kg) obtained from Chinese Moutan Cortex (10.0 kg) was partitioned into an AcOEt and H_2O mixture to give the AcOEt-soluble fraction and the H_2O-soluble portion (1.55 kg). The H_2O-soluble portion (960 g) was subjected to XAD-2 (Organo Co. Ltd., 2 kg, $H_2O\rightarrow$MeOH) column chromatography to give a H_2O-eluted fraction (781 g) and a MeOH-eluted fraction (179 g).[1] The MeOH-eluted fraction (118 g) was separated by ordinary-silica gel column chromatography (3 kg, $CHCl_3$–MeOH–H_2O) and reversed-phase silica gel column chromatography (3 kg, MeOH–H_2O) to provide suffruticosides A (**1**, 0.0004%), B (**2**, 0.0003%), C (**4**, 0.0002%), D (**5**, 0.0004%), E (**7**, 0.0005%), and galloyl-oxypaeoniflorin (**8**, 0.0010%) together with apiopaeonoside (**3**, 0.0002%), paeonolide (**6**, 0.0002%), paeonoside (**13**, 0.0001%), galloyl-paeoniflorin (**9**, 0.0005%), oxypaeoniflorin (**10**, 0.26%), and paeoniflorin (**11**, 0.52%).

Known glycosides were identified by comparison of their physical data with authentic samples and reported values.[3—9]

Suffruticoside A (**1**): A white powder, $[\alpha]_D^{20}$ −57.8° (c=1.1, MeOH). High-resolution FAB-MS: Calcd for $C_{27}H_{32}O_{16}Na$ $(M+Na)^+$: 635.1588. Found: 635.1592. UV [EtOH, nm (ε)]: 219 (30300), 272 (16200). IR (KBr) cm^{-1}: 3450, 1703, 1651, 1605, 1205, 1070, 1035. ^{1}H-NMR (pyridine-d_5) δ: 3.01 (3H, s, Ac), 3.84 (3H, s, OCH_3), 4.73 (1H, d, J=2.3 Hz, 2″-H), 4.78,

4.84 (2H, ABq, J=10.0 Hz, 5″-H_2), 6.67 (1H, dd, J=2.0, 8.8 Hz, 5-H), 7.40 (1H, d, J=2.0 Hz, 3-H), 7.87 (2H, s, galloyl-H), 8.12 (1H, d, J=8.8 Hz, 6-H). ^{13}C-NMR: given in Table 1. Positive-ion FAB-MS: m/z 651 $(M+K)^+$, 635 $(M+Na)^+$, 613 $(M+H)^+$, 167, 153, 133.

Suffruticoside B (**2**): A white powder, $[\alpha]_D^{20}$ −32.7° (c=0.8, MeOH). High-resolution FAB-MS: Calcd for $C_{27}H_{32}O_{16}Na$ $(M+Na)^+$: 635.1588. Found: 635.1586. UV: [EtOH, nm (ε)]: 217 (19900), 271 (10760). IR (KBr) cm^{-1}: 3450, 1707, 1655, 1602, 1448, 1361, 1037. ^{1}H-NMR (pyridine-d_5) δ: 2.94 (3H, s, Ac), 3.85 (3H, s, OCH_3), 4.13 (2H, br s, 5″-H_2), 4.26, 4.51 (2H, ABq, J=9.3 Hz, 4″-H_2), 4.47 (1H, t-like, 3′-H), 4.69 (1H, d, J=2.0 Hz, 2″-H), 5.63 (1H, d, J=7.3 Hz, 1′-H), 5.56 (1H, d, J=2.0 Hz, 1″-H), 5.77 (1H, dd, J=9.5, 9.5 Hz, 4′-H), 6.67 (1H, dd, J=2.0, 8.8 Hz, 5-H), 7.30 (1H, d, J=2.0 Hz, 3-H), 7.87 (2H, s, galloyl-H_2), 8.08 (1H, d, J=8.8 Hz, 6-H). ^{13}C-NMR: given in Table 1. Poitive-ion FAB-MS: m/z 635 $(M+Na)^+$, 613 $(M+H)^+$, 167, 153, 133.

Suffruticoside C (**4**): A white powder, $[\alpha]_D^{20}$ −8.8° (c=0.5, MeOH). High-resolution FAB-MS: Calcd for $C_{27}H_{32}O_{16}Na$ $(M+Na)^+$: 635.1588. Found: 635.1588. UV [EtOH, nm (ε)]: 217 (29400), 271 (15600). IR (KBr) cm^{-1}: 3450, 1700, 1657, 1603, 1254, 1072. ^{1}H-NMR (pyridine-d_5) δ: 2.92 (3H, s, Ac). 3.81 (3H, s, OCH_3), 4.89 (1H, d, J=7.3 Hz, 1″-H), 5.65 (1H, d, J=7.6 Hz, 1′-H), 5.82 (1H, br s, 4″-H), 6.65 (1H, dd, J=2.2, 8.8 Hz, 5-H), 7.29 (1H, d, J=2.2 Hz, 3-H), 7.97 (2H, s, galloyl-H_2), 8.06 (1H, d, J=8.8 Hz, 6-H). Positive-ion FAB-MS: m/z 651 $(M+K)^+$, 635 $(M+Na)^+$, 613 $(M+H)^+$, 167.

Suffruticoside D (**5**): A white powder, $[\alpha]_D^{20}$ −5.3° (c=0.4, H_2O). High-resolution FAB-MS: Calcd for $C_{27}H_{32}O_{16}Na$ $(M+Na)^+$: 635.1588. Found: 635.1569. UV [EtOH, nm (ε)]: 217 (16600), 272 (9060). IR (KBr) cm^{-1}: 3450, 1707, 1655, 1601, 1203, 1072. ^{1}H-NMR (pyridine-d_5) δ: 2.93 (3H, s, Ac), 3.86 (3H, s, OCH_3), 4.64 (1H, d, J=7.1 Hz, 1″-H), 5.66 (1H, d, J=7.6 Hz, 1′-H), 5.83 (1H, dd, J=9.5, 9.5 Hz, 4′-H), 6.69 (1H, dd, J=2.2, 8.8 Hz, 5-H), 7.29 (1H, d, J=2.2 Hz, 3-H), 7.92 (2H, s, galloyl-H_2), 8.08 (1H, d, J=8.8 Hz, 6-H). Positive-ion FAB-MS: m/z 635 $(M+Na)^+$, 619 $(M+Li)^+$, 613 $(M+H)^+$, 167, 153, 133.

Suffruticoside E (**7**): A white powder, $[\alpha]_D^{20}$ −47.8° (c=0.49, H_2O). High-resolution FAB-MS: Calcd for $C_{26}H_{38}O_{17}Na$: 645.2007. Found: 645.2015. UV [EtOH, nm (ε)]: 215 (17000), 268 (9550). IR (KBr) cm^{-1}: 3450, 1661, 1605, 1264, 1076. ^{1}H-NMR (pyridine-d_5) δ: 2.89 (3H, s, Ac), 3.68 (1H, d, J=10.7 Hz, 5‴-H), 3.83 (3H, s, OCH_3), 4.56 (1H, dd, J=2.0, 11.5 Hz, 6″-H), 4.70 (1H, br d, J=9.9 Hz, 6′-H), 4.81 (1H, d, J=8.0 Hz, 1‴-H), 5.32 (1H, d, J=7.9 Hz, 1″-H), 5.60 (1H, d, J=7.3 Hz, 1′-H), 6.77 (1H, dd, J=2.2, 8.8 Hz, 5-H), 7.25 (1H, d, J=2.2 Hz, 3-H), 8.07 (1H, d, J=8.8 Hz, 6-H). ^{13}C-NMR: given in Table 1. Positive-ion FAB-MS: m/z 645 $(M+Na)^+$, 629 $(M+Li)^+$. Negative-ion FAB-MS: m/z 621 $(M-H)^-$.

Galloyl-oxypaeoniflorin (**8**): A white powder, $[\alpha]_D^{20}$ −27.3° (c=0.42, EtOH). High-resolution FAB-MS: Calcd for $C_{30}H_{33}O_{16}$ $(M+H)^+$: 649.1769. Found: 649.1732. UV [EtOH, nm (ε)]: 215 (17000), 267 (9550). IR (KBr) cm^{-1}: 3450, 1701, 1653, 1609, 1230, 1076. ^{1}H-NMR (pyridine-d_5) δ: 1.67 (3H, s, 10-H_3), 5.11 (1H, d, J=7.5 Hz, 1′-H), 5.93 (1H, s, 9-H), 7.09, 8.17 (2H each, d, J=8.8 Hz, p-hydroxybenzoyl-H_4), 7.93 (2H, s, galloyl-H_2). Positive-ion SIMS: m/z 649 $(M+H)^+$, 631 $(M+H-H_2O)^+$, 613 $(M+H-2H_2O)^+$, 315, 153, 121.

Alkaline Treatment of 1 and 2 A solution of **1** and **2** (21 mg each) in 0.3% MeONa–MeOH (8 ml) was stirred for 1 h. The whole mixture was neutralized with Dowex 50 WX8 (H^+ form) and the resin was filtered. After removal of the solvent *in vacuo* from the filtrate, the residue was purified by ODS column chromatography (H_2O–MeOH) to give **3** (17 mg) and methyl gallate (2 mg), which were shown to be identical with authentic samples by TLC, IR, ^{1}H-NMR, and ^{13}C-NMR.

Alkaline Treatment of 4 and 5 A solution of **4** and **5** (30 mg each) in 0.3% MeONa–MeOH (10 ml) was stirred for 1 h, then neutralized with Dowex 50 WX8 (H^+ form) and filtered. Workup of the filtrate yielded a residue which was subjected to ODS column chromatography (H_2O–MeOH) to furnish **6** (19 mg) and methyl gallate (2 mg). These products were identified by comparison of their TLC behavior and physical date with authentic samples.

Acid Hydrolysis of 7 A solution of **7** (5 mg) in 5% aqueous H_2SO_4–1,4-dioxane (1 : 1, v/v, 1 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was removed by filtration. Removal of the solvent *in vacuo* gave a residue, which was transferred to a Sep-Pak C_{18} cartridge with H_2O and MeOH. The H_2O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (3 mg) in pyridine (0.5 ml) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivatives of D-glucose (i) and L-arabinose (ii): GLC conditions: Supelco TM-1, 0.25 mm (i.d.)×30 m capillary column, column temperature 230 °C, He flow rate 15 ml/min, t_R: i (24.2 min); ii (15.1 min).

Partial Alkaline Hydrolysis of 8 A solution of **8** (20 mg) in MeOH (5 ml) was treated with 1% aqueous KOH solution (1 ml) and the mixture was stirred for 10 h at room temperature. The reaction solution was neutralized with Dowex 50WX8 (H^+ form) and the resin was removed by filtration. Removal of the solvent from the filtrate under reduced pressure gave a residue, which was separated by silica gel column chromatography [1 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer)] to give **8** (5 mg) and **10** (6 mg), which were identified with authentic samples by TLC and ^{1}H-NMR comparisons.

DPPH Radical Scavenging Activity DPPH Radical scavenging activities of test samples were investigated according to the method reported previously.[1,12] 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 concentrations required for 50% reduction (50% scavenging concentration, SC_{50}) of 40 μM DPPH radical were determined graphically. Results are shown in Table 2.

References and Notes

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