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Antioxidative phenylpropanoids from berries of *Pimenta dioica*

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Abstract

A phenylpropanoid, *threo*-3-chloro-1-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol, was isolated from the berries of *Pimenta dioica* together with five known compounds, eugenol, 4-hydroxy-3-methoxycinnamaldehyde, 3,4-dimethoxycinnamaldehyde, vanillin and 3-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol. In addition, the stereochemistry of 3-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol was determined. The phenylpropanoids inhibited autoxidation of linoleic acid in a water-alcohol system.

Keywords: Pimenta dioica; Myrtaceae; Allspice; Phenylpropanoid; Antioxidant activity

1. Introduction

As a part of our studies on the antioxidative components of spices and herbs (Nakatani & Inatani, 1984; Kikuzaki & Nakatani, 1989, 1993), we investigated the antioxidants present in allspice, berries of *Pimenta dioica*. Allspice is known to have antioxidant activity and contains eugenol as its main active component (Chipault, Mizuno, Hawkins & Lundberg, 1952; Fujio, Hiyoshi, Asari & Suminoe, 1969). We now report on the isolation and structural elucidation of a new phenylpropanoid and the antioxidative effects of the isolated compounds.

2. Results and discussion

Antioxidant activities of the extracts with different polarities from allspice were determined by inhibition of autoxidation of linoleic acid in a water-alcohol assay system. As shown in Fig. 1, all extracts exhibited potent activities.

The *n*-hexane and dichloromethane extracts were subjected to a combination of column chromatography on silica gel and Sephadex LH-20 to give two compounds (**1**, **2**) together with eugenol (**3**), 4-hydroxy-3-methoxycinnamaldehyde (**4**), 3,4-dimethoxycinnamaldehyde (**5**) and vanillin (**6**). Compounds **3**–**6** were identified by comparison of their physical and spectral data with those of authentic samples.

Compound **1**, colorless needles, has ten carbons, including one methylene (δ 47.4), one methoxyl (δ 56.2), two oxygenated methines (δ 74.9 and 76.7) and six aromatic (δ 111.0, 115.3, 120.2, 134.1, 146.9 and 148.1) in accordance with a phenylpropanoid skeleton. In the ^{1}H-NMR spectrum, three aromatic protons were observed at δ 6.79 (1H, *d*, J = 8.0 Hz), 6.85 (1H, *dd*, J = 2.0, 8.0 Hz) and 7.04 (1H, *d*, J = 2.0 Hz), corresponding to a 1,3,4-trisubstituted phenyl group. An NOE between the signal at δ 7.04 and the methoxyl proton signal at δ 3.84 (3H, *s*) indicated the presence of a 4-hydroxy-3-methoxyphenyl group. The propane chain was defined in the ^{1}H-NMR spectrum by a double doublet signal at δ 4.64 (1H, J = 4.5, 5.0 Hz), a multiplet at δ 3.81 and two double doublets at δ 3.37 (J = 6.5, 11.5 Hz) and 3.62 (J = 4.5, 11.5 Hz). Double resonance ^{1}H-NMR and HMQC measurements allowed assignments of all protons and carbons. Acetylation of **1** showed the presence of a phenolic hydroxyl group and two aliphatic hydroxyl groups, based on the signals at δ 2.31 (3H, *s*), 2.08 (3H, *s*) and 2.11

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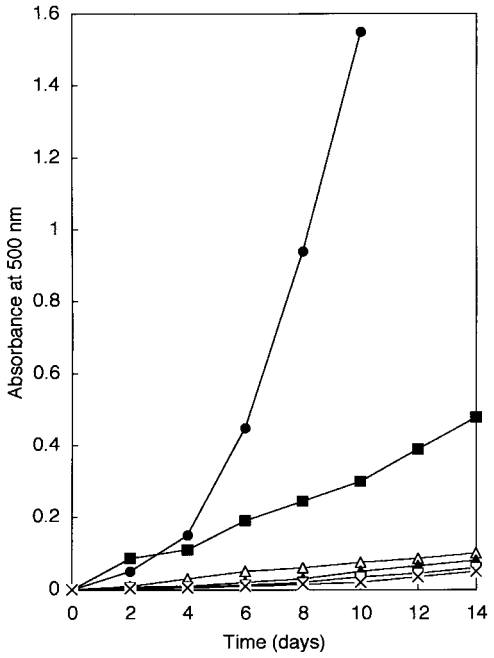


Fig. 1. Antioxidant activities of extracts with different polarities from allspice using the ferric thiocyanate method: Each sample, concentration 0.02%. ● - ● control (without additives); ■ - ■ α-tocopherol; × - × *n*-hexane extract; □ - □ CH_2Cl_2 extract; ▲ - ▲ EtOAc soluble part; △ - △ water soluble part.

(3H, *s*) in the ^{1}H-NMR spectrum of the product, **1a**. The lower field shifts of H-1 (δ 6.04) and H-2 (δ 5.38) indicated that **1** had two hydroxyl groups at C-1 and C-2. The large coupling constant between H-1 and H-2 (7.9 Hz) indicated a *threo* form (Ludwig, Nist & McCarthy, 1964). The EI mass spectrum of **1** exhibited a $[M]^+$ peak at *m*/*z* 232 together with a $[M+2]^+$ peak at *m*/*z* 234 in the ratio 1: 0.41. These data showed that **1** has a chlorine atom in the molecule, which was supported by the high resolution EI mass measurement ($[M]^+$ at *m*/*z* 232.0547 for $C_{10}H_{13}O_4Cl$). The chemical shifts of H-3 (δ 3.37 and 3.62) and C-3 (δ 47.4) revealed the chlorine to be on C-3. Consequently, **1** was determined to be *threo*-3-chloro-1-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol.

To determine whether **1** is a natural metabolite or an artifact derived during the extraction and separation process using dichloromethane, re-extraction of allspice and isolation of **1** were carried out in the absence of chlorinated solvents. The ethyl acetate fraction afforded colorless needles whose HPLC retention time and ^{1}H-NMR spectrum were identical with those of **1**. Thus, it was confirmed that **1** is a natural metabolite.

Compound **2** showed the exact mass ion peak at *m*/*z* 198.0914 in agreement with the molecular formula of $C_{10}H_{14}O_4$. The spectral data of **2** revealed that it was also a phenylpropanoid. The presence of a 4-hydroxy-3-methoxyphenyl group was supported by three aromatic proton signals (δ 6.67, 6.72 and 6.85) and a methoxyl proton signal at δ 3.83 in the ^{1}H-NMR spectrum and an NOE between the signals at δ 6.85 and 3.83. Two double doublets at δ 2.59 and 2.70 assignable to the benzylic methylene protons were coupled with an oxymethine proton signal at δ 3.80. The latter was also coupled with the remaining two signals (*ddd*) at δ 3.43 and 3.51 belonging to an oxymethylene group attached to C-2. Acetylation of **2** gave a triacetate **2a** ($[M]^+$, 324), ^{1}H-NMR spectrum suggested the presence of a phenolic acetate group and two aliphatic acetate groups, supported by three 3H singlets at δ 2.30, 2.04 and 2.08, respectively. The downfield shifts of the resonances of H-1 (δ 4.05 and 4.25) and H-2 (δ 5.27) confirmed the propane skeleton to be a 1,2-diol. Thus, **2** was determined to be 3-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol.This compound has previously been found in kraft black liquor of spruce (Lowendahl, Petersson & Samuelson, 1978) and identified as a metabolite of eugenol (Fischer, Von Unruh & Dengler, 1990). Recently, after our submission of this paper to this journal, Greca, Ferrara, Fiorentino, Monaco and Previtera (1998) reported that **2** was isolated from *Zantedeschia aethiopica* and had a negative optical rotation ($[\alpha]_D - 6°$), while our compound, **2**, showed $[\alpha]_D^{25} - 12°$ (EtOH; *c* 0.99).

The absolute configuration of **2** at C-2 was determined by first methylating the compound with methyl iodide to give its monomethyl ether, **2b**. The ^{1}H-NMR spectrum of **2b** showed an additional three proton singlet at δ 3.88 attributed to a phenolic methoxyl group. The EI mass spectrum revealed a $[M]^+$ peak at *m*/*z* 212, 14 mass units larger than that of **2** and a stable fragment ion peak at *m*/*z* 151 corresponding to $[CH_2C_6H_3(OMe)_2]^+$. Compound **2b** was then treated with (*S*)-*O*-methylmandelic acid and DCC in the presence of dimethylaminopyridine in CH_2Cl_2 to give **2c**. In the ^{1}H-NMR spectrum of **2c**, the benzyl methylene protons gave a doublet signal at δ 2.57, and two double doublet signals at δ 2.69 and 2.73 in approximately a 1:4 ratio. In addition, while the oxymethylene protons assigned to C-1 in **2** were observed as a pair of doublet of doublet of doublet signals at δ 3.43 and δ 3.51, these protons were observed in **2c** as two pairs of double doublet signals (δ 4.06, 4.35 and δ 3.92, 4.22) in a 1:4 ratio. This suggested that **2c** was a mixture of the *R* and *S* isomers at C-2. To confirm this structure, synthesis of (±)-**2b** was carried out using eugenol as a starting material. Eugenol was treated with *m*-chloroperbenzoic acid in CH_2Cl_2 to give an epoxide, **7**. In the ^{1}H-NMR spectrum of **7**, epoxy protons were observed at δ 2.54 (1H, *dd*, J = 2.4, 4.9 Hz, H-1a), 2.79 (1H, *dd*, J = 2.4, 4.9 Hz, H-1b) and 3.13 (1H, *dddd*, J = 2.4, 2.4, 5.5, 5.5 Hz, H-2). Hydrolysis of **7** with dilute H_2SO_4 gave a racemic mixture of diol **2**. The spectroscopic characteristics of this synthetic

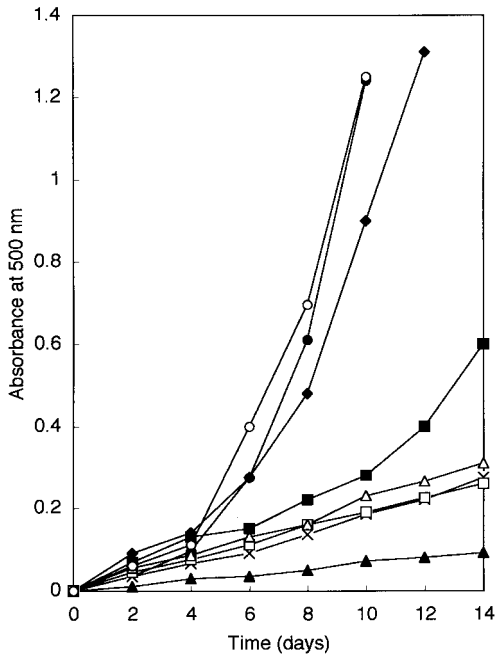
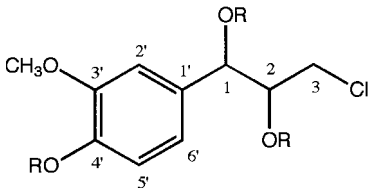


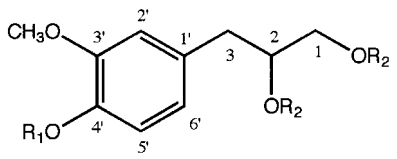
Fig. 2. Antioxidant activities of compounds **1**–**6** using the ferric thiocyanate method: Each sample, concentration 200 μM. ● - ● control (without additives); ■ - ■ α-tocopherol; × - × **1**; □ - □ **2**; ▲ - ▲ **3**; △ - △ **4**; ◆ - ◆ **5**; ○ - ○ **6**.

diol were consistent with those of the natural diol **2** except for the optical rotation. The synthetic diol was methylated with methyl iodide, followed by esterification with (*S*)-*O*-methylmandelic acid, to afford a mixture of (*S*)-*O*-methylmandelates (**8** and **9**), which were separated by silica gel column chromatography with isopropylether as eluent. ^{1}H-NMR measurements based on the method reported by Trost, Belletire, Godleski, McDougal and Balkovec (1986) allowed the determination of the absolute configurations of **8** and **9**. Comparison of the ^{1}H-NMR spectra of **8** and **9** with that of **2b** revealed that in **8**, the (*S*)-*O*-methylmandelic acid did not exert any shielding effect on the benzylmethylene proton signals, while in **9**, the benzylmethylene signal (δ 2.57) showed an upfield shift. Furthermore, the aromatic proton signals of **9** appeared at higher field than those of **8**. On the other hand, the oxymethylene protons of **8** resonated at higher field (δ 3.92 and 4.22) than those of **9** (δ 4.06 and 4.35). These shielding effects indicated that **8** had an *S* configuration while **9** had an *R* configuration. Each mandelate was hydrolyzed under alkaline conditions to give the 2*S* and 2*R* isomers of **2b**, which showed a negative ($[\alpha]_D^{26} - 23^\circ$ (EtOH; *c* 0.69)) and a positive optical rotation ($[\alpha]_D^{26} + 18^\circ$ (EtOH; *c* 0.73)), respectively. **2b** derived from the natural diol **2** showed a negative optical rotation ($[\alpha]_D^{25} - 14^\circ$ (EtOH; *c* 0.66)). These results confirmed that **2** was a mixture of 2*S*-3-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol and 2*R*-3-(4-hydroxy-3-methoxyphenyl)propane-1,2-diol, the *S* isomer being the major compound present.

Fig. 2 shows the antioxidant activities of **1**–**6**. Compounds **1**–**4** exhibited potent activities while **5** and **6** had no activity. Eugenol (**3**) was a major component of the *n*-hexane extract, thus the antioxidative effect of the *n*-hexane extract was probably due to eugenol (**3**). Concerning the dichloromethane extract, phenylpropanoids with a 4-hydroxy-3-methoxyphenyl group in the molecule (**1**–**4**) may contribute to the antioxidative effect of this extract.



1 R=H
1a R=Ac



2 R_1=R_2=H
2a R_1=R_2=Ac
2b R_1=CH_3, R_2=H
2c R_1=CH_3, R_2=(*S*)-*O*-methylmandelyl

3. Experimental

3.1. General

Melting points: uncorr. ^{1}H-NMR: 500 MHz, ^{13}C-NMR: 125 MHz, TMS as int. standard (Varian). EI and HR-EIMS: 70 or 20 eV, direct inlet (HITACHI).

3.2. Extraction and isolation

Berries of *Pimenta dioica* from Jamaica were kindly supplied by Taiyo Koryo, Osaka Japan. These were dried and ground (500 g) and then successively extracted with *n*-hexane (6 × 1.5 l), CH_2Cl_2 (6 × 1.5 l) and 70% aq. Me_2CO (6 × 1.5 l) at room temp. For each extraction, the plant material was soaked in the solvent and allowed to stand overnight. The combined *n*-hexane fractions and the combined CH_2Cl_2 fractions were evaporated separately in vacuo to give the *n*-hexane extract (37.4 g) and the CH_2Cl_2 extract (17.6 g). The Me_2CO from the combined 70% aq. Me_2CO fraction was also evaporated in vacuo, and the resulting

aq. residue was partitioned with EtOAc to give the EtOAc soluble and H_2O soluble parts. The *n*-hexane extract (5.0 g) was dissolved with *n*-hexane (200 ml) and the soln. was extracted two times with 0.5 N NaOH aq. (200 ml). Acidification of the alkaline soln. with 4 N HCl, followed by extraction with EtOAc, gave the acidic fraction (2.6 g), which was subjected to CC on silica gel eluted with CH_2Cl_2 to afford eugenol (**3**, 1.6 g). The CH_2Cl_2 extract (5.3 g) was chromatographed on silica gel and eluted with CH_2Cl_2 and MeOH mixture of increasing polarity to give 12 frs. Fr. 2 which was eluted with CH_2Cl_2, was identified as eugenol (1.6 g). Fr. 3, eluted with CH_2Cl_2, was recrystallized with water to afford vanillin (**6**, 230 mg). Fr. 4, eluted with CH_2Cl_2, was rechromatographed on silica gel (C_6H_6–Me_2CO) to give **4** (20 mg) and **5** (7 mg). Fr. 7, eluted with CH_2Cl_2–MeOH (99:1), was rechromatographed on Sephadex LH-20 (*iso*-PrOH), followed by repeated CC on silica gel (CH_2Cl_2–MeOH, C_6H_6–Me_2CO, isopropylether) and recrystallization with $CHCl_3$, to give **1** (29 mg). Fr. 8, eluted with CH_2Cl_2–MeOH (97:3), was rechromatographed on Sephadex LH-20 (*iso*-PrOH) and silica gel (CH_2Cl_2–MeOH) to give **2** (37 mg).

3.3. Compound *1*

Colorless needles, mp 121° ($CHCl_3$). $[\alpha]_D^{25}$ − 2° (EtOH; *c* 0.52). IR ν_{max}^{nujol} cm^{-1}: 3400–3200, 1609, 1522. ^{1}H-NMR ($(CD_3)_2CO$): δ 3.37 (1H, *dd*, *J* = 6.5, 11.5 Hz, H-3a), 3.62 (1H, *dd*, *J* = 4.5, 11.5 Hz, H-3b), 3.81 (1H, *m*, H-2), 3.84 (3H, *s*, OCH_3), 4.32 (1H, *d*, *J* = 5.0 Hz, 2-OH), 4.45 (1H, *d*, *J* = 4.5 Hz, 1-OH), 4.64 (1H, *dd*, *J* = 4.5, 5.0 Hz, H-1), 6.79 (1H, *d*, *J* = 8.0 Hz, H-5′), 6.85 (1H, *dd*, *J* = 2.0, 8.0 Hz, H-6′), 7.04 (1H, *d*, *J* = 2.0 Hz, H-2′), 7.55 (1H, *s*, 4′-OH). ^{13}C-NMR ($(CD_3)_2CO$): δ 47.4 (C-3), 56.2 (OCH_3), 74.9 (C-1), 76.7 (C-2), 111.0 (C-2′), 115.3 (C-5′), 120.2 (C-6′), 134.1 (C-1′), 146.9 (C-4′), 148.1 (C-3′). EIMS 20 eV *m*/*z* (rel. int.): 234 $[M+2]^+$ (0.9, $[M+2]/[M]$ = 0.41), 232 $[M]^+$ (2.2), 216 (1), 214 (4), 196(4), 178 (11), 151 (100), 137 (47). HR-EIMS 20 eV: found: *m*/*z* 232.0547 $[M]^+$, $C_{10}H_{13}O_4Cl$ requires: 232.0501.

3.4. Acetylation of *1*

A soln. of **1** (3.3 mg) in pyridine (0.5 ml) and Ac_2O (0.5 ml) was allowed to stand overnight at room temp. 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** (5.7 mg), IR ν_{max}^{film} cm^{-1}: 1735, 1609, 1510, 1219. ^{1}H-NMR ($CDCl_3$): δ 2.08 (3H, *s*, OAc), 2.11 (3H, *s*, OAc), 2.31 (3H, *s*, OAc), 3.33 (1H, *dd*, *J* = 4.9, 12.2 Hz, H-3a), 3.60 (1H, *dd*, *J* = 4.0, 12.2 Hz, H-3b), 3.85 (3H, *s*, OCH_3), 5.38 (1H, *ddd*, *J* = 4.0, 4.9, 7.9 Hz, H-2), 6.04 (1H, *d*, *J* = 7.9 Hz, H-1), 6.99 (1H, *dd*, *J* = 2.0, 8.5 Hz, H-6′), 7.00 (1H, *d*, *J* = 2.0 Hz, H-2′), 7.04 (1H, *d*, *J* = 8.5 Hz, H-5′). EIMS 20 eV *m*/*z* (rel. int.): 360 $[M+2]^+$ (3.1, $[M+2]/[M]$ = 0.39), 358 $[M]^+$ (7.9), 318 (23), 316 (68), 256 (99), 214 (100), 195 (99), 179 (99), 153 (89).

3.5. Compound *2*

Colorless oil, $[\alpha]_D^{25}$ − 12° (EtOH; *c* 0.99). IR ν_{max}^{film} cm^{-1}: 3600–3200, 1605, 1516, 1274. ^{1}H-NMR ($(CD_3)_2CO$): δ 2.59 (1H, *dd*, *J* = 4.4, 13.2 Hz, H-3a), 2.70 (1H, *dd*, *J* = 3.4, 13.2 Hz, H-3b), 3.43 (1H, *ddd*, *J* = 5.8, 5.8, 10.8 Hz, H-1a), 3.51 (1H, *ddd*, *J* = 4.4, 6.0, 10.8 Hz, H-1b), 3.80 (1H, *m*, H-2), 3.83 (3H, *s*, OCH_3), 6.67 (1H, *dd*, *J* = 2.0, 8.0 Hz, H-6′), 6.72 (1H, *d*, *J* = 8.0 Hz, H-5′), 6.85 (1H, *d*, *J* = 2.0 Hz, H-2′), 7.31 (1H, *s*, 4′-OH). ^{13}C-NMR ($(CD_3)_2CO$): δ 40.4 (C-3), 56.1 (OCH_3), 66.5 (C-1), 74.2 (C-2), 113.7 (C-2′), 115.5 (C-5′), 122.6 (C-6′), 131.4 (C-1′), 145.7 (C-4′), 148.0 (C-3′). EIMS 70 eV *m*/*z* (rel. int.): 198 $[M]^+$ (20), 180 (9), 167 (17), 137 (100). HR-EIMS 70 eV: found *m*/*z* 198.0914 $[M]^+$, $C_{10}H_{14}O_4$, requires 198.0892.

3.6. Acetylation of *2*

2 (5.0 mg) was treated in the same manner as described above to give **2a** (8.5 mg), IR ν_{max}^{film} cm^{-1}: 1735, 1606, 1510, 1231. ^{1}H-NMR ($CDCl_3$): δ 2.04 (3H, *s*, OAc), 2.08 (3H, *s*, OAc), 2.30 (3H, *s*, OAc), 2.86 (1H, *dd*, *J* = 6.7, 14.0 Hz, H-3a), 2.93 (1H, *dd*, *J* = 6.7, 14.0 Hz, H-3b), 3.82 (3H, *s*, OCH_3), 4.05 (1H, *dd*, *J* = 6.1, 11.6 Hz, H-1a), 4.25 (1H, *dd*, *J* = 3.4, 11.6 Hz, H-1b), 5.27 (1H, *dddd*, *J* = 3.4, 6.1, 6.7, 6.7 Hz, H-2), 6.78 (1H, *dd*, *J* = 1.8, 7.9 Hz, H-6′), 6.82 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.95 (1H, *d*, *J* = 7.9 Hz, H-5′). EIMS 70 eV *m*/*z*: (rel. int.): 324 (4), 282 (6), 264 (3), 222 (100), 180 (22), 179 (32), 137 (48).

3.7. Methylation of *2*

To a soln. of **2** (10.5 mg) in dried Me_2CO (5 ml) were added dry K_2CO_3 (0.50 g) and CH_3I (0.94 g). After stirring overnight at room temp., CH_2Cl_2 (20 ml) was added and the mixture was filtered. After removing the solvent from the filtrate, the residue was submitted to CC on silica gel (CH_2Cl_2:MeOH = 97:3) to give **2b** (8.4 mg), $[\alpha]_D^{25}$ − 14° (EtOH; *c* 0.66). ^{1}H-NMR ($CDCl_3$): δ 2.69 (1H, *dd*, *J* = 8.0, 12.0 Hz, H-3a), 2.76 (1H, *dd*, *J* = 5.0, 12.0 Hz, H-3b), 3.53 (1H, *dd*, *J* = 7.0, 11.0 Hz, H-1a), 3.71 (1H, *dd*, *J* = 3.0, 11.0 Hz, H-1b), 3.86 (3H, *s*, OCH_3), 3.88 (3H, *s*, OCH_3), 3.92 (1H, *m*, H-2), 6.75 (1H, *br s*, H-2′), 6.76 (1H, *dd*, *J* =

2.0, 8.0 Hz, H-6′), 6.82 (1H, *d*, *J* = 8.0 Hz, H-5′). EIMS 70 eV *m*/*z*: (rel. int.): 212 $[M]^+$ (9), 151 (100).

3.8. (*S*)-*O*-Methylmandelate (**2c**) of **2b**

Compound **2b** (11.8 mg), (*S*)-*O*-methylmandelic acid (20 mg) and DCC (26 mg) were dissolved in CH_2Cl_2 (3 ml). To the soln. was added DMAP (14.6 mg) at 0°C. After stirring overnight, the mixture was filtered and then concentrated to give **2c** (23 mg), ^{1}H-NMR ($CDCl_3$): δ 2.57 (*d*, *J* = 6.7 Hz), 2.69 (*dd*, *J* = 6.1, 13.5 Hz), 2.73 (*dd*, *J* = 6.7, 13.5 Hz), 3.73 (*s*), 3.77 (*s*), 3.81 (*s*), 3.82 (*s*), 3.92 (*dd*, *J* = 6.1, 11.6 Hz), 4.06 (*dd*, *J* = 5.5, 11.6 Hz), 4.22 (*dd*, *J* = 3.1, 11.6 Hz), 4.35 (*dd*, *J* = 3.1, 11.6 Hz), 5.24 (*m*), 5.29 (*dddd*, *J* = 3.1, 6.1, 6.1, 6.7 Hz), 6.35 (*dd*, *J* = 1.8, 7.9 Hz), 6.44 (*d*, *J* = 1.8 Hz), 6.48 (*dd*, *J* = 1.8, 8.5 Hz), 6.55 (*d*, *J* = 1.8 Hz), 6.57 (*d*, *J* = 7.9 Hz), 6.67 (*d*, *J* = 8.5 Hz).

3.9. Eugenol epoxide (7)

To a soln. of eugenol (**3**) (1.64 g) in CH_2Cl_2 (15 ml) was added dropwise *m*-chloroperbenzoic acid (2.58 g) in CH_2Cl_2 (30 ml) at 25°C. After stirring for 3 h, 10% Na_2SO_3 aq. (10 ml) was added to the mixture and the soln. was washed two times with 5% $NaHCO_3$ (25 ml). The CH_2Cl_2 layer was dried (Na_2SO_4) and concentrated. The residue was purified by silica gel CC with C_6H_6–Me_2CO (98:2) to give **7** (1.00 g), ^{1}H-NMR ($CDCl_3$): δ 2.54 (1H, *dd*, *J* = 2.4, 4.9 Hz, H-1a), 2.78 (1H, *dd*, *J* = 5.5, 14.7 Hz, H-3a), 2.79 (1H, *dd*, *J* = 2.4, 4.9 Hz, H-1b), 2.82 (1H, *dd*, *J* = 5.5, 14.7 Hz, H-3b), 3.13 (1H, *dddd*, *J* = 2.4, 2.4, 5.5, 5.5 Hz, H-2), 6.74 (1H, *dd*, *J* = 1.8, 7.9 Hz, H-6′), 6.76 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.86 (1H, *d*, *J* = 7.9 Hz, H-5′). ^{13}C-NMR ($CDCl_3$): δ 38.4 (C-3), 46.8 (C-1), 52.7 (C-2), 55.9 (OCH_3), 111.6 (C-2′), 114.4 (C-5′), 121.6 (C-6′), 129.0 (C-1′), 144.4 (C-4′), 146.5 (C-3′). EIMS 70 eV *m*/*z*: (rel. int.): 180 $[M]^+$ (56), 137 (100).

3.10. Racemate of 2

To a soln. of **7** (489 mg) in CH_2Cl_2 (10 ml) were added water (20 ml) and 5% H_2SO_4 (1.7 ml). After stirring overnight at 25°C, the organic solvent was evaporated in vacuo. The aq. residue was extracted with EtOAc and the EtOAc layer was dried (Na_2SO_4) and concentrated. The residue was purified by silica gel CC with CH_2Cl_2–MeOH (95:5) to give the racemate of **2** (228 mg). Its spectral data (IR, NMR and EIMS) were consistent in all respects with those of natural diol **2**.

3.11. Synthesis of 8 and 9

The racemate of **2** (91 mg) was methylated in the same manner as described above to give racemate of **2b** (98 mg), which was identified as natural **2b** by analytical TLC (CH_2Cl_2–MeOH = 95:5). The racemate of **2b** (52 mg) was esterified with (*S*)-*O*-methylmandelic acid (94 mg), DCC (89 mg) and DMAP (69 mg) in CH_2Cl_2. The reaction product was purified by silica gel CC with isopropyl ether to give two diastereomers, **8** (a higher R_f compound: 34 mg) and **9** (a lower R_f compound: 33 mg). **8**, $[\alpha]_D^{26} + 68°$ ($CHCl_3$; *c* 1.03). ^{1}H-NMR ($CDCl_3$): δ 2.69 (1H, *dd*, *J* = 6.1, 13.5 Hz, H-3a), 2.73 (1H, *dd*, *J* = 6.7, 13.5 Hz, H-3b), 3.77 (3H, *s*, OCH_3), 3.82 (3H, *s*, OCH_3), 3.92 (1H, *dd*, *J* = 6.1, 11.6 Hz, H-1a), 4.22 (1H, *dd*, *J* = 3.1, 11.6 Hz, H-1b), 5.29 (1H, *dddd*, *J* = 3.1, 6.1, 6.1, 6.7 Hz, H-2), 6.48 (1H, *dd*, *J* = 1.8, 8.5 Hz, H-6′), 6.55 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.67 (1H, *d*, *J* = 8.5 Hz, H-5′), for (*S*)-*O*-methylmandelyl part: 3.29 (3H, *s*), 3.35 (3H, *s*), 4.55 (1H, *s*), 4.68 (1H, *s*), 7.35 (10H, *m*). **9**, $[\alpha]_D^{26} + 65°$ ($CHCl_3$; *c* 1.30). ^{1}H-NMR ($CDCl_3$): δ 2.57 (2H, *d*, *J* = 6.7 Hz, H-3), 3.73 (3H, *s*, OCH_3), 3.81 (3H, *s*, OCH_3), 4.06 (1H, *dd*, *J* = 5.5, 11.6 Hz, H-1a), 4.35 (1H, *dd*, *J* = 3.1, 11.6 Hz, H-1b), 5.24 (1H, *m*, H-2), 6.35 (1H, *dd*, *J* = 1.8, 7.9 Hz, H-6′), 6.44 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.57 (1H, *d*, *J* = 7.9 Hz, H-5′), for the (*S*)-*O*-methylmandelyl part: 3.35 (3H, *s*), 3.40 (3H, *s*), 4.59 (1H, *s*), 4.73 (1H, *s*), 7.2–7.5 (10H, *m*).

3.12. (−)- and (+)-3-(3,4-Dimethoxyphenyl)propane-1,2-diol (2b)

To a soln. of **8** (22 mg) in MeOH (1 ml) was added 1 N NaOH in MeOH (0.4 ml). After stirring for 8 h at room temp., the reaction mixture was acidified with 1 N HCl and extracted two times with EtOAc. The organic layer was dried (Na_2SO_4) and evaporated. The residue was purified by silica gel CC with CH_2Cl_2–MeOH (95:5) to give the (−)-isomer of **2b** (7.0 mg), $[\alpha]_D^{26} - 23°$ (EtOH; *c* 0.69). By the same manner, the (+)-isomer of **2b** (7.3 mg) was obtained from **9** (22 mg), $[\alpha]_D^{26} + 18°$ (EtOH; *c* 0.73).

3.13. Re-extraction and fractionation

Dried and ground allspice (1 kg) was successively extracted with *n*-hexane (5 × 2 l) and 70% aq. Me_2CO (5 × 2 l) at room temp. For each extraction, the plant material was soaked in the solvent and allowed to stand overnight. The Me_2CO from the combined 70% aq. Me_2CO fraction was evaporated in vacuo, and the resulting aq. residue was extracted succesively with *n*-hexane (3 × 1 l) and EtOAc (3 × 1 l) to give the *n*-hexane soluble (8.8 g), the EtOAc soluble (29.9 g) and water soluble parts. The EtOAc soluble part (13.5 g) was subjected to CC on Sephadex LH-20 and eluted with *iso*-PrOH to give 7 frs.

3.14. HPLC analysis

HPLC analysis was carried out with a pump with a system controller (Jasco PU-980, Tokyo, Japan) connected to a photodiode array detector (Jasco MD-910, Tokyo, Japan) operating in the wavelength range 195–350 nm. The column (4.6 × 250 nm) was a 5 μm Develosil ODS HG-5 (Nomura Chem., Japan), the mixture of MeCN and H_2O (1:4, v/v) was used as a solvent with a flow rate of 0.5 ml/min. Compound **1** showed the R_t at 12.5 min. The third fr. of the EtOAc soluble part showed six major peaks (R_t: 4.8, 6.7, 8.5, 10.9, 12.5, 24.6 min).

3.15. Isolation of compound *1*

The third fr. was rechromatographed on ODS eluted with MeCN-H_2O (1:4), followed by CC on silica gel (C_6H_6–Me_2CO) to give colorless needles (24 mg). Its ^{1}H-NMR spectrum was compared with that of compound **1**.

3.16. Antioxidant assay (Kikuzaki & Nakatani, 1993)

A mixture of 4 ml of a weighed sample in 99.5% EtOH, 4.1 ml of 2.51% linoleic acid in 99.5% EtOH, 8 ml of 0.05 M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in a vial ($\phi = 38$, $h = 75$ mm) with a screw cap and then placed in an oven at 40°C in the dark. To 0.1 ml of this soln. was added 9.7 ml of 75% EtOH and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after addition of 0.1 ml of 2×10^{-2} M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance of red color was measured at 500 nm every 24 h. All tests were run in duplicate.

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