Medicinal Flowers. VIII.[1] Radical Scavenging Constituents from the Flowers of *Prunus mume*: Structure of Prunose III

Hisashi MATSUDA,[a] Toshio MORIKAWA,[a] Tomoko ISHIWADA,[a] Hiromi MANAGI,[a] Masatomo KAGAWA,[a] Yoshihiko HIGASHI,[b] and Masayuki YOSHIKAWA*,[a]

[a] *Kyoto Pharmaceutical University; Misasagi, Yamashina-ku, Kyoto 607–8412, Japan: and* [b] *Research Laboratories, Azumanoen Co., Ltd.; 836–1 Higashihonjo, Minabegawamura, Hidakagun, Wakayama 645–0021, Japan.*
Received November 18, 2002; accepted January 14, 2003

The methanolic extract and its fractions from the fresh flowers of *Prunus mume* SIEB. et ZUCC. were found to show scavenging effects on 1,1-diphenylpicryl-2-hydrazyl (DPPH) radical and superoxide. The fragrance constituents of *P. mume* were analyzed by GC-MS and a new polyacylated sucrose, prunose III, was isolated from the ethyl acetate-soluble fraction. The structure of prunose III was determined on the basis of chemical and physicochemical evidence as 4,3′,4′,6′-tetra-*O*-acetyl-6-*O*-*p*-coumaroylsucrose. In addition, the scavenging effects of the principal constituents on DPPH radical and superoxide were examined.

Key words *Prunus mume*; prunose III; radical scavenging effect; fragrance constituent; polyacylated sucrose; medicinal flower

Japanese apricot, *Prunus* (*P.*) *mume* SIEB. et ZUCC. (Rosaceae), has been widely cultivated as an ornamental plant and its fruit is consumed as a garnish foodstuff (pickled ume) and drink (ume brandy) in Japan. In Chinese traditional medicine, various parts of this plant (ex. an immature fruit, leaf, branch, seed, and root) are used as herbal medicines. Particularly, the flowers of *P. mume* (Chinese name "白梅花") have been prescribed for detoxification, stomachic, expectorant, and sedative purposes and for the remedy of eye pain, dipsia, and skin disorders in Chinese traditional preparations. The distilled water of the flowers (Chinese name "梅露") is also used for the treatment of dipsia and heliosis and as a beauty lotion.

In the course of our characterization studies on the bioactive constituents of medicinal flowers,[1—7] we have reported that the methanolic extract of *P. mume* exhibited inhibitory effects on rat lens aldose reductase and platelet aggregation. From the methanolic extract, we have isolated two flavonol oligoglycosides called 2″-*O*-acetylrutin and 2″-*O*-acetyl-3′-*O*-methylrutin and two polyacylated sucroses termed prunoses I and II together with 15 known compounds, and have characterized their structures as well as their inhibitory activities on aldose reductase and platelet aggregation.[1]

As a continuation of the characterization studies on the flowers of *P. mume*, we have found that the methanolic extract and its fractions showed potent scavenging effects on 1,1-diphenylpicryl-2-hydrazyl (DPPH) radical and superoxide anion radical ($\cdot O_2^-$). From the methanolic extract, a new polyacylated sucrose called prunose III (**1**) was isolated and the radical scavenging effects of the principle constituents were examined.

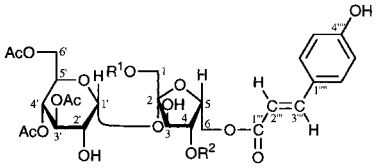
This paper deals with the isolation and structure elucidation of a new polyacylated sucrose (**1**) from the fresh flowers of *P. mume.* We also describe GC-MS analysis of the nonpolar constituents and the radical scavenging activities of the principle constituents.

Isolation of Prunose III (1) from the Fresh Flowers of *P. mume* Fresh flowers of *P. mume* cultivated in Wakayama Prefecture (Japan) were extracted with methanol under reflux. The methanolic extract was partitioned into an ethyl acetate (AcOEt)–water mixture to furnish the AcOEt-soluble portion and aqueous phase. The aqueous phase was further extracted with *n*-butanol (*n*-BuOH) to give *n*-BuOH- and H_2O-soluble portions. To clarify the fragrance constituents, which were the component of the distilled water from the flowers of the plant, the AcOEt-soluble portion was subjected to GC-MS analysis and 22 non-polar constituents were identified: acetic acid, benzaldehyde, methyl benzoate, dimethyl succinate, diethyl succinate, benzyl acetate, ethyl dodecanate, benzyl alcohol, methyl eugenol, ethyl tetradecanate, 6,10,14-trimethyl-2-pentadecanone, eugenol, methyl hexadecanate, ethyl hexadecanate, 4-vinyl phenol, benzoic acid, ethyl octadecanate, 5-hydroxyl methylfurfural, ethyl linolate, methyl linolenate, ethyl linolenate, and phytol (Chart 2). Prunose III (**1**) was also isolated using normal- and reversed-phase column chromatography and finally HPLC from the AcOEt-soluble portion.

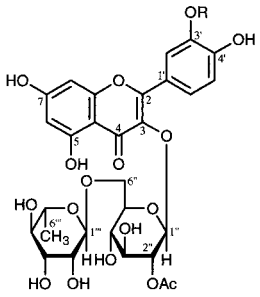
Structure of Prunose III (1) Prunose III (**1**) was isolated as a white powder with positive optical rotation ($[\alpha]_D^{26}$ +27.8°). In the positive-ion fast atom bombardment (FAB)-MS of **1**, a quasimolecular ion peak was observed at *m/z* 679 $(M+Na)^+$, while the negative-ion FAB-MS showed a quasimolecular ion peak at *m/z* 655 $(M-H)^-$ as well as a fragment ion peak at *m/z* 367 $(M-C_{12}H_{17}O_8)^-$, which was thought to be derived by cleavage of the glycosidic linkage at the 1′-position of the 3′,4′,6′-triacetyl D-glucosyl moiety. The molecular formula $C_{29}H_{36}O_{17}$ of **1** was determined from the quasimolecular ion peak $(M+Na)^+$ and by high-resolution MS measurement. The IR spectrum of **1** showed absorption bands assignable to hydroxyl, acetyl, and α,β-unsaturated ester functions at 3453, 1731, 1712, and 1036 cm^{-1}, while the UV spectrum showed absorption maxma at 228 nm (log ε 3.96) and 314 nm (4.24), suggestive of a coumaroyl group.

Alkaline treatment of **1** with 0.1% sodium methoxide in methanol liberated sucrose and methyl *p*-coumarate. The ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (Table 1) spectra[8] of **1** showed signals due to a *p*-coumaroyl group [δ 6.34, 7.61 (both d, J=15.8 Hz, 2‴ and 3‴-H), 6.80, 7.56 (both d, J=8.6 Hz, 3⁗, 5⁗ and 2⁗, 6⁗-H)] and a sucrose moiety [δ 3.42 (m, 6-H_2), 3.63 (m, 1-H_2 and 2′-H), 4.05 (m, 3-H), 4.08 (m, 6′-H_2), 4.28 (m, 5′-H), 4.82 (t-like, 4′-H), 5.14 (t-like, 3′-H), 5.37 (t-like, 4-H), 5.40 (d, J=3.5 Hz, 1′-H), 5.61 (d,

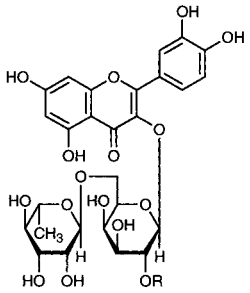
* To whom correspondence should be addressed. e-mail: shoyaku@mb.kyoto-phu.ac.jp



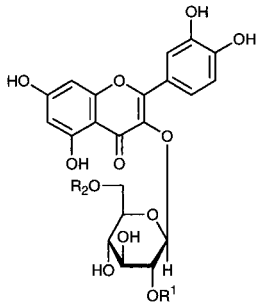
Prunose III (**1**): R^1=H, R^2=Ac
Prunose I (**2**): R^1=Ac, R^2=Ac
Prunose II (**3**): R^1=Ac, R^2=H



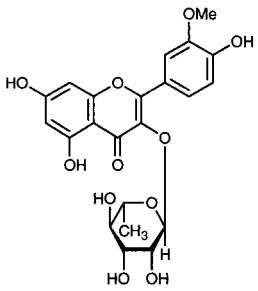
2"-*O*-acetylrutin (**4**):R=H
2"-*O*-acetyl-3'-*O*-methylrutin (**5**):R=Me



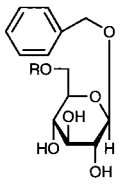
quercetin 3-*O*-rhamnopyranosyl (1→6)galactopyranoside (**6**): R=H
quercetin 3-*O*-(2",6"-α-L-dirhamnopyranosyl)-β-D-galactopyranoside (**7**): R=Rha



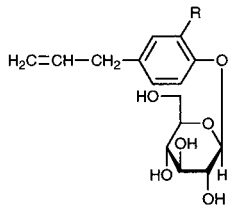
rutin (**8**): R^1=H, R^2=Rha
quercetin 3-*O*-neohesperidoside (**9**) : R^1=Rha, R^2=H



isorhamnetin 3-rhamnoside (**10**)



benzyl glucopyranoside (**11**): R=H
benzyl alcohol xylosyl(1→6)glucoside (**12**): R=Xyl



chavicol β-D-glucoside (**13**): R=H
eugenyl glucoside (**14**): R=OMe

Rha: α-L-rhamnopyranosyl
Xyl: β-D-xylopyranosyl

Chart 1

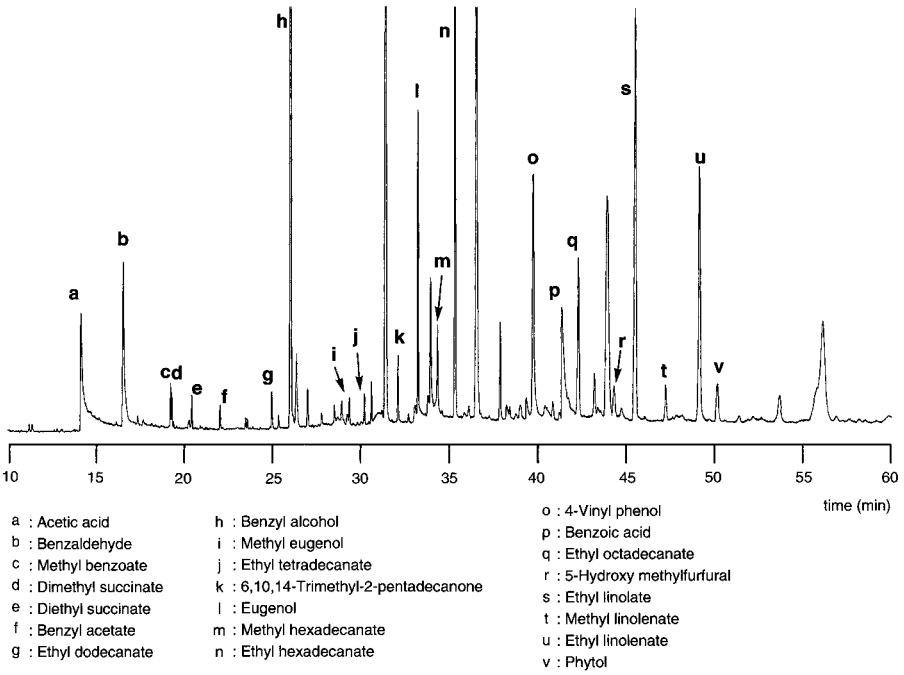


Chart 2. GC-MS Chromatogram of the AcOEt-Soluble Portion of the Methanolic Extract from the Flowers of *P. mume*

Table 1. ^{13}C-NMR Data for Prunose III (**1**)

	1		**1**
C-1	61.5	C-1‴′	124.6
C-2	104.4	C-2‴′, 6‴′	130.1
C-3	80.8	C-3‴′, 5‴′	115.5
C-4	75.0	C-4‴′	159.7
C-5	74.9		
C-6	62.3	$CH_3\underline{C}OO$–	169.7
C-1′	90.9		169.4
C-2′	68.5		169.3
C-3′	72.3		169.0
C-4′	68.3		
C-5′	67.5	$\underline{C}H_3COO$–	20.6
C-6′	62.0		20.5
C-1‴	164.8		20.3
C-2‴	113.0		20.0
C-3‴	145.4		

68 MHz, DMSO-d_6.

Table 2. Radical Scavenging Activities of MeOH Extract and AcOEt-, *n*-BuOH-, and H_2O-Soluble Portions from *P. mume*

Treatment	DPPH radical SC_{50} (μg/ml)[a]	$\cdot O_2^-$ IC_{50} (μg/ml)
MeOH extract	9.2	1.7
AcOEt-soluble portion	10	3.1
n-BuOH-soluble portion	6.5	1.5
H_2O-soluble portion	15	4.4
α-Tocopherol	4.7	—
(+)-Catechin	1.7	0.49

a) Concentration required for 50% reduction of 40 μM DPPH radical.

J=7.0 Hz, 5-H)] together with four acetyl groups [δ 1.83, 1.97, 2.00, 2.03 (all s)]. The bonding positions of four acetyl and *p*-coumaroyl groups in **1** were determined by heteronuclear multiple bond correlation (HMBC) experiment, which showed long-range correlations between the 6-protons and the *p*-coumaroyl carbonyl carbon and between the 4,3′,4′,6′-protons and four acetyl carbonyl carbons. Consequently, the structure of prunose III was elucidated to be 4,3′,4′,6′-tetra-*O*-acetyl-6-*O*-*p*-coumaroylsucrose (**1**).

Radical Scavenging Activities of Constituents from the Fresh Flowers of *P. mume* The DPPH radical, which is stable and shows an absorption at 517 nm, has been used as a convenient tool for the radical scavenging assay, and this assay is independent of any enzyme activity.[9,10] When this compound accepts an electron or hydrogen radical and becomes a more stable compound, the absorption vanishes. The xanthine–xanthine oxidase system was conventionally used for generation of $\cdot O_2^-$, which was detected by reduction of 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate sodium salt (WST-1) in the present study.[11] We previously reported the DPPH radical and $\cdot O_2^-$ scavenging activities of several natural medicines, such as the rhizome of *Rheum undulatum*[12] and the fruit hulls of *Garcinia mangostana*.[13] In our continuing studies on antioxidative principles from natural medicines, the methanolic extract, fractions, and principal constituents from *P. mume* were examined. The methanolic extract exhibited a potent scavenging effect on DPPH radical and $\cdot O_2^-$, and its AcOEt-, *n*-BuOH-, and H_2O-soluble portions also exhibited the inhibitory activity (Table 2).

Table 3. Radical Scavenging Activities of Constituents from *P. mume*

Treatment	DPPH radical SC_{50} (μM)[a]	$\cdot O_2^-$ IC_{50} (μM)
Prunose III (**1**)	>40 (−1)[b]	>100 (12)[c]
2″-*O*-Acetylrutin (**4**)	4.9	8.7
2″-*O*-Acetyl-3′-*O*-methylrutin (**5**)	>40 (32)[b]	22
6	9.8	12
7	5.9	14
Rutin (**8**)	4.3	4.5
9	5.7	13
10	>40 (33)[b]	>100 (45)[c]
Benzyl glucoside (**11**)	>40 (−4)[b]	>100 (22)[c]
12	>40 (6)[b]	>100 (47)[c]
Chavicol β-D-glucoside (**13**)	>40 (4)[b]	50
Eugenyl glucoside (**14**)	>40 (5)[b]	>100 (25)[c]

a) Concentration required for 50% reduction of 40 μM DPPH radical. Values in parentheses represent the inhibition (%) at *b*) 40 and *c*) 100 μM.

As shown in Table 3, 2″-*O*-acetylrutin (**4**, SC_{50}=4.9 μM), quercetin 3-*O*-rhamnopyranosyl(1→6)galactopyranoside (**6**, 9.8 μM), quercetin 3-*O*-(2″,6″-α-L-dirhamnopyranosyl)-β-D-galactopyranoside (**7**, 5.9 μM), rutin (**8**, 4.3 μM), and quercetin 3-*O*-neohesperidoside (**9**, 5.7 μM) were found to show the potent DPPH radical scavenging activity, while **4** (IC_{50}=8.7 μM), **6** (12 μM), **7** (14 μM), **8** (4.5 μM), and **9** (13 μM) also inhibited $\cdot O_2^-$ scavenging activity.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu UV-1200 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; EI-MS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz) spectrometer; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) spectrometer with tetramethylsilane as an internal standard; HPLC detector, Shimadzu RID-6A refractive index detector.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150—350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); TLC, pre-coated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, pre-coated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4, followed by heating.

Isolation of Prunose III (1) Fraction 4 (5.2 g), obtained from the AcOEt-soluble portion of the fresh flowers of *P. mume* (cultivated in Wakayama Prefecture, Japan), and isolated prunoses I (**2**) and II (**3**), as reported previously,[1] were further purified by reversed-phase silica gel column chromatography [150 g, MeOH–H_2O (65 : 35)→MeOH] and HPLC [YMC-pack ODS-A 250×20 mm i.d., MeOH–H_2O (55 : 45)] to give prunose III (**1**, 209 mg, 0.0084%).

Prunose III (**1**): A white powder, $[\alpha]_D^{26}$ +27.8° (c=0.90, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{29}H_{36}O_{17}Na$ $(M+Na)^+$: 679.1850. Found: 679.1840. UV [MeOH, nm (log ε)]: 228 (3.96), 314 (4.24). IR (KBr): 3453, 1731, 1712, 1036 cm^{-1}. ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 1.83, 1.97, 2.00, 2.03 (3H each, all s, Ac×4), 3.42 (2H, m, 6-H_2), 3.63 (3H, m, 1-H_2, 2′-H), 4.05 (1H, m, 3-H), 4.08 (2H, m, 6′-H_2), 4.28 (1H, m, 5′-H), 4.82 (1H, t-like, 4′-H), 5.14 (1H, t-like, 3′-H), 5.37 (1H, t-like, 4-H), 5.40 (1H, d, J=3.5 Hz, 1′-H), 5.61 (1H, d, J=7.0 Hz, 5-H), 6.34, 7.61 (1H each, both d, J=15.8 Hz, 2‴,3‴-H), 6.80, 7.56 (2H each, both d, J=8.6 Hz, 3‴′,5‴′-H and 2‴′,6‴′-H). ^{13}C-NMR (68 MHz, DMSO-d_6) δ_C: given in Table 1. Negative-ion FAB-MS: m/z 655 $(M-H)^-$, 367 $(M-C_{12}H_{17}O_8)^-$. Positive-ion FAB-MS: m/z 679 $(M+Na)^+$.

Alkaline Hydrolysis of Prunose III (1) A solution of prunose III (**1**, 11.1 mg) in 0.1% NaOMe–MeOH (1.0 ml) was stirred at room temperature (25 °C) for 1 h. The reaction mixture was neutralized with Dowex HCR W2 (H^+ form) and the residue was removed by filtration. After removal of the solvent from the filtrate *in vacuo*, the residue was separated by normal-phase silica gel column chromatography [500 mg, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer→6 : 4 : 1)] to give methyl *p*-coumarate (2.7 mg, 90%) and sucrose (4.1 mg, 71%), which were identified by comparison of physical data (^{1}H-NMR, IR, MS, $[\alpha]_D$) with authentic samples.

Bioassay

DPPH Radical Scavenging Activity The free radical scavenging activity of the constituents of *P. mume* was assessed using the DPPH radical.[9,10] An ethanol solution of DPPH (200 μM, 0.5 ml) radical was mixed with different concentrations of each test compound (0—100 μM, 1.0 ml) in ethanol and 0.1 M acetate buffer (pH 5.5, 1.0 ml), and the absorbance change at 517 nm was measured 30 min later. The reaction solution without DPPH was used as a blank test. Measurements were performed in duplicate, and the concentration required for a 50% reduction (50% scavenging concentration, SC_{50}) of 40 μM DPPH radical solution was determined graphically.

$\cdot O_2^-$ Scavenging Activity Spectrophotometric assay for $\cdot O_2^-$ scavenging activity based on the reduction of WST-1 by the xanthine-xanthine oxidase system was performed using a commercial kit (SOD Assay Kit-WST, Dojindo Molecular Technologies, Inc., Gaithersburg, MD, U.S.A.).[11] Measurements were performed in quadruplicate, and IC_{50} values were determined graphically.

References and Notes

1) Part VII.: Yoshikawa M., Murakami T., Ishiwada T., Morikawa T., Kagawa M., Higashi Y., Matsuda H., *J. Nat. Prod.*, **65**, 1151—1155 (2002).
2) Yoshikawa M., Morikawa T., Murakami T., Toguchida I., Harima S., Matsuda H., *Chem. Pharm. Bull.*, **47**, 340—345 (1999).
3) Yoshikawa M., Morikawa T., Toguchida I., Harima S., Matsuda H., *Chem. Pharm. Bull.*, **48**, 651—656 (2000).
4) Yoshikawa M., Murakami T., Kishi A., Kageura T., Matsuda H., *Chem. Pharm. Bull.*, **49**, 863—870 (2001).
5) Murakami T., Kishi A., Yoshikawa M., *Chem. Pharm. Bull.*, **49**, 974—978 (2001).
6) Matsuda H., Ninomiya K., Shimoda H., Yoshikawa M., *Bioorg. Med. Chem.*, **10**, 707—712 (2002).
7) Matsuda H., Morikawa T., Toguchida I., Harima S., Yoshikawa M., *Chem. Pharm. Bull.*, **50**, 972—975 (2002).
8) The ^{1}H- and ^{13}C-NMR spectra of **1** were assigned with the aid of homo- and hetero-correlation spectroscopy (^{1}H–^{1}H, ^{13}C–^{1}H COSY), distortionless enhancement by polarization transfer (DEPT), and heteronuclear multiple bond connectivity (HMBC) experiments.
9) Uchiyama M., Suzuki Y., Fukuzawa K., *Yakugaku Zasshi*, **88**, 678—683 (1968).
10) Blois M. S., *Nature* (London), **181**, 1199—1200 (1958).
11) Ukeda H., Kawana D., Maeda S., Sawamura M., *Biosci. Biotechnol. Biochem.*, **63**, 485—488 (1999).
12) Matsuda H., Morikawa T., Toguchida I., Park J.-Y., Harima S., Yoshikawa H., *Bioorg. Med. Chem.*, **9**, 41—50 (2001).
13) Yoshikawa M., Harada E., Miki A., Tsukamoto K., Liang S. Q., Yamahara J., Murakami N., *Yakugaku Zasshi*, **114**, 129—133 (1994).