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Abscisic Acid Related Compounds and Lignans in Prunes (*Prunus domestica* L.) and Their Oxygen Radical Absorbance Capacity (ORAC)

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Four new abscisic acid related compounds (**1**−**4**), together with (+)-abscisic acid (**5**), (+)-β-D-glucopyranosyl abscisate (**6**), (6*S*,9*R*)-roseoside (**7**), and two lignan glucosides ((+)-pinoresinol mono-β-D-glucopyranoside (**8**) and 3-(β-D-glucopyranosyloxymethyl)-2- (4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-(2*R*,3*S*)-dihydrobenzofuran (**9**)) were isolated from the antioxidative ethanol extract of prunes (*Prunus domestica* L.). The structures of **1**−**4** were elucidated on the basis of NMR and MS spectrometric data to be *rel*-5-(3*S*,8*S*-dihydroxy-1*R*,5*S*-dimethyl-7-oxa-6-oxobicyclo[3,2,1]-oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic acid (**1**), *rel*-5-(3*S*,8*S*-dihydroxy-1*R*,5*S*-dimethyl-7-oxa-6-oxobicyclo[3,2,1]oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic acid 3′-*O*-β-D-glucopyranoside (**2**), *rel*-5-(1*R*,5*S*-dimethyl-3*R*,4*R*,8*S*-trihydroxy-7-oxa-6-oxobicyclo[3,2,1]oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic acid (**3**), and *rel*-5-(1*R*,5*S*-dimethyl-3*R*,4*R*,8*S*-trihydroxy-7-oxabicyclo[3,2,1]- oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic acid (**4**). The antioxidant activities of these isolated compounds were evaluated on the basis of oxygen radical absorbance capacity (ORAC). The ORAC values of abscisic acid related compounds (**1**−**7)** were very low. Two lignans (**8** and **9)** were more effective antioxidants whose ORAC values were 1.09 and 2.33 μmol of Trolox equiv/μmol, respectively.

KEYWORDS: Prunes (Prunus domestica L.); abscisic acid related compounds; lignan glucoside; oxygen radical absorbance capacity (ORAC)

INTRODUCTION

It is generally accepted that fruits and vegetables are the best available natural sources of antioxidants such as flavonoids and phenolic acid related compounds because of their high consumption. Such dietary antioxidants play an important role for defense systems from oxidative stress (*1*). Some vegetables and fruits have been investigated for their antioxidant activity based on the oxygen radical absorbance capacity (ORAC), and prunes were found to show considerably higher antioxidant activity than other fruits and vegetables (*2*). Prunes are the dried fruits of some cultivars of *Prunus domestica* L. (Rosaceae) originating from the Caucasus region in western Asia. Thus, prunes are recognized as a healthy food (*3*).

The major antioxidant components in prunes are known to be caffeoylquinic acids such as 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, and 5-*O*-caffeoylquinic acid (*4, 5*). It is well-known that these acids are antioxidants for human LDL, scavengers for reactive oxygen and nitrogen species, and inhibitors against the formation of conjugated diene from linoleic acid oxidation (*5*−*9*). In our previous study, the degree of contribution of these caffeoylquinic acids to the antioxidant activity of prunes was estimated to be 28.4% on the basis of their total contents and ORAC values in the ethanol extract of prunes (*10*), which suggested that other antioxidants might exist in prunes. This prompted us to undertake a study on elucidation of undiscovered antioxidants in prunes. *o*-Diphenol compounds such as caffeic acid, protocatechuic acid, rutin and (−)-epicatechin, which are well-known antioxidants, were previously identified in prunes, and 4-amino-4-carboxychroman-2-one was isolated as an antioxidative synergist to caffeoylquinic acid isomers (*10, 11*). This investigation deals with the structural elucidation of some additional prune components and their ORAC.

MATERIALS AND METHODS

General Procedures. NMR spectra were obtained on a Varian Unity Plus 500 spectrometer (Varian Inc., Palo Alto, CA) at 500 MHz (^{1}H)

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Table 1. $^{1}H^{a}$- and $^{13}C^{b}$-NMR Data of Compounds **1**–**4**

	1		2		3		4	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1		171.0		169.8		169.5		169.6
2	5.83 (*brs*)	122.2	5.81 (*brs*)	120.8	5.81 (*brs*)	120.7	5.76 (*brs*)	119.4
3		148.5		150.2		150.5		151.3
4	7.97 (*dd*, 0.7, 16.1)	133.5	8.01 (*dd*, 0.5, 15.9)	133.2	8.02 (*brd*, 16.1)	133.4	7.99 (*dd*, 0.7, 15.9)	132.1
5	6.38 (*dd*, 0.5, 16.1)	131.3	6.43 (*brd*, 15.9)	131.9	6.27 (*brd*, 16.1)	132.0	6.31 (*brd*, 15.9)	134.8
3-CH_3	2.06 (3H, *d*, 1.2)	20.9	2.08 (3H, *d*, 1.5)	21.1	2.06 (3H, *d*, 1.2)	21.0	2.08 (3H, *d*, 1.5)	21.2
1′		89.8		89.7		89.1		86.9
2′ax	1.84 (*dd*, 10.1, 14.3)	42.3	1.94 (*dd*, 10.0, 14.2)	39.4	1.83 (*dd*, 9.8, 14.5)	40.5	1.68 (*dd*, 10.5, 14.2)	43.8
eq	2.23 (*ddd*, 1.7, 7.1, 14.3)		2.44 (*ddd*, 1.2, 7.2, 14.2)		2.31 (*dd*, 7.3, 14.5)		2.03 (*dd*, 7.8, 14.2)	
3′ax	3.83 (*dddd*, 7.1, 7.1, 10.1, 11.1)	65.2	3.99 (*dddd*, 7.2, 7.8, 10.0, 11.2)	73.0	3.58 (*ddd*, 7.3, 9.8, 9.8)	72.3	3.76 (*ddd*, 7.8, 8.5, 10.5)	72.5
4′ax	1.72 (*dd*, 11.1, 13.6)	41.0	1.88 (*dd*, 11.2, 13.9)	39.5	3.53 (*d*, 9.8)	76.6	3.48 (*dd*, 1.2, 8.5)	78.1
eq	1.89 (*ddd*, 1.7, 7.1, 13.6)		2.06 (*ddd*, 1.2, 7.8, 13.9)					
5′		53.5		53.5		59.1		55.0
6′		181.1		180.9		178.9	3.57 (*dd*, 1.5, 7.6)	72.3
8′		82.8		82.7		82.6	3.91 (*d*, 7.6)	82.9
1′-CH_3	1.34 (3H, *s*)	18.5	1.36 (3H, *s*)	18.5	1.33 (3H, *s*)	18.5	1.14 (3H, *s*)	19.4
5′-CH_3	1.07 (3H, *s*)	14.5	1.08 (3H, *s*)	14.5	1.18 (3H, *s*)	10.3	0.99 (3H, *s*)	12.2
Glc-1			4.33 (*d*, 7.8)	103.2				
2			3.12 (*dd*, 7.8, 9.0)	75.0				
3			3.31 (*dd*, 9.0, 9.5)	78.0				
4			3.27 (*dd*, 9.5, 9.5)	71.5				
5			3.26 (*m*)	78.0				
6a			3.65 (*dd*, 5.4, 12.0)	62.5				
6b			3.83 (*dd*, 2.2, 12.0)					

[a] ^{1}H NMR data were obtained at 500 MHz with methanol *d*-4 (25 °C). Chemical shifts are shown in δ values relative to solvent peak. Multiplicity and coupling constant(s) in Hz are in parentheses. [b] ^{1}C NMR data were obtained at 125 MHz with methanol *d*-4 (25 °C). Chemical shifts are shown in δ values relative to solvent peak.

and 125 MHz (^{13}C) in CD_3OD and referenced to the residual solvent resonance (CD_3OD at 3.30 ppm for ^{1}H and 49.0 ppm for ^{13}C NMR). Optical rotations were measured using a P-1030 automatic digital polarimeter (Jasco Co., Tokyo, Japan). UV spectra were recorded on a UV-2500PC UV−vis spectrophotometer (Shimadzu Co., Kyoto, Japan). IR spectra were run on a model 1800 instrument (Perkin-Elmer Inc., Wellesley, MA). A fast atom bombardment (FAB) MS and HR-FABMS were performed on a JMS 700T mass spectrometer (JEOL Ltd., Tokyo, Japan). Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), Chromatorex ODS DM1020T, 100−200 mesh, (Fuji Silysia Chemical, Tokyo, Japan), and silica gel 60, 70−230 mesh, (Merck, Darmstadt, Germany) were used for column chromatography. Thin-layer chromatography (TLC) was carried out on silica gel F-254 (Merck) and RP-18F254s (Merck) plates, and spots were detected by ultraviolet (UV) illumination.

Plant Material. Prunes, the d′Agen cultivar, were supplied by Miki Foods Co., Ltd. (Hyogo, Japan), and were imported from the United States as the material for a commercial prune extract (concentrated prune juice). It is called "natural condition prune (NC prune)", with moisture levels adjusted to 21% (*12*).

Chemicals. β-Phycoerythrin from *Porphyridium cruentum* was obtained from Molecular Probes, Inc. (Eugene, OR), and 2,2′-azobis-(2-amidinopropane)dihydrochloride (AAPH) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Aldrich Chemical Co. (Milwaukee, WI). All solvents and chemicals were of the highest analytical grade.

Extraction and Isolation. Pitted prune fruits (7.08 kg) were swelled with 14 L of 90% aqueous ethanol (EtOH) followed by homogenizing. After filtration, another 6-L portion of 95% aqueous EtOH was added to the residue. This procedure was repeated five times. The combined extracts were evaporated in vacuo to remove EtOH, and the residual extract was partitioned between hexane (5 L) and water five times. The hexane-soluble fractions were combined and evaporated in vacuo to give concentrate (74.2 g). The water-soluble fraction was separated by Diaion HP-20, using H_2O as the eluent followed by MeOH and acetone, to give the dried H_2O eluate (2806 g), the MeOH eluate (44.3 g), and the acetone eluate (0.9 g). The MeOH eluate (44.0 g) was rechromatographed on Sephadex LH-20 gel using 80% aqueous acetone as a mobile phase to give four fractions (fractions 1−4) by monitoring with silica gel and ODS TLC analysis. Fraction 2 (6.77 g) was further subjected to ODS column chromatography eluted with H_2O/CH_3CN (95:5, v/v) to afford fifteen fractions (fractions 2-1 to 2-15). Fraction 2-4 (0.50 g) was rechromatographed over ODS gel eluted with H_2O/CH_3CN (90:10) to give five fractions (fractions 2-4-1 to 2-4-5). Each fraction of 2-4-2 and 2-4-3 was purified on ODS column chromatography [H_2O/CH_3CN (90:10)] to give compound **2** (1.7 mg) and **4** (2.8 mg), respectively. Fraction 2-4-4 was applied to a Sephadex LH-20 column eluted with 80% aqueous acetone to give compound **3** (5.7 mg). Fraction 2-8 (0.60 g) was rechromatographed over successive column of ODS [H_2O/CH_3CN (80:20)] and silica gel [EtOAc/MeOH/H_2O (70:20:10)] to afford compound **1** (3.8 mg) and compound **7** (7.0 mg). Rechromatography of fraction 2-11 (1.30 g) over Sephadex LH-20 eluted with MeOH gave compound **6** (34.1 mg). Fraction 2-12 (0.25 g) was subjected to a Sephadex LH-20 column eluted with MeOH to give five fractions (fractions 2-12-1 to 2-12-5). Fraction 2-12-3 was further purified on silica gel column chromatography [CH_2Cl_2/MeOH (80:20)] to afford compound **8** (14.7 mg). Fraction 2-12-4 was purified by successive column chromatography using ODS gel [H_2O/CH_3CN (80:20)] and silica gel [CH_2Cl_2/MeOH (80:20)] to give compound **9** (4.6 mg). Fraction 2−14 (0.21 g) was purified on a silica gel column eluted with CH_2Cl_2/MeOH (90:10), and then Sephadex LH-20 column (*i*-PrOH) to afford compound **5** (2.4 mg).

***rel*-5-(3*S*,8*S*-Dihydroxy-1*R*,5*S*-dimethyl-7-oxa-6-oxobicyclo[3,2,1]-oct-8-yl)-3-methyl- 2*Z*,4*E*-pentadienoic Acid (1).** Colorless solid. ^{1}H and ^{13}C NMR: see **Table 1**. $[\alpha]^{25}_{D}$ −56.7° (*c* 0.26, MeOH). UV (MeOH) λ_{max} (log ϵ): 258.4 (4.27) nm. IR (Nujol) ν_{max}: 3600−2500, 1757, 1685, 1654, 1602, 1169 cm^{-1}. FABMS: *m*/*z* 387 [M + G − H]$^-$, 295 [M − H]$^-$. HRMS-FAB (*m*/*z*): [M − H]$^-$calcd for $C_{15}H_{19}O_6$, 295.1182; found, 295.1196.

***rel*-5-(3*S*,8*S*-Dihydroxy-1*R*,5*S*-dimethyl-7-oxa-6-oxobicyclo[3,2,1]-oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic Acid 3′-*O*-β-D-glucopyranoside (2).** Colorless viscous liquid. ^{1}H and ^{13}C NMR: see **Table 1**. $[\alpha]^{22}_{D}$ −33.2° (*c* 0.09, MeOH). UV (MeOH) λ_{max} (log ϵ): 258.6 (4.12) nm. FABMS: *m*/*z* 457 [M − H]$^-$, 277 [M − H − Glc]$^-$. HRMS-FAB (*m*/*z*): [M − H]$^-$ calcd for $C_{21}H_{29}O_{11}$, 457.1716; found, 457.1710.

***rel*-5-(1*R*,5*S*-Dimethyl-3*R*,4*R*,8*S*-trihydroxy-7-oxa-6-oxobicyclo-[3,2,1]oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic Acid (3).** Colorless viscous liquid. ^{1}H and ^{13}C NMR: see **Table 1**. $[\alpha]^{23}_D$ −66.0° (*c* 0.56, MeOH). UV (MeOH) λ_{max} (log ϵ): 259.0 (4.07) nm. IR (Nujol) ν_{max}: 3600−2500, 1757, 1685, 1654, 1603, 1169 cm^{-1}. FABMS: *m*/*z* 403 [M + G − H]$^-$, 311 [M − H]$^-$. HRMS-FAB (*m*/*z*): [M − H]$^-$ calcd for $C_{15}H_{19}O_7$, 311.1125; found, 311.1128.

***rel*-5-(1*R*,5*S*-Dimethyl-3*R*,4*R*,8*S*-trihydroxy-7-oxabicyclo[3,2,1]-oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic Acid (4).** Colorless viscous liquid. ^{1}H and ^{13}C NMR: see **Table 1**. $[\alpha]^{23}_D$ −15.0° (*c* 0.21, MeOH). UV (MeOH) λ_{max} (log ϵ): 260.2 (4.21) nm. IR (Nujol) ν_{max}: 3600−2500, 1685, 1639, 1602 cm^{-1}. FABMS: *m*/*z* 389 [M + G − H]$^-$, 297 [M − H]$^-$. HRMS-FAB (*m*/*z*): [M − H]$^-$ calcd for $C_{15}H_{21}O_6$, 297.1338; found, 297.1340.

(+)-Abscisic Acid (5) (*13*). Colorless solid. $[\alpha]^{23}_D$ +278.3° (*c* 0.21, MeOH). FABMS: *m*/*z* 263 [M − H]$^-$.

(+)-β-D-Glucopyranosyl Abscisate (6) (*13*, *14*). Colorless viscous liquid, $[\alpha]^{23}_D$ +153.7° (*c* 0.44, MeOH). FABMS: *m*/*z* 425 [M − H]$^-$.

(6*S*,9*R*)-Roseoside (7) (*15*). White powder. $[\alpha]^{26}_D$ +64.1° (*c* 0.46, MeOH). FABMS: *m*/*z* 385 [M − H]$^-$.

(+)-Pinoresinol Mono-β-D-glucopyranoside (8) (*16*). White powder. $[\alpha]^{20}_D$ +6.5° (*c* 0.20, MeOH). FABMS: *m*/*z* 519 [M − H]$^-$.

3-(β-D-Glucopyranosyloxymethyl)-2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-(2*R*,3*S*)-dihydrobenzofuran (9). White powder. ^{1}H NMR (CD_3OD): δ_H 1.80 (2H, m, H-2′), 2.61 (2H, t, *J* = 7.5 Hz, H-1′), 3.22 (1H, dd, *J* = 8.0, 9.0 Hz, Glc-2), 3.26−3.36 (3H, m, Glc-3,4,5), 3.55 (2H, t, *J* = 6.5 Hz, H-3′), 3.62 (1H, m, H-3), 3.65 (1H, dd, *J* = 5.0, 12.0 Hz, Glc-6a), 3.74 (1H, m, 3-CH_2), 3.81 (3H, s, 3"-OCH_3), 3.84 (3H, s, 7-OCH_3), 3.84 (1H, dd, *J* = 2.0, 12.0 Hz, Glc-6b), 4.20 (1H, dd, *J* = 6.0, 9.5 Hz, 3-CH_2), 4.34 (1H, d, *J* = 8.0 Hz, Glc-1), 5.60 (1H, d, *J* = 6.5 Hz, H-2), 6.72 (1H, br d, *J* = 1.0 Hz, H-6), 6.74 (1H, d, *J* = 8.0 Hz, H-5"), 6.78 (1H, br t, *J* = 1.0 Hz, H-4), 6.86 (1H, dd, *J* = 2.0, 8.0 Hz, H-6"), 6.98 (1H, d, *J* = 2.0 Hz, H-2"). ^{13}C NMR (CD_3OD): δ_C 32.9 (C-1′), 35.8 (C-2′), 53.3 (C-3), 56.4 (3"-CH_3), 56.7 (7-CH_3), 62.2 (C-3′), 63.1 (Glc-6), 71.6 (Glc-4), 72.1 (3-CH_2), 75.2 (Glc-2), 78.2 (Glc-3,5), 89.0 (C-2), 104.6 (Glc-1), 110.7 (C-2"), 114.2 (C-6), 116.1 (C-5"), 118.2 (C-4), 119.7 (C-6"), 129.6 (C-3a), 134.8 (C-1"), 137.0 (C-5), 145.2 (C-7), 147.0 (C-1a, 4"), 149.0 (C-3"). $[\alpha]^{20}_D$ +38.7° (*c* 0.08, MeOH) (*17*); FABMS: *m*/*z* 521 [M − H]$^-$.

Measurement of ORAC. The ORAC values of isolated compounds were measured according to a method described in an earlier paper (*18*). This assay is based on the principle that antioxidant compounds delay the decrease of β-phycoerythrin fluorescence induced by AAPH, a peroxyl radical generator. In this study, an Arvo 1420sx microplate reader (Wallac Berthold Japan Co., Tokyo, Japan) was used for the ORAC assay at an excitation wavelength of 530 nm and an emission wavelength of 570 nm.

In previous papers concerning ORAC assay (*19*, *20*), Ou and coworkers showed that β-phycoerythrin is photobleached by repeated irradiation of excited light on their microplate reader. They also indicated that β-phycoerythrin bound with grape seed extract and the fluorescence of it apparently decreased in a few minutes. In this study, we confirmed that the fluorescence of β-phycoerythrin without AAPH remained >95% after 35 times of repeated irradiation of excited light over a 70 min period. Furthermore, we also confirmed that the fluorescence of β-phycoerythrin showed no decrease during the incubation with test compounds for 30 min before the addition of AAPH.

The test mixture was prepared with 170 μL of 19.6 nM β-phycoerythrin in 75 mM phosphate buffer, pH 7.0, and 10 μL of sample in 75 mM phosphate buffer or acetone at 20 μM for pure compounds in 96-well, black microwell plates (Corning Coaster Co., Cambridge, MA). Phosphate buffer alone was used as a blank, and 50−400 μM Trolox was used as a control. After 30 min of incubation at 37 °C, 20 μL of 300 mM AAPH solution was added to the mixture to initiate the assay, and the fluorescence of each well was read every 2 min over a 70-min period at 37 °C. The area under the fluorescence curve was calculated, and the ORAC value of each sample was expressed as 1 unit for 1 μmol equivalent of Trolox. Each sample was measured in triplicate, and the data were determined as the mean values ± the standard deviations. The ORAC value of each isolated compound was compared to that of Trolox, and was statistically analyzed by *t*-test.

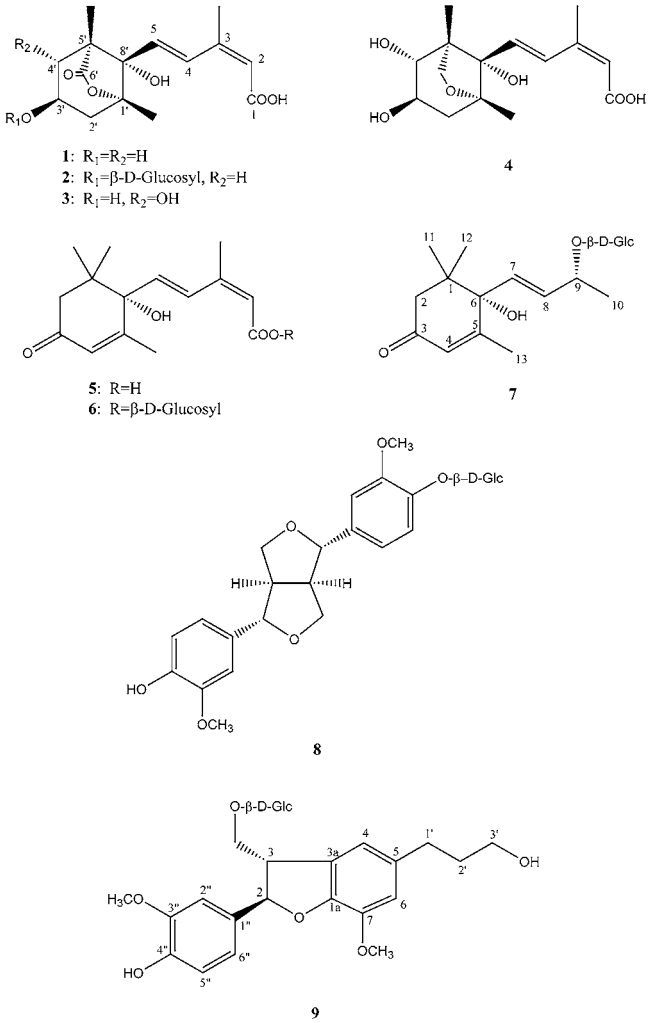

Figure 1. Structures of the isolated prune components.

RESULTS AND DISCUSSION

Structural Elucidation of Prune Components. The ethanol extract of prunes was separated into the hexane-soluble and water-soluble parts, and the latter was fractionated by Diaion HP-20 column chromatography to give the H_2O, methanol, and acetone eluates. A previous study indicated that the methanol eluate exhibited the strongest antioxidant activity (*11*), so that the methanol eluate was further purified using chromatographic techniques to result in the isolation of four new compounds (**1**−**4**) together with five known compounds (**5**−**9**) (**Figure 1**).

Compound **1** exhibited an optical rotation of −56.7° and an [M − H]$^-$ peak in good agreement with the molecular formula $C_{15}H_{20}O_6$ by HRMS-FAB in the negative mode. The UV spectrum of **1** showed an absorption maximum at 258 nm, suggesting the presence of a conjugated dienone group. The IR spectrum revealed a broad absorption band in the region of 3600−2500 cm^{-1} due to alcoholic and carboxylic acid functions. In addition, the characteristic absorption bands of ester (1757 and 1169 cm^{-1}), conjugated carboxyl (1685 cm^{-1}) and olefinic (1654 and 1602 cm^{-1}) functions were observed in the IR spectrum. The ^{1}H NMR spectrum of **1** showed three tertiary methyls (δ_H 1.07, 1.34, and 2.06), two methylenes (δ_H1.84 (dd,

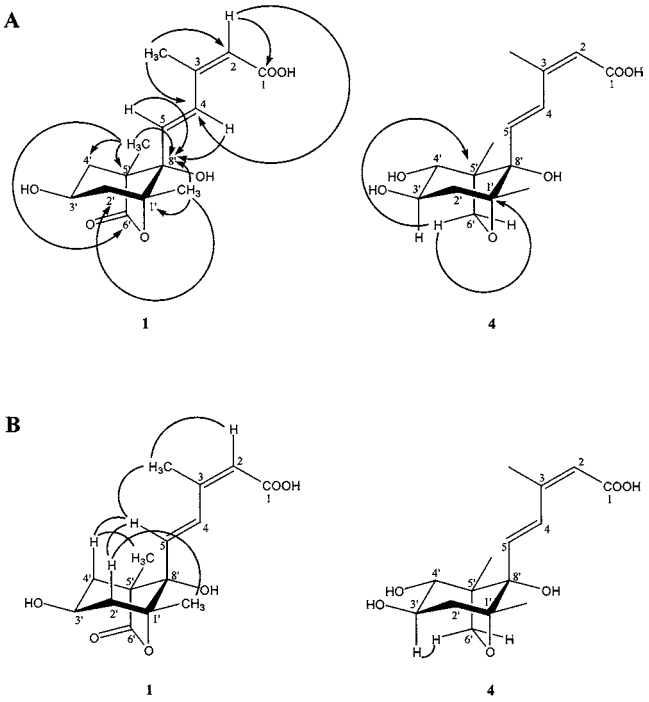

Figure 2. (A) Significant long-range correlations in the HMBC spectra; (B) Significant NOEs observed in the NOESY spectra of **1** and **4**.

J = 10.1, 14.3 Hz) and 2.23 (ddd, J = 1.7, 7.1, 14.3 Hz), δ_H 1.72 (dd, J = 11.1, 13.6 Hz) and 1.89 (ddd, J = 1.7, 7.1, 13.6 Hz)), one oxymethine (δ_H 3.83 (dddd, J = 7.1, 7.1, 10.1, 11.1 Hz)), and three olefinic protons (δ_H 5.83 (brs), 6.38 (dd, J = 0.5, 16.1 Hz), and 7.97 (dd, J = 0.7, 16.1 Hz)) (**Table 1**). The ^{13}C NMR spectrum indicated six nonprotonated carbons, consistent with an olefinic (δ_C 148.5), two carboxyl (δ_C 171.0 and 181.1), a quaternary (δ_C 53.5), and two oxygenated quaternary (δ_C 82.8 and 89.8) carbons together with nine protonated carbons (**Table 1**). ^{1}H−^{1}H correlation spectroscopy (^{1}H−^{1}H COSY) measurement established the sequence of CH_2−CH(O)−CH_2 and an *E*-form of an olefin moiety in the molecule. In a heteronuclear multiple bond correlation (HMBC) experiment, long-range correlations were observed between the olefinic proton (δ_H 5.83) and both the carboxyl (δ_C 171.0) and the olefinic carbons (δ_C 133.5) and between the methyl protons (δ_H 2.06) and both of the olefinic carbons (δ_C 122.2 and 133.5), indicating the presence of a 3-methyl-2,4-pentadienoic acid moiety (**Figure 2**). The nuclear Overhauser exchange spectroscopy (NOESY) correlations between the 3-methyl protons and both H-2 and H-5 confirmed the 2*Z*,4*E*-form (**Figure 2**). The protons of one of the tertiary methyls at δ_H 1.07 correlated with the methylene carbon (δ_C 41.0, C-4′), the quaternary carbons at δ_C 53.5 (C-5′) and 82.8 (C-8′) and the carboxyl carbon at δ_C 181.1, while another set of tertiary methyl protons (δ_H 1.34) correlated with the methylene carbon (δ_C 42.3, C-2′), C-8′, and the oxygenated quaternary carbon at δ_C 89.8 (C-1′) in the HMBC spectrum. These findings resulted in a 3,8-dihydroxy-1,5-dimethyl-7-oxa- 6-oxobicyclo[3,2,1]octane skeleton in **1**. In addition, the HMBC correlations between both the olefinic protons at δ_H 6.38 and 7.97 and C-8′ indicated that the pentadienoic acid moiety was linked to C-8′. The relative configuration of **1** was confirmed by NOESY measurement, in which the correlations between H-2′ax and both 1′-CH_3 and H-5, and between H-4′ax and both 5′-CH_3 and H-5 were observed to establish the relative configuration as shown in **Figure 2**. Thus, **1** was determined to be *rel*-5-(3*S*,8*S*-dihydroxy-1*R*,5*S*-dimethyl-7-oxa-6-oxobicyclo[3,2,1]oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic acid, a derivative of phaseic acid, which is known as a metabolite of abscisic acid (*21*, *22*).

Compound **2** showed an [M − H]$^-$ corresponding to the molecular formula $C_{21}H_{30}O_{11}$ by HRMS-FAB in the negative mode. The ^{1}H and ^{13}C NMR spectra of **2** revealed similar signals concerning the sesquiterpene moiety to those of **1**, except for the proton and carbon signals due to C-2′, 3′, and 4′ (**Tables 1**). Compound **2** showed six additional carbon signals (δ_C 62.5, 71.5, 75.0, 78.0 (2C), and 103.2) attributable to a glucopyranose moiety. The anomeric proton signal at δ_H 4.33 (dd, J = 7.8 Hz) indicated a β-configuration. The downfield shift of C-3′ (Δ7.8 ppm) with respect to **1** in the ^{13}C NMR spectrum, as well as the HMBC correlation between the anomeric proton and C-3′ and the NOESY correlation between the anomeric proton and H-3′ax, revealed that compound **2** was a 3-*O*-glucoside of **1**. Furthermore, the characteristic upfield shifts of C-2′ (Δ2.9 ppm, pro-*S*) and C-4′ (Δ1.5 ppm, pro-*R*) in comparison with those of **1** suggested that the configuration of C-3′ was S (*23*, *24*). Also, the J value of 9.8 Hz indicates an axial−axial coupling between H-4′ and H-3′.

On the basis of HRMS-FAB in the negative mode, the molecular formula of **3** was assigned as $C_{15}H_{20}O_7$, indicating that **3** had one more oxygen than **1**. The spectroscopic characteristics of **3** resembled those of **1**. Oxymethine proton (δ_H 3.53) and carbon (δ_C 76.6) signals appeared in the ^{1}H and ^{13}C NMR spectra of **3** instead of the disappearance of the methylene proton and carbon signals at the 4′ position of **1**. H-4′ correlated with H-2′ax (δ_H 1.83) in the NOESY spectrum, which indicated that H-4′ was axial-oriented. Thus, **3** was concluded to be *rel*-5-(1*R*,5*S*-dimethyl-3*R*,4*R*,8*S*-trihydroxy-7-oxa-6-oxobicyclo[3,2,1]- oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic acid.

Compound **4** had a molecular formula $C_{15}H_{22}O_6$, which was determined by HRMS-FAB in the negative mode. Comparing the ^{1}H and ^{13}C NMR spectra of **3**, signals due to an oxymethylene group were observed at δ_H 3.57 (dd, J = 1.5, 7.6 Hz) and 3.91 (d, J = 7.6 Hz) in the ^{1}H NMR and at δ_C 72.3 in the ^{13}C NMR spectra of **4**, with the disappearance of the carboxyl carbon at δ_C 178.9 assignable to C-6′. In the HMBC spectrum of **4**, the oxymethylene proton at δ_H 3.91 was correlated with C-1′ (δ_C 86.9) and C-5′ (δ_C 55.0), indicating that C-6′ was an oxymethylene carbon. The NOESY correlation between H-6′ (δ_H 3.91) and H-3′ax (δ_H 3.76) confirmed that the orientation of the oxymethylene was axial. Thus, **4** was determined to be *rel*-5-(1*R*,5*S*-dimethyl-3*R*,4*R*,8*S*-trihydroxy-7- oxabicyclo-[3,2,1]-oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic acid.

Compounds **5**−**9** were identified on the basis of their NMR and MS data, and their optical rotations to be (+)-abscisic acid (**5**) (*13*), (+)-β-D-glucopyranosyl abscisate (**6**) (*13*, *14*), (6*S*, 9*R*)-roseoside (**7**) (*15*), (+)-pinoresinol mono-β-D-glucopyranoside (**8**) (*16*) and 3-(β-D-glucopyranosyloxymethyl)-2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-(2*R*,3*S*)-dihydrobenzofuran (**9**) (*17*), respectively. This is the first report on the isolation of compounds **1**−**9** from prunes.

ORAC of Prune Components. Antioxidant activity of the isolated compounds was evaluated on the basis of their ORAC values. Abscisic acid related compounds, **1**−**7**, showed ORAC values in the range of 0.34−0.77 units/μmol. On the other hand, lignan compounds **8** and **9** were more active than **1**−**7**, showing 1.09 unit/μmol that was comparable to Trolox, and 2.33 unit/μmol that was about twice as high as Trolox, respectively (**Table 2**).

Table 2. ORAC[a] of the Isolated Prune Components

compound	ORAC
rel-5-(3*S*,8*S*-dihydroxy-1*R*,5*S*-dimethyl-7-oxa-6-oxobicyclo[3,2,1]oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic acid (**1**)	0.50 ± 0.01[b,c]
rel-5-(3*S*,8*S*-dihydroxy-1*R*,5*S*-dimethyl-7-oxa-6-oxobicyclo[3,2,1]oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic acid 3′-*O*-β-D-glucopyranoside (**2**)	0.72 ± 0.01[c]
rel-5-(1*R*,5*S*-dimethyl-3*R*,4*R*,8*S*-trihydroxy-7-oxa-6-oxobicyclo[3,2,1]oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic acid (**3**)	0.34 ± 0.00[c]
rel-5-(1*R*,5*S*-dimethyl-3*R*,4*R*,8*S*-trihydroxy-7-oxabicyclo[3,2,1]oct-8-yl)-3-methyl-2*Z*,4*E*-pentadienoic acid (**4**)	0.67 ± 0.01[d]
(+)-abscisic acid (**5**)	0.77 ± 0.02[c]
(+)-β-D-glucopyranosyl abscisate (**6**)	0.41 ± 0.01[c]
(6*S*,9*R*)-roseoside (**7**)	0.49 ± 0.00[c]
(+)-pinoresinol mono-β-D-glucopyranoside (**8**)	1.09 ± 0.03[e]
3-(β-D-glucopyranosyloxymethyl)-2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy- (2*R*,3*S*)-dihydrobenzofuran (**9**)	2.33 ± 0.02[e]

[a] ORAC, oxygen radical absorbance capacity. [b] ORAC values are presented as 1 unit for 1 μmol of Trolox equivalent per μmol of compound. Data are expressed as the mean values ± the standard deviations ($n = 3$). [c] Significantly lower than Trolox ($p < 0.01$). [d] Significantly lower than Trolox ($p < 0.05$). [e] Significantly higher than Trolox ($p < 0.01$).

In previous studies, *o*-diphenols such as caffeic acid, chlorogenic acid, protocatechuic acid, (−)-epicatechin, (+)-catechin, luteolin, myricetin, and cyanidin chloride showed 2−4 times higher ORAC value than Trolox. Non *o*-diphenol compounds such as kaempferol, naringenin, malvidin chloride, and genistein also indicated higher ORAC values than Trolox (>2 unit/μmol), and other non *o*-diphenol components such as *p*-coumaric acid, vanillic acid, and pelargonidin-3,5-diglucoside exhibited almost the same ORAC values as Trolox (*25*, *26*). The ORAC value of compound **9** was relatively high and was comparable to other non *o*-diphenol antioxidants, and the activity of **8** was as same as the latter compounds. The abscisic acid related compounds, although they are not phenolics, showed certain ORAC activity. However, we found that these abscisic acid related compounds showed no scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (data not shown). To our knowledge, there are no data of antioxidant activity related to this kind of constituents; therefore, further investigation on antioxidant activity of abscisic acid related compounds are required. Consequently, it is found that lignans **8** and **9** probably contribute to the antioxidant activity of prunes, and abscisic acid related compounds **1**−**7** might associate with the antioxidant capacity of prunes.

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