Galloylglucosides from Berries of *Pimenta dioica*

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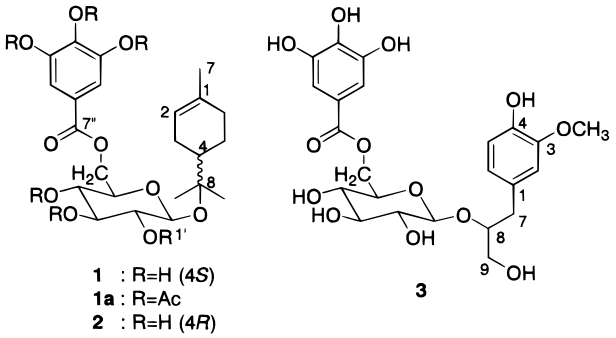
Three new galloylglucosides, (4*S*)-α-terpineol 8-*O*-*β*-D-(6-*O*-galloyl)glucopyranoside (**1**); (4*R*)-α-terpineol 8-*O*-*β*-D-(6-*O*-galloyl)glucopyranoside (**2**), and 3-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol 2-*O*-*β*-D-(6-*O*-galloyl)glucopyranoside (**3**), were isolated from the berries of *Pimenta dioica* together with three known compounds, gallic acid (**4**), pimentol (**5**), and eugenol 4-*O*-*β*-D-(6-*O*-galloyl)glucopyranoside (**6**). The structures of **1**−**3** were elucidated on the basis of MS and NMR spectral data and enzymatic hydrolysis. These galloylglucosides (**1**−**3**, **5**, and **6**) showed radical-scavenging activity nearly equivalent to that of gallic acid (**4**) against 1,1-diphenyl-2-picrylhydrazyl radical.

As a part of studies on the chemical components of spices and herbs,[1−3] we investigated allspice, that is, berries of *Pimenta dioica* (Myrtaceae). The plant contains various essential oils,[4,5] phenolic acids,[6] flavonoids,[7] and catechins.[8] In a previous study, we isolated several phenylpropanoids from allspice.[9] We now report the isolation and characterization of three new galloylglucosides (**1**−**3**) from allspice and a study of their free radical-scavenging effects on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.



Results and Discussion

The EtOAc-soluble fraction obtained from a 70% aqueous acetone extract of allspice was subjected to repeated column chromatography using Sephadex LH-20, Si gel, and Chromatorex ODS to give three new compounds (**1**−**3**) together with gallic acid (**4**), pimentol (**5**), and eugenol 4-*O*-*β*-D-(6-*O*-galloyl)glucopyranoside (**6**). Compound **4** was identified by comparing its TLC behavior and its spectral data with those of an authentic sample, and identification of **5** and **6** was carried out by comparing their physical and spectral data with those in the previous reports.[10−12]

Compound **1** exhibited an optical rotation of −7.0°and an $[M + H]^+$ peak at *m*/*z* 469.2053 in good agreement with the molecular formula $C_{23}H_{33}O_{10}$. The IR spectrum revealed hydroxyl (3600−3100 cm^{-1}) and conjugated ester (1697, 1234, and 1083 cm^{-1}) functions and an aromatic ring (1613 and 1536 cm^{-1}). The presence of a galloyl group was supported by a two-proton singlet at *δ* 7.14 in the ^{1}H NMR spectrum and five characteristic carbon signals (*δ* 166.7, 145.9, 138.6, 121.8, and 109.8) in the ^{13}C NMR spectrum (Tables 1 and 2). The remaining 16 signals indicated three methyl (*δ* 25.1, 23.5, and 22.9), three methylene (*δ* 31.5, 27.6, and 24.5), one methine (*δ* 44.5), one quaternary (*δ* 79.8), and two olefinic (*δ* 134.2 and 121.6) carbons, as well as six carbons corresponding to a glucose moiety (*δ* 98.2, 78.0, 75.0, 74.5, 71.5, and 64.9). Double resonance ^{1}H NMR and HMQC measurements allowed assignments of the glucose signals and indicated esterification at C-6′ based on the deshielded 6′-methylene protons [*δ* 4.52 (dd, *J* = 11.7, 2.2 Hz) and 4.30 (dd, *J* = 11.7, 6.6 Hz)]. The observation of an anomeric proton signal at *δ* 4.56 as a doublet with a coupling constant of 7.6 Hz indicated the *β*-configuration. Three methyl (*δ* 1.52, 1.20, and 1.15), one olefinic (*δ* 5.30), and one methine (*δ* 1.64) proton and three pairs of geminal proton signals corresponding to three methylenes were observed in the ^{1}H NMR spectrum, showing the presence of an α-terpinyl moiety. Furthermore, a fragment ion peak at *m*/*z* 315 in the SIMS indicated that the α-terpinyl moiety formed a glycosidic linkage with a glucose moiety. The 6′-methylene protons showed HMBC correlation with the carbonyl carbon (*δ* 166.7) of a galloyl group, and the anomeric proton (*δ* 4.56) showed correlation with a quaternary carbon (*δ* 79.8) attributed to C-8 of an α-terpinyl moiety (Figure 1). Acetylation of **1** gave a hexaacetate (**1a**). The ^{1}H NMR spectrum of **1a** showed one 6H singlet at *δ* 2.242 and one 3H singlet at *δ* 2.239, which were attributed to three phenolic acetate groups, consistent with a galloyl moiety. Furthermore, three 3H singlets (*δ* 1.959, 1.956, and 1.94) were observed and assigned to three aliphatic acetate groups. The deshielding of H-2′ (*δ* 4.93), H-3′ (*δ* 5.18), and H-4′ (*δ* 4.98) of the glucose moiety in **1a**, compared to **1**, confirmed that the C-6′ hydroxyl group was esterified with a galloyl group. Consequently, compound **1** was defined as α-terpineol 8-*O*-*β*-D-(6-*O*-galloyl)glucopyranoside.

Compound **2** exhibited an optical rotation of +31.0° and an $[M + H]^+$ peak at *m*/*z* 469.2077, indicating that **2** had the same molecular formula ($C_{23}H_{33}O_{10}$) as **1**. The ^{1}H and ^{13}C NMR, SIMS, and UV data of **2** were very close to those of **1**, except for the optical rotation. These data suggested that **2** was a diastereomer of **1**. Comparing the ^{1}H and ^{13}C NMR spectra of **1** and **2**, some differences were observed in the signals corresponding to the α-terpinyl moiety. The C-4 methine proton was observed at *δ* 1.64 in **1**, while the corresponding signal in **2** was observed at *δ* 1.60. The C-7 methyl proton appeared at *δ* 1.52 in **1**, while in **2** it appeared at *δ* 1.57. In the ^{13}C NMR of **1**, C-9 and C-10 were observed at *δ* 22.9 and 25.1, while they were observed at *δ* 23.9 and 24.1 in the case of **2**. These findings suggested that **1** and **2** were epimers at C-4.

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Table 1. ^{1}H NMR Data of Compounds **1**, **2**, **7**, and **8** in $(CD_3)_2CO$ at 500 MHz[a]

H	1	2	7	8
2	5.30 (m)	5.32 (m)	5.34 (m)	5.35 (m)
3eq	2.02 (m)	2.03 (m)	2.02 (m)	2.03 (m)
4	1.64 (dddd, 12.0, 12.0, 4.4, 2.2)	1.60 (dddd, 12.0, 12.0, 4.6, 2.0)	1.64 (dddd, 12.0, 12.0, 4.6, 2.0)	1.60 (dddd, 11.7, 11.7, 4.9, 2.2)
5ax	1.15 (dddd, 12.0, 12.0, 12.0, 5.4)	1.18 (dddd, 12.0, 12.0, 12.0, 3.9)	1.19 (dddd, 12.0, 12.0, 12.0, 5.1)	1.21 (dddd, 11.7, 11.7, 11.7, 3.9)
5eq	1.98 (m)	1.97 (m)	2.00 (m)	1.99 (m)
6ax	1.90 (m)	1.92 (m)	2.00 (m)	1.95 (m)
6eq	1.82 (br d, 16.6)	1.86 (br d, 16.4)	1.88 (br d, 16.6)	1.89 (br d, 15.9)
7	1.52 (br s)	1.57 (br s)	1.61 (br s)	1.61 (br s)
9	1.15 (s)[b]	1.16 (s)[b]	1.16 (s)[b]	1.18 (s)[b]
10	1.20 (s)[b]	1.19 (s)[b]	1.21 (s)[b]	1.20 (s)[b]
1′	4.56 (d, 7.6)	4.543 (d, 7.6)	4.50 (d, 7.6)	4.50 (d, 7.6)
2′	3.19 (dd, 8.8, 7.6)	3.18 (dd, 8.8, 7.6)	3.12 (dd, 9.0, 7.6)	3.12 (dd, 8.5, 7.6)
3′	3.45 (apparent triplet, 8.8)	3.45 (apparent triplet, 8.8)	3.39 (apparent triplet, 9.0)	3.39 (apparent triplet, 8.5)
4′	3.39 (apparent triplet, 8.8)	3.37 (apparent triplet, 8.8)	3.32 (apparent triplet, 9.0)	3.31 (apparent triplet, 8.5)
5′	3.60 (ddd, 8.8, 6.6, 2.2)	3.59 (ddd, 8.8, 7.1, 2.0)	3.28 (ddd, 9.0, 6.6, 2.9)	3.28 (ddd, 8.5, 6.1, 2.7)
6′a	4.52 (dd, 11.7, 2.2)	4.536 (dd, 11.7, 2.0)	3.79 (dd, 11.5, 2.9)	3.79 (dd, 11.2, 2.7)
6′b	4.30 (dd, 11.7, 6.6)	4.28 (dd, 11.7, 7.1)	3.62 (dd, 11.5, 6.6)	3.61 (dd, 11.2, 6.1)
2″, 6″	7.14 (s)	7.14 (s)		

[a] Chemical shifts are shown in δ values (ppm) relative to solvent peak. Multiplicity and coupling constant(s) in Hz are in parentheses. [b] Assignments are interchangeable in each column.

Table 2. ^{13}C NMR Data of Compounds **1**, **2**, **7**, and **8** in $(CD_3)_2CO$ at 125 MHz[a]

C	1	2	7	8
1	134.2	134.1	134.3	134.2
2	121.6	121.7	121.7	121.8
3	27.6	27.5	27.6	27.5
4	44.5	44.5	44.6	44.6
5	24.5	24.4	24.5	24.5
6	31.5	31.6	31.6	31.7
7	23.5	23.5	23.6	23.5
8	79.8	79.7	79.7	79.5
9	22.9[b]	23.9[b]	23.0[b]	23.9[b]
10	25.1[b]	24.1[b]	25.0[b]	24.1[b]
1′	98.2	98.1	98.1	98.1
2′	75.0	75.0	75.1	75.1
3′	78.0	78.1	78.2	78.2
4′	71.5	71.7	72.0	72.0
5′	74.5	74.5	76.9	76.9
6′	64.9	64.9	63.2	63.2
1″	138.6	138.6		
2″,6″	109.8	109.8		
3″,5″	145.9	145.9		
4″	121.8	121.9		
7″	166.7	166.6		

[a] Chemical shifts are shown in δ values (ppm) relative to solvent peak. [b] Assignments are interchangeable in each column.

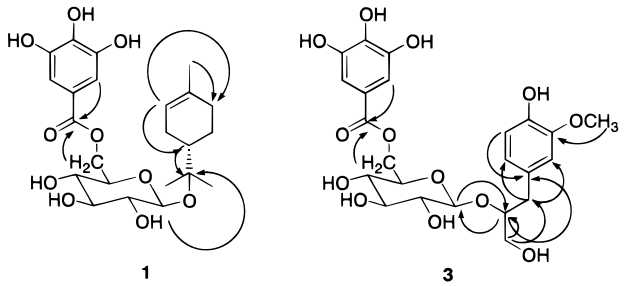
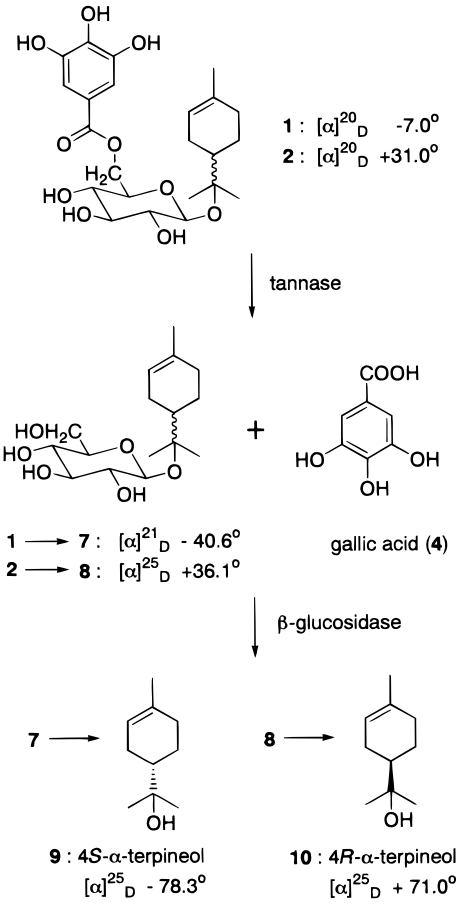


Figure 1. HMBC correlations for compounds **1** and **3**.

The absolute configuration of **1** and **2** at C-4 was determined by enzymatic hydrolysis. Treatment of **1** and **2** with tannase gave **7** ($[\alpha]^{21}_D$ −40.6°) and **8** ($[\alpha]^{25}_D$ +36.1°), respectively, along with gallic acid (**4**) (Scheme 1). Each compound showed an $[M + H]^+$ peak at *m/z* 317 in the SIMS spectrum, which was 170 mass units smaller than those of **1** and **2**. The two proton singlets observed in the ^{1}H NMR spectra of **1** and **2**, attributed to a galloyl group, were absent in the spectra of **7** and **8**. In addition, the 6′-methylene resonances were shielded in **7** and **8** compared to **1** and **2** (Table 1). α-Terpinyl glucoside is distributed widely in fruits and vegetables and is known as a precursor of flavor component.[13,14] However, to our knowledge, there has been no report dealing with the full structure elucidation of α-terpinyl glucoside.

Scheme 1. Enzymatic Hydrolysis of Compounds **1** and **2**



Compounds **7** and **8** were separately hydrolyzed with *β*-glucosidase to give **9** and **10**, respectively (Scheme 1). Both **9** and **10** showed the same ^{1}H NMR spectrum and were identified as α-terpineol by comparison with the ^{1}H NMR spectrum of an authentic sample. Compound **9** showed a negative optical rotation ($[\alpha]^{25}_D$ −78.3°), while **10** exhibited a positive optical rotation ($[\alpha]^{25}_D$ +71.0°). Thus, it was concluded that **9** was (4*S*)-α-terpineol ($[\alpha]_D$ −100.5°) [15] and **10** was (4*R*)-α-terpineol ($[\alpha]_D$ +101.8°).[15]

Table 3. Scavenging Effects of Compounds **1**−**6** on the DPPH Radical[a]

compound	radical-scavenging efficacies expressed as percentage at concentration[b]					
	80.0 μM	40.0 μM	20.0 μM	10.0 μM	5.0 μM	2.5 μM
1	95.4 ± 0.06^{c}	95.4 ± 0.05^{c}	88.9 ± 1.38^{efg}	48.4 ± 0.64^{f}	26.2 ± 0.72^{e}	14.8 ± 0.66^{de}
2	95.3 ± 0.02^{c}	95.3 ± 0.05^{c}	90.1 ± 0.46^{df}	46.2 ± 1.74^{d}	24.5 ± 0.32^{e}	12.9 ± 0.55^{e}
3	95.6 ± 0.02^{c}	95.3 ± 0.11^{c}	92.4 ± 0.37^{d}	56.9 ± 0.33^{de}	33.1 ± 0.40^{cd}	17.7 ± 0.80^{cd}
4	95.7 ± 0.20^{c}	94.8 ± 0.25^{c}	86.8 ± 0.69^{g}	61.6 ± 1.04^{c}	34.9 ± 0.15^{c}	19.0 ± 0.30^{c}
5	95.8 ± 0.26^{c}	95.7 ± 0.09^{c}	91.2 ± 0.91^{de}	53.6 ± 1.11^{e}	26.8 ± 0.93^{e}	12.2 ± 0.41^{e}
6	95.7 ± 0.05^{c}	95.2 ± 0.07^{c}	95.2 ± 0.21^{c}	60.9 ± 1.50^{cd}	31.7 ± 0.63^{d}	15.0 ± 1.49^{de}
α-tocopherol	95.8 ± 0.07^{c}	95.7 ± 0.18^{c}	48.8 ± 0.92^{h}	23.4 ± 1.69^{g}	14.0 ± 0.63^{f}	8.3 ± 0.66^{f}
BHT	68.0 ± 2.04^{d}	47.5 ± 3.60^{d}	28.6 ± 0.66^{i}	16.7 ± 1.14^{h}	11.8 ± 1.75^{f}	6.3 ± 1.92^{f}

[a] The concentration of DPPH ethanolic solution was 100 μM. [b] The results are expressed in % = [(*A* in the absence of compound − *A* in the presence of compound)/*A* in the absence of compound] × 100. Values with standard deviations are from three independent experiments. [c−i] Values in each column with the different superscripts are significantly different ($p < 0.05$).

Based on all the above data, compound **1** was identified as (4*S*)-α-terpineol 8-*O*-*β*-D-(6-*O*-galloyl)glucopyranoside and **2** as (4*R*)-α-terpineol 8-*O*-*β*-D-(6-*O*-galloyl)glucopyranoside.

Compound **3** showed an $[M + H]^+$ at *m*/*z* 513 and a stable fragment ion peak at *m*/*z* 315 in the positive SIMS. The spectra of **3** were very similar to those of **1**, except for the signals attributed to the monoterpene moiety, and indicated that **3** was also a related 6-*O*-galloylglucose compound. An aglycon moiety having six aromatic (δ 148.0, 145.7, 130.6, 122.6, 115.5, and 113.8), one methylene (δ 38.3), one methoxyl (δ 56.1), one oxygenated methylene (δ 64.8), and one oxygenated methine (δ 83.9) carbons was revealed by ^{13}C NMR and HMQC measurements. Three aromatic protons were observed in the ^{1}H NMR spectrum at δ 6.94, 6.71, and 6.69, corresponding to a 1,2,4-trisubstituted phenyl group. An NOE between the proton at δ 6.94 and the methoxy protons at δ 3.81 (3H, s) indicated this to be a 4-hydroxy-3-methoxyphenyl group. A 2H-broad doublet signal at δ 2.77 assignable to benzylic methylene protons was coupled with an oxymethine proton at δ 3.89 (tt), the latter was also coupled with a 2H-broad doublet signal at δ 3.52 corresponding to an oxymethylene group. These data suggested that the aglycon moiety was a 3-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol, which had previously been isolated from allspice.[9] The shielded anomeric proton (δ 4.39) compared with **5** (δ 4.74)[11] and **6** (δ 4.93)[12] suggested that a glucosyl unit was linked with an aliphatic hydroxyl, not with a phenolic hydroxyl group. The C-8 oxymethine carbon of **3** resonated at lower field (δ 83.9) than that of the previously reported aglycon (δ 74.2),[9] suggesting that the glucose moiety was attached to C-8. This was confirmed by HMBC, in which an anomeric proton showed correlation with C-8, and H-8 (δ 3.89) showed correlation with C-1′ (δ 103.9) (Figure 1). Thus, compound **3** was characterized as 3-(4-hydroxy-3-methoxyphenyl)-propane-1,2-diol 2-*O*-*β*-D-(6-*O*-galloyl)glucopyranoside. Determination of the absolute configuration at C-8 has not been achieved due to the small amount of **3** obtained. The aglycon of **3**, 3-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol, was a mixture of *R* and *S* isomers in a 1:4 ratio.[9] However, compound **3** might have either configuration at C-8 because its NMR spectrum showed no signals corresponding to C-8 diastereoisomers.

The radical-scavenging properties of compounds **1**−**6** were evaluated against the DPPH radical in a spectrophotometric assay.[16,17] Two antioxidants, α-tocopherol and butylated hydroxytoluene (BHT), were used as reference compounds. As shown in Table 3, compounds **1**−**6** showed higher activity than α-tocopherol and BHT. Recent investigations on the DPPH radical-scavenging effects of phenolic compounds indicated that the presence of an additional hydroxyl group in the ortho position plays an important role in the activity, and the *ortho*-diphenols additionally increase the capacity for quenching DPPH radicals.[18,19] Galloylglucosides (**1**−**3**, **5**, and **6**) exhibited almost the same activity as gallic acid (**4**), which had been reported to be one of the strong scavengers.[19]

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Union PM-101 automatic digital polarimeter. UV spectra were recorded on a Shimadzu UV-2500PC UV−vis spectrophotometer. IR spectra were run on a Perkin-Elmer 1800 instrument. The ^{1}H, ^{13}C, and 2D NMR spectra were recorded on a Varian Unity plus 500 (500 MHz) spectrometer. Positive SIMS (matrix: glycerol) and EIMS (70 eV) were measured on a Hitachi M-2000 mass spectrometer, and HRSIMS were obtained by a Hitachi M-4100 mass spectrometer [internal standard: poly(ethylene glycol)]. Si gel 60 (70−230 mesh, Merck), Sephadex LH-20 (Pharmacia), and Chromatorex ODS DM1020T (100−200 mesh, Fuji Silysia Chemical) were used for column chromatography, and Si gel 60 F_{254} plates (Merck) and ODS plates (Merck) were used for TLC. HPLC analysis was carried out with a pump and a system controller (Hitachi) connected to a UV detector (Hitachi) operating at 280 nm. The column (4.6 × 250 nm) was a 5-μm Develosil ODS HG-5 (Nomura Chemicals), a mixture of MeCN, H_2O, and HOAc (29:70:1, v/v/v) was used as a solvent with a flow rate of 0.75 mL/min. For measuring the DPPH radical scavenging activity, a UV-2500PC UV−vis spectrophotometer (Shimadzu) was employed. DPPH, α-tocopherol, and BHT were purchased from Wako Pure Chemical Industries, Ltd.

Plant Material. Berries of *P. dioica* from Jamaica were kindly supplied by Taiyo Koryo Co., Ltd., Osaka, Japan.

Extraction and Isolation. Dried and ground berries of *P. dioica* (500 g) were successively extracted with *n*-hexane (6 × 1.5 L), CH_2Cl_2 (6 × 1.5 L), and 70% aqueous Me_2CO (6 × 1.5 L) at room temperature. For each extraction, the plant material was soaked in the solvent and allowed to stand overnight. Acetone from the combined 70% aqueous Me_2CO extract was evaporated in vacuo, and the resulting aqueous residue was partitioned with EtOAc to give the EtOAc-soluble and H_2O-soluble fractions. The EtOAc-soluble fraction (6.3 g) was subjected to Sephadex LH-20 column chromatography using 2-propanol to give six fractions. Fraction 2 was rechromatographed over ODS gel (MeCN−H_2O, 3:7) to give nine fractions (A−I). Fraction B was recrystallized with H_2O to afford gallic acid (**4**, 337 mg). Fraction D was rechromatographed on Si gel (CH_2Cl_2−MeOH, 9:1), then over ODS (MeCN−H_2O−HOAc, 15:84:1) to give **3** (3.4 mg). Fraction H was rechromatographed over Si gel (CH_2Cl_2−MeOH, 9:1) to give a mixture of **1** and **2**, which was further subjected to repeated column chromatography over ODS gel eluted with MeCN−H_2O−HOAc, 15:84:1 to separate **1** (28.8 mg) and **2** (12.0 mg). Fraction 3 was subjected to ODS chromatography eluted with MeCN−H_2O (3:7) to give pimentol (**5**, 611 mg) and crude compound **6**, which was purified by Si gel column chromatography (CH_2Cl_2−MeOH, 9:1) to give **6** (42 mg).

(4*S*)-α-Terpineol 8-*O*-*β*-D-(6-*O*-galloyl)glucopyranoside (1): colorless amorphous powder; δ_H, see Table 1; δ_C, see Table 2; $[\alpha]^{20}_D$ −7.0° (*c* 1.00, EtOH); UV (EtOH) λ_{max} (log ϵ 277.0 (4.05) nm; IR (Nujol) ν_{max} 3600−3100, 1697, 1613, 1536, 1234, 1083 cm^{-1}; SIMS *m/z* 469 $[M + H]^+$, 315, 153, 137; HRSIMS *m/z* 469.2053 $[M + H]^+$ (calcd for $C_{23}H_{33}O_{10}$, 469.2071), *m/z* 491.1886 $[M + Na]^+$ (calcd for $C_{23}H_{32}O_{10}Na$, 491.1892).

Acetylation of 1. A solution of **1** (4.7 mg) in pyridine (1.0 mL) and Ac_2O (1.0 mL) was allowed to stand overnight at room temperature. The reaction mixture was poured into cold 2 N HCl, and then extracted with EtOAc. The organic layer was washed with water, dried over anhydrous $MgSO_4$, and evaporated to dryness to give **1a** (6.5 mg): ^1H NMR [$(CD_3)_2CO$, 500 MHz] δ 7.71 (2H, s, H-2″, 6″), 5.25 (1H, m, H-2), 5.18 (1H, apparent triplet, *J* = 9.5 Hz, H-3′), 4.98 (1H, apparent triplet, *J* = 9.5 Hz, H-4′), 4.93 (1H, dd, *J* = 9.5 Hz, 8.1 Hz, H-2′), 4.64 (1H, d, *J* = 8.1 Hz, H-1′), 4.37 (1H, dd, *J* = 12.0 Hz, 2.6 Hz, H-6′a), 4.25 (1H, dd, *J* = 12.0 Hz, 6.6 Hz, H-6′b), 3.73 (1H, ddd, *J* = 9.5 Hz, 6.6 Hz, 2.7 Hz, H-5′), 2.242 (6H, s, OAc), 2.239 (3H, s, OAc), 1.959 (3H, s, OAc), 1.956 (3H, s, OAc), 1.94 (3H, s, OAc), 1.91 (1H, m, H-3eq), 1.88 (1H, m, H-5eq), 1.85 (1H, m, H-6a), 1.79 (1H, m, H-6b), 1.64 (1H, m, H-3ax), 1.53 (3H, br s, H-7), 1.51 (1H, m, H-4), 1.11 (1H, dddd, *J* = 12.0 Hz, 12.0 Hz, 12.0 Hz, 5.5 Hz, H-5ax), 1.09, 1.04 (each 3H, s, H-9, 10); EIMS *m/z* 720 (0.5), 567 (8), 525 (4), 483 (1), 440 (2), 405 (3), 398 (2), 380 (2), 363 (1), 338 (4), 320 (3), 279 (26), 237 (40), 195 (21), 153 (20), 152 (26), 136 (100).

Enzymatic Hydrolysis of 1. A solution of **1** (10.7 mg) in H_2O (3 mL) was incubated with 5 mg of tannase (49 units/mg, *Aspergillus oryzae*, Wako) at 30 °C for 3 h. HPLC analysis showed that **1** (t_R 19.9 min) disappeared, and gallic acid (t_R 3.9 min) was yielded. The reaction mixture was extracted with EtOAc (3 × 3 mL), and the EtOAc extract (10.2 mg) was chromatographed on Si gel (CH_2Cl_2−MeOH, 9:1) to obtain **7** (5.5 mg); δ_H, see Table 1; δ_C, see Table 2; $[\alpha]^{21}_D$ −40.6° (*c* 0.55, MeOH); SIMS *m/z* 317 $[M + H]^+$. A solution of **7** (5.0 mg) in H_2O (2 mL) was incubated with *β*-glucosidase (10 mg) at 37 °C for 24 h. The reaction mixture was extracted with Et_2O (3 × 2 mL), and the organic layer was dried and evaporated in vacuo to give **9** (1.4 mg). TLC (SiO_2, *n*-hexane−acetone, 3:1, R_f 0.5) and ^1H NMR of **9** was compared with authentic α-terpineol; $[\alpha]^{25}_D$ −78.3° (*c* 0.14, $CHCl_3$) [lit.[15] −100.5°].

(4*R*)-α-Terpineol 8-*O*-*β*-D-(6-*O*-galloyl)glucopyranoside (2): colorless amorphous powder; δ_H, see Table 1; δ_C, see Table 2; $[\alpha]^{20}_D$ +31.0° (*c* 0.21, EtOH); UV (EtOH) λ_{max} (log ϵ) 276.0 (3.95) nm; SIMS *m/z* 469 $[M + H]^+$, 315, 153, 137; HRSIMS *m/z* 469.2077 $[M + H]^+$ (calcd for $C_{23}H_{33}O_{10}$, 469.2071), *m/z* 491.1897 $[M + Na]^+$ (calcd for $C_{23}H_{32}O_{10}Na$, 491.1892).

Enzymatic Hydrolysis of 2. Compound **2** (8.5 mg) was treated with tannase in the same manner as **1** (t_R 20.9 min) to give **8** (3.1 mg): δ_H, see Table 1; δ_C, see Table 2; $[\alpha]^{25}_D$ +36.1° (*c* 0.31, MeOH); SIMS *m/z* 317 $[M + H]^+$. Compound **8** (2.5 mg) was hydrolyzed in the same manner as **7** to give **10** (0.5 mg). TLC (SiO_2, *n*-hexane−acetone, 3:1, R_f, 0.5) and ^1H NMR of **10** was identical with authentic α-terpineol, $[\alpha]^{25}_D$ +71.0° (*c* 0.05, $CHCl_3$) [lit.[15] +101.8°].

3-(4-Hydroxy-3-methoxyphenyl)propane-1,2-diol 2-*O*-*β*-D-(6-*O*-galloyl)glucopyranoside (3): colorless viscous liquid; $[\alpha]^{20}_D$ −27.7° (*c* 0.15, MeOH); ^1H NMR [$(CD_3)_2CO$, 500 MHz] δ 7.16 (2H, s, H-2″, 6″), 6.94 (1H, br d, *J* = 1.5 Hz, H-2), 6.71 (1H, d, *J* = 7.3 Hz, H-5), 6.69 (1H, br dd, *J* = 7.3 Hz, 1.5 Hz, H-6), 4.65 (1H, dd, *J* = 12.0 Hz, 2.0 Hz, H-6′a), 4.39 (1H, d, *J* = 7.8 Hz, H-1′), 4.27 (1H, dd, *J* = 12.0 Hz, 6.3 Hz, H-6′b), 3.89 (1H, tt, *J* = 6.1 Hz, 5.6 Hz, H-8), 3.81 (3H, s, 3-OCH_3), 3.63 (1H, ddd, *J* = 9.0 Hz, 6.3 Hz, 2.0 Hz, H-5′), 3.52 (2H, br d, *J* = 5.6 Hz, H-9a,b), 3.43 (1H, apparent triplet, *J* = 9.0 Hz, H-4′), 3.40 (1H, apparent triplet, *J* = 9.0 Hz, H-3′), 3.24 (1H, dd, *J* = 9.0 Hz, 7.8 Hz, H-2′), 2.77 (2H, br d, *J* = 6.1 Hz, H-7a,b); ^{13}C NMR [$(CD_3)_2CO$, 125 MHz] δ 166.6 (C-7″), 148.0 (C-3), 145.9 (C-3″, 5″), 145.7 (C-4), 138.7 (C-1″), 130.6 (C-1), 122.6 (C-6), 121.6 (C-4″), 115.5 (C-5), 113.8 (C-2), 109.9 (C-2″,6″), 103.9 (C-1′), 83.9 (C-8), 77.4 (C-3′), 75.1 (C-5′), 74.7 (C-2′), 71.2 (C-4′), 64.8 (C-9), 64.4 (C-6′), 56.1 (OCH_3), 38.3 (C-7); SIMS *m/z* 605 $[M + G]^+$, 513 $[M + H]^+$, 315.

Determination of the Scavenging Effect on DPPH Radicals.[16,17] Test compounds were added to an EtOH solution of DPPH radical (final concentration was 100 μM), the reaction mixtures were shaken vigorously on a vortex stirrer and then incubated for 30 min in a H_2O bath at 25 °C in the dark. The absorbance of the remaining DPPH was determined colorimetrically at 517 nm. The scavenging activity of the tested compounds was measured as the decrease in absorbance of the DPPH expressed as a percentage of the absorbance of a control DPPH solution without test compounds. Six different concentrations of each of the isolated compounds were prepared for DPPH tests. All analyses were carried out in triplicate, and the values were averaged. A factorial analysis of variance (ANOVA) with multiple comparisons was carried out. Significance was established at $p < 0.05$.

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References and Notes

(1) Kikuzaki, H.; Kawabata, M.; Ishida, E.; Akazawa, Y.; Takei, Y.; Nakatani, N. *Biosci. Biotech. Biochem*. **1993**, *57*, 1329−1333.
(2) Kikuzaki, H.; Nakatani, N. *Phytochemistry* **1996**, *43*, 273−277.
(3) Wang, M.; Kikuzaki, H.; Lin, C.-C.; Kahyaoglu, A.; Huang, M.-T.; Nakatani, N.; Ho, C.-T. *J. Agric. Food Chem*. **1999**, *47*, 1911−1914.
(4) Pino, J.; Rosado, A.; Gonzalez, A. *Nahrung* **1989**, *33*, 717−720.
(5) Oberdieck, R. *Fleischwirtschaft*. **1989**, *69*, 320−330.
(6) Schulz, J. M.; Herrmann, K. *Z. Lebensm.-Unters. Forsch*. **1980**, *171*, 193−199.
(7) Vösgen, B.; Herrmann, K. *Z. Lebensm.-Unters. Forsch*. **1980**, *170*, 204−207.
(8) Schulz, J. M.; Herrmann, K. *Z. Lebensm.-Unters. Forsch*. **1980**, *171*, 278−280.
(9) Kikuzaki, H.; Hara, S.; Kawai, Y.; Nakatani, N. *Phytochemistry* **1999**, *52*, 1307−1312.
(10) Matsumoto, A.; Matsumoto, T.; Tokuda, H. *Japan Kokai Tokkyo Koho*, 04041499 (Feb. 12, 1992); *Chem. Abstr*. **1992**, *117*, 14407.
(11) Oya, T.; Osawa, T.; Kawakishi, S. *Biosci. Biotech. Biochem*. **1997**, *61*, 263−266.
(12) Tanaka, T.; Orii, Y.; Nonaka, G.; Nishioka, I. *Chem. Pharm. Bull*. **1993**, *41*, 1232−1237.
(13) Williams, P. J.; Strauss, C. R.; Wilson, B.; Massy-Westropp, R. A. *J. Agric. Food Chem*. **1982**, *30*, 1219−1223.
(14) Sakho, M. J.; Chassagne, D.; Crouzet, J. *J. Agric. Food Chem*. **1997**, *45*, 883−888.
(15) *Beilsteins Handbuch Der Organischen Chemie*; Springer-Verlag: Berlin, 1944; H6, pp 55−57 (E II 6, pp 66−67).
(16) Blois, M. S. *Nature* **1958**, *181*, 1199−1200.
(17) Brand-Willams, W.; Cuvelier, M. E.; Berset, C. *Food Sci. Technol*. (*London*) **1995**, *28*, 25−30.
(18) Chen, J. H.; Ho, C.-T. *J. Agric. Food Chem*. **1997**, *45*, 2374−2378.
(19) Chevalley, I.; Marston, A.; Hostettmann, K. *Phytochemistry* **1999**, *50*, 151−154.

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