Bioactive Constituents from Chinese Natural Medicines. XVII.[1] Constituents with Radical Scavenging Effect and New Glucosyloxybenzyl 2-Isobutylmalates from *Gymnadenia conopsea*

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The methanol-eluted fraction of the methanolic extract from the tubers of *Gymnadenia conopsea* was found to show radical scavenging activities for DPPH and super oxide anion ($\cdot O_2^-$) radicals. Three new glucosyloxybenzyl 2-isobutylmalates, gymnosides VIII, IX, and X, were isolated from this natural medicine together with 58 known constituents. The stereostructures of gymnosides were elucidated on the basis of chemical and physicochemical evidence. In addition, the phenanthrene and dihydrostilbene constituents showed radical scavenging activities and suggested the following structural requirements on radical scavenging activities; a) phenanthrenes: 1) dihydrogenation at the 9,10-positions enhances the activities, 2) the 1 or 3-*p*-hydroxybenzyl group enhances the activities; b) dihydrostilbenes: 1) methylation of the 3′-position reduces the activities, 2) the 2- and/or 6-*p*-hydroxybenzyl groups enhance the activities.

Key words *Gymnadenia conopsea*; gymnoside; glucosyloxybenzyl 2-isobutylmalate; radical scavenging activity; phenanthrene; stilbene

The Orchidaceae plant, *Gymnadenia conopsea* R. BR., is a perennial herb widely distributed in China. The tubers of this plant have been used as a traditional Chinese medicine for the treatment of asthma, neurasthenia, and chronic hepatitis. In the course of our studies on bioactive constituents from Chinese natural medicines,[2—12] we found that the methanolic extract of this natural medicine showed an antiallergic activity on ear passive cutaneous anaphylaxis reactions in mice. From the methanolic extract, three dihydrophenanthrenes and a dihydrostilbene named gymconopins A—D were isolated together with 10 known phenanthrene and stilbene constituents and their inhibitory effects on antigen-induced degranulation in RBL-2H3 cells were characterized.[13] Furthermore, we reported the isolation and structure elucidation of seven glucosyloxybenzyl 2-isobutylmalates termed gymnosides I—VII from the tubers of *G. conopsea*.[1] As a continuing study of this natural medicine, the methanolic extract was found to show redical scavenging activities for DPPH radical and $\cdot O_2^-$, and also we additionally isolated three new glycosyloxybenzyl 2-isobutylmalates called gymnosides VIII (**1**), IX (**2**), and X (**3**). This paper deals with the isolation and absolute stereostructure elucidation of three new glycosides (**1**—**3**) as well as the radical scavenging activities of the phenanthrene and stilbene constituents.

The tubers of *G. conoposa* were extracted with methanol under reflux. The methanolic extract was subjected to Diaion HP-20 column chromatography to give H_2O-, MeOH-, and acetone-eluted fractions.[1,13] As shown in Table 1, the MeOH-eluted fraction was found to show radical scavenging activities for DPPH radical (SC_{50}=59.1 μg/ml) and $\cdot O_2^-$ (IC_{50}=13.3 μg/ml) without exhibiting any inhibitory activity on xanthine oxidase (IC_{50}>100 μg/ml). The MeOH-eluted fraction was subjected to ordinary and reversed-phase silica gel column chromatography and finally HPLC to furnish gymnosides VIII (**1**, 0.0003% from the natural medicine), IX (**2**, 0.013%), and X (**3**, 0.0005%) together with dactylorhin A (**4**, 0.12%),[1,14] gymconopins A (**5**, 0.0006%)[13] and B (**6**, 0.0003%),[13] 2-methoxy-9,10-dihydrophenanthrene-4,5-diol (**7**, 0.0010%),[13] 4-methoxy-9,10-dihydrophenanthrene-2,7-diol (**8**, 0.0012%),[13] 1-(4-hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene-2,7-diol (**9**, 0.0013%),[13] 1-(4-hydroxybenzyl)-4-methoxyphenanthrene-2,7-diol (**10**, 0.0003%),[13] blestriarenes A (**11**, 0.0004%),[13] B[15] (0.0001%), and C[15] (0.0001%), batatacin III (**12**, 0.0016%),[13] 3′,5-dihydroxy-2-(4-hydroxybenzyl)-3-methoxybibenzyl (**13**, 0.0009%),[13] 3,3′-dihydroxy-2-(4-hydroxybenzyl)-5-methoxybibenzyl (**14**, 0.0008%),[13] 3,3′-dihydroxy-2,6-bis(4-hydroxybenzyl)-5-methoxybibenzyl (**15**, 0.0003%),[13] militarine[1,14] (0.072%), lo-

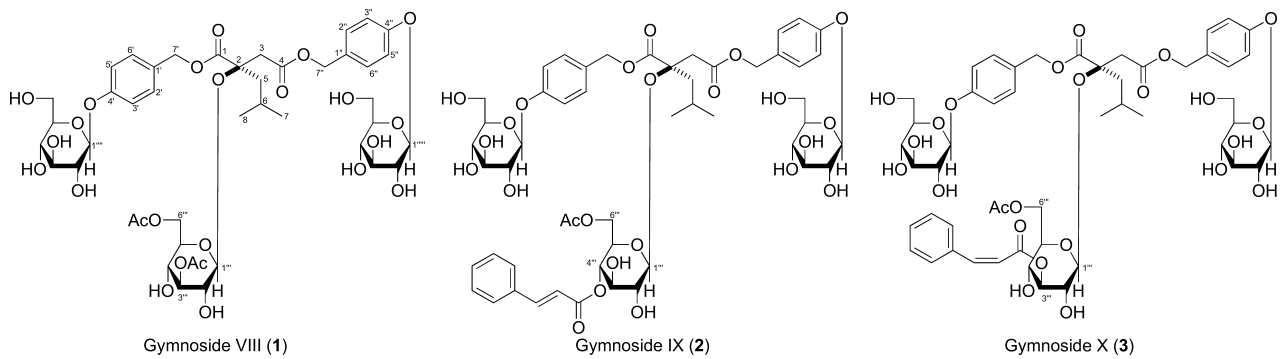

Chart 1

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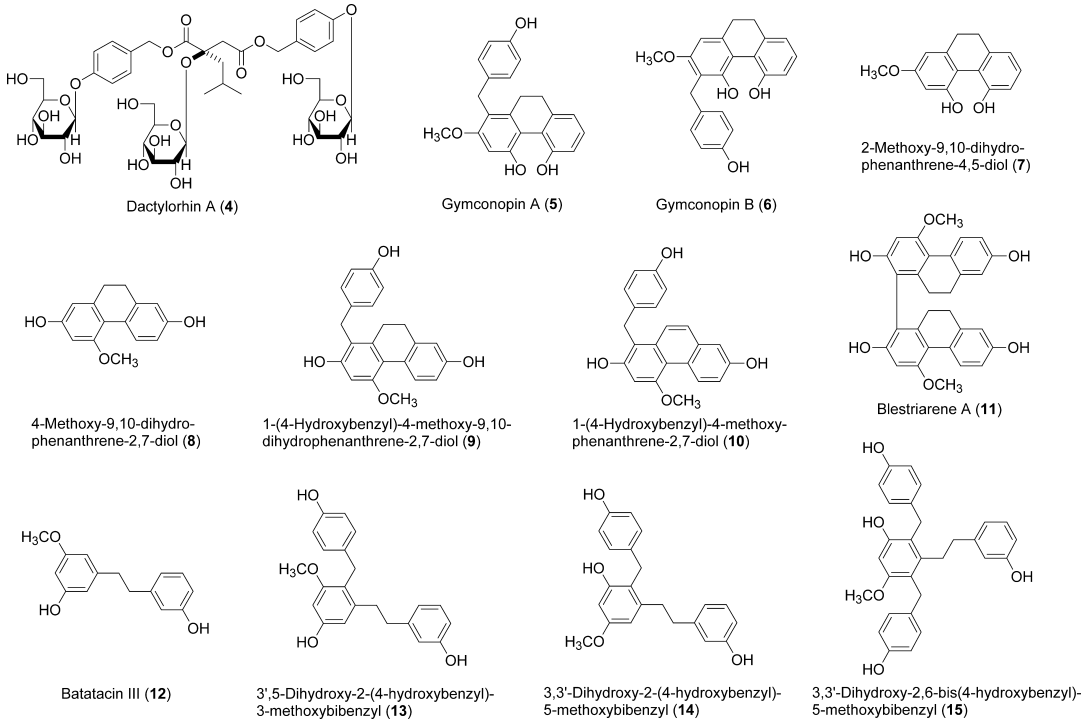

Chart 2

Table 1. DPPH Radical and $\cdot O_2^-$ Scavenging Activities and Xanthine Oxidase Inhibitory Activity of the MeOH Extract from the Tubers of *G. conopsea* and H_2O-, MeOH-, and Acetone-Eluted Fractions

	DPPH radical SC_{50} (μg/ml)[a]	$\cdot O_2^-$	
		Formozan formation IC_{50} (μg/ml)	Xanthine oxidase IC_{50} (μg/ml)
G. conopsea MeOH Ext.	>100 (28.7%)[b]	89.5	>100 (10.3%)[b]
H_2O-eluted fraction	>100 (10.1%)[b]	>100 (31.3%)[b]	—
MeOH-eluted fraction	59.1	13.3	>100 (1.9%)[b]
Acetone-eluted fraction	55.4	33.2	29.7

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

roglossin[14] (0.011%), dactylorhin B[14] (0.021%), 5-*O*-methylbatatacin III[16] (0.0001%), bulbocodins C[17] (0.0001%) and D[17] (0.0001%), bulbocol[18] (0.0003%), 3,3′-dihydroxy-4-(4-hydroxybenzyl)-5-methoxybibenzyl[19] (0.0003%), *p*-coumaric acid[20,21] (0.0006%), ferulic acid[20,21] (0.0002%), (−)-pinoresinol[22,23] (0.0002%), 2-*C*-(4-hydroxybenzyl)-α-L-*xylo*-3-ketohexulofuranosono-1,4-lactone[14] (0.0008%), pyrocatechol[24] (0.0001%), syringol[25] (0.0003%), 4-hydroxybenzyl alcohol[20,26] (0.0027%), 4-hydroxybenzyl methyl ether[26] (0.0082%), 4-hydroxybenzyl ethyl ether[27] (0.0024%), bis(4-hydroxybenzyl) ether[26] (0.0003%), 4-hydroxybenzaldehyde[20,26] (0.0022%), 2,4-dihydroxybenzaldehyde[20,28] (0.0003%), syringaldehyde[25] (0.0002%), 4-hydroxybenzoic acid[29] (0.0009%), vanillic acid[20,30] (0.0011%), phenyl β-D-glucopyranoside[31] (0.0002%), 4-methylphenyl β-D-glucopyranoside[32] (0.0007%), 4-hydroxymethylphenyl β-D-glucopyranoside[26] (0.0005%), 4-formylphenyl β-D-glucopyranoside[33] (0.0004%), 4-methoxyphenyl β-D-glucopyranoside[34] (0.0003%), benzyl β-D-glucopyranoside[35] (0.0007%), 4-hydroxybenzyl β-D-glucopyranoside[36] (0.0009%), dactyloses A[14] (0.0006%) and B[14] (0.0003%), thymidine[20,37] (0.0005%), and 5-hydroxymethylfuraldehyde[38] (0.0004%).

Structures of Gymnosides VIII (1), IX (2), and X (3) Gymnoside VIII (**1**) was obtained as a white powder and exhibited a negative optical rotation ($[\alpha]_D^{24}$ −37.3° in MeOH). In the UV spectrum of **1**, absorption maxima were observed at 224 (log ε 4.36) and 271 (3.41) nm. The IR spectrum of **1** showed absorption bands at 1734, 1617, 1516, 1458, and 1237 cm^{-1} assignable to ester carbonyl and carboxyl functions and aromatic ring in addition to strong absorption bands at 3432 and 1076 cm^{-1} suggestive of a glycoside moiety. In the positive- and negative-ion FAB-MS of **1**, quasi-molecular ion peaks were observed at *m/z* 995 $(M+Na)^+$ and 971 $(M-H)^-$, and high-resolution FAB-MS analysis revealed the molecular formula of **1** to be $C_{44}H_{60}O_{24}$. The acid hydrolysis of **1** with 1.0 M hydrochloric acid (HCl) liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[1,3,4,6—10,12] The ^{1}H- (pyridine-d_5, Table 2) and ^{13}C-NMR (Table 3) spectra of **1**, which were assigned by various NMR experiments,[39] showed signals assignable to two *sec.*-methyls [δ 0.88, 0.90 (3H each, both d, J=6.7 Hz, 7, 8-H_3)], four methylenes and a methine [δ 1.81,

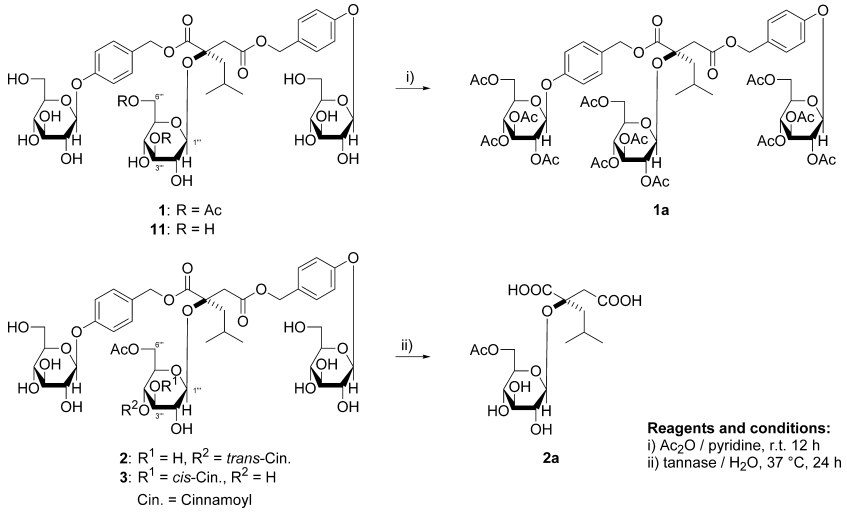

Chart 3

1.88 (1H each, m, 5-H_2), 2.08 (1H, m, H-6), 3.38, 3.56 (1H each, both d, J=17.4 Hz, 3-H_2), 5.11, 5.28 (1H each, both d, J=11.9 Hz, 7″-H_2), 5.26, 5.34 (1H each, both d, J=11.9 Hz, 7′-H_2)], two acetyls [δ 1.94, 2.15 (3H each, both s, Ac-H_3)], and two *ortho*-coupled A_2B_2 type aromatic protons [δ 7.40, 7.45 (2H each, both d, J=8.9 Hz, 3′,5′,2′, 6′-H), 7.40, 7.45 (2H each, both d, J=8.9 Hz, 3″,5″, 2″,6″-H)] together with three β-glucopyranosyl moieties [δ 5.59 (1H, d, J=7.3 Hz, 2-*O*-Glc-1-H), 5.65 (1H, d, J=7.6 Hz, 4″-*O*-Glc-1-H), 5.68 (1H, d, J=7.6 Hz, 4′-*O*-Glc-1-H)]. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **1** were similar to those of 1,4-diglucosylbenzyl 2-*O*-glucosyl 2-isobutylmalate such as dactylorhin A (**4**) and gymnosides,[1] except for the signals due to the 2-*O*-glucosyl part with two acetyl groups. That is, the ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) and homonuclear Hartmann-Hahn spectroscopy (^{1}H–^{1}H HOHAHA) experiments on **1** indicated the presence of partial structures written in bold lines, and in the heteronuclear multiple-bond correlations (HMBC) experiment, long-range correlations were observed between the following protons and carbons [3-H_2, 5-H_2, 7′-H_2 and 1-C; 6-H, 1‴-H and 2-C; 5-H_2 and 3-C; 3-H_2, 7″-H_2 and 4-C; 7-H_3, 8-H_3 and 5-C; 6-H, 8-H_3 and 7-C; 6-H, 7-H_3 and 8-C; 3′,5′-H, 7′-H_2 and 1′-C; 7′-H_2 and 2′-C; 2′,6′-H, 1⁗-H and 4′-C; 3″,5″-H, 7″-H_2 and 1″-C; 7″-H_2 and 2″-C; 2″,6″-H, 1⁗-H and 4″-C, 3‴-H, acetyl methyl proton (δ 2.15) and acetylcarbonyl carbon (δ_C 170.4), 6‴-H, acetyl methyl proton (δ 1.94) and acetylcarbonyl carbon (δ_C 171.1)] (Fig. 1). Furthermore, comparison of the ^{1}H- and ^{13}C-NMR spectra of **1** with those of **4**, whose absolute configuration of the 2-position was determined to be *R* orientation,[14] showed acetylation shifts around the 3- and 6-positions of the 2-*O*-glucosyl part in **4**. Finally, acetylation of **1** by acetic anhydride (Ac_2O) and pyridine gave the acetyl derivative (**1a**), which was also obtained by the similar acetylation of **4**. On the basis of those findings, the structure of gymnoside VIII (**1**) was determined to be the 3‴,6‴-di-*O*-acetate of **4**.

Gymnoside IX (**2**) was also isolated as a white powder with negative optical rotation ($[\alpha]_D^{24}$ −26.5° in MeOH). The UV spectrum of **2** showed absorption maxima at 224 (log ε 4.56) and 278 (4.36) nm. The IR spectrum of **2** showed absorption bands at 3432, 1734, 1636, 1516, 1235, and 1075 cm^{-1} ascribable to hydroxyl, ester carbonyl, carboxyl, and ether functions and aromatic ring. The molecular formula $C_{51}H_{64}O_{24}$ of **2** was determined by the quasimolecular ion peaks in positive and negative ion FAB-MS and by high-resolution FAB-MS. The acid hydrolysis of **2** with 1.0 M HCl liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[1,3,4,6—10,12] The enzymatic hydrolysis of **2** with tannase gave **2a**[1] and *trans*-cinnamic acid.[20] The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **2** were superimposable on those of **1** and **4**, expect for the signals due to the 2-*O*-glucosyl part with acetyl and *trans*-cinnamoyl groups. Namely, the proton and carbon signals in the ^{1}H- (pyridine-d_5, Table 2) and ^{13}C-NMR (Table 3) spectra[39] of **2** showed signal assignable to two *sec*.-methyls [δ 0.89, 0.91 (3H each, both d, J=5.8 Hz, 7, 8-H_3)], four methylenes and a methine [δ 1.99, 2.02 (1H each, m, 5-H_2), 2.02 (1H, m, 6-H), 3.39, 3.54 (1H each, both d, J=17.7 Hz, 3-H_2), 5.16, 5.32 (1H each, both d, J=11.3 Hz, 7″-H_2), 5.31, 5.36 (1H each, both d, J=11.9 Hz, 7′-H_2)], an acetyl [δ 2.06 (3H, s, Ac-H)], two *ortho*-coupled A_2B_2 type aromatic protons [δ 7.39, 7.44 (2H each, both d, J=8.2 Hz, 3′,5′, 2′,6′-H), 7.39, 7.48 (2H each, both d, J=8.2 Hz, 3″,5″, 2″,6″-H)], and a *trans*-cinnamoyl ester moiety [δ 6.71, 7.91 (1H each, both d, J=16.0 Hz, Cin.-8, 7-H), 7.37 (2H, ddd, J=2.1, 7.9, 7.9 Hz, Cin.-3,5-H), 7.38 (1H, tt, J=2.1, 7.9 Hz, Cin.-4), 7.54 (2H, dd, J=2.1, 7.9 Hz, Cin.-2,6-H)] together with three β-glucopyranosyl moiety [δ 5.54 (1H, d, J=7.6 Hz, 2-*O*-Glc-1-H), 5.63 (1H, d, J=7.6 Hz, 4″-*O*-Glc-1-H), 5.64 (1H, d, J=7.6 Hz, 4′-*O*-Glc-H)]. Comparison of the ^{1}H- and ^{13}C-NMR data of **2** with those of **4** indicated two acylation shifts around the 4‴- and 6‴-positions. The positions of acyl moieties in **2** were confirmed by the HMBC experiment, which showed long-range correlations between the 4-proton of the 2-*O*-glucosyl part and the *trans*-cinnamoyl carbonyl carbon (δ_C 166.4) and between the 6-protons of the 2-*O*-glucosyl part and the actyl carbonyl carbon (δ_C 170.7), as shown in Fig. 1. Consequently, the structure of gymnoside IX (**2**) was constructed to be 4‴-*O*-*trans*-cinnamoyl-6‴-*O*-acetyldactylorhin A.

Gymnoside X (**3**) was isolated as a white powder with

Table 2. ^{1}H-NMR (500 MHz, Pyridine-d_5) Data of Gymnosides VIII (**1**), IX (**2**), and X (**3**)

H-	δ (J Hz)		
	1	**2**	**3**
3	3.38 (d, 17.4)	3.39 (d, 17.7)	3.35 (d, 17.2)
	3.56 (d, 17.4)	3.54 (d, 17.7)	3.52 (d, 17.2)
5	1.81 (m)	1.99 (m)	1.93 (m)
	1.88 (m)	2.02 (m)	2.01 (m)
6	2.08 (m)	2.02 (m)	2.01 (m)
7	0.88 (d, 6.7)	0.89 (d, 5.8)	0.86 (d, 6.4)
8	0.90 (d, 6.7)	0.91 (d, 5.8)	0.91 (d, 6.4)
2′,6′	7.45 (d, 8.9)	7.44 (d, 8.2)	7.40 (d, 8.5)
3′,5′	7.40 (d, 8.9)	7.39 (d, 8.2)	7.37 (d, 8.5)
7′	5.26 (d, 11.9)	5.31 (d, 11.9)	5.26 (d, 12.2)
	5.34 (d, 11.9)	5.36 (d, 11.9)	5.30 (d, 12.2)
2″,6″	7.45 (d, 8.9)	7.48 (d, 8.2)	7.44 (d, 8.5)
3″,5″	7.40 (d, 8.9)	7.39 (d, 8.2)	7.41 (d, 8.5)
7″	5.11 (d, 12.2)	5.16 (d, 11.3)	5.12 (d, 11.9)
	5.28 (d, 12.2)	5.32 (d, 11.3)	5.20 (d, 11.9)
2-*O*-Glc-1‴	5.59 (d, 7.3)	5.54 (d, 7.6)	5.54 (d, 7.6)
2‴	4.24 (dd, 7.3, 8.9)	4.11 (m)	4.06 (dd, 7.6, 9.2)
3‴	5.56 (dd, 8.9, 8.9)	4.36 (dd, 7.6, 9.5)	5.92 (dd, 9.2, 9.5)
4‴	4.15 (m)	5.71 (dd, 9.5, 9.8)	4.23 (dd, 9.5, 9.5)
5‴	3.80 (m)	3.96 (m)	3.86 (m)
6‴	4.68 (dd, 5.2, 11.9)	4.43 (dd, 5.0, 12.2)	4.71 (dd, 4.9, 12.2)
	4.76 (dd, 2.3, 12.2)	4.61 (dd, 2.1, 12.2)	4.76 (dd, 2.1, 12.2)
3‴-Ac	2.15 (s)		
6‴-Ac	1.94 (s)	2.06 (s)	1.96 (s)
3‴-Cin.-2,6			7.90 (dd, 1.9, 7.6)
3,5			7.30 (ddd, 1.9, 7.3, 7.6)
4			7.25 (tt, 1.9, 7.3)
7			6.90 (d, 12.5)
8			6.17 (d, 12.5)
4‴-Cin.-2,6		7.54 (dd, 2.1, 7.9)	
3,5		7.37 (ddd, 2.1, 7.9, 7.9)	
4		7.38 (tt, 2.1, 7.9)	
7		7.91 (d, 16.0)	
8		6.71 (d, 16.0)	
4′-*O*-Glc-1⁗	5.68 (d, 7.6)	5.64 (d, 7.6)	5.67 (d, 7.6)
2⁗	4.36 (dd, 7.6, 8.5)	4.33 (dd, 7.6, 9.1)	4.32 (dd, 7.6, 7.9)
3⁗	4.39 (m)	4.38 (m)	4.38 (m)
4⁗	4.37 (m)	4.36 (m)	4.36 (m)
5⁗	4.14 (m)	4.11 (m)	4.13 (m)
6⁗	4.41 (dd, 5.2, 11.9)	4.39 (dd, 4.8, 12.2)	4.41 (dd, 4.9, 12.2)
	4.56 (dd, 2.1, 11.9)	4.53 (dd, 2.1, 12.2)	4.54 (dd, 2.5, 12.2)
4″-*O*-Glc-1⁗′	5.65 (d, 7.6)	5.63 (d, 7.6)	5.63 (d, 7.6)
2⁗′	4.36 (dd, 7.6, 8.5)	4.33 (dd, 7.6, 9.1)	4.32 (dd, 7.6, 7.9)
3⁗′	4.39 (m)	4.38 (m)	4.38 (m)
4⁗′	4.37 (m)	4.36 (m)	4.36 (m)
5⁗′	4.14 (m)	4.11 (m)	4.13 (m)
6⁗′	4.41 (dd, 5.2, 11.9)	4.39 (dd, 4.8, 12.2)	4.41 (dd, 4.9, 12.2)
	4.54 (dd, 2.1, 11.9)	4.53 (dd, 2.1, 12.2)	4.54 (dd, 2.5, 12.2)

negative optical rotation ($[\alpha]_D^{24}$ −11.2° in MeOH). In the positive- and negative-ion FAB-MS of **3**, the quasimolecular ion peaks were observed at *m*/*z* 1083 $(M+Na)^+$ and *m*/*z* 1059 $(M-H)^-$, and the molecular formula $C_{51}H_{64}O_{24}$, which was the same as that of **2**, was determined by high resolution FAB-MS measurement. Acid hydrolysis of **3** with 1.0 M HCl liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[1,3,4,6—10,12] Treatment of **3** with tannase gave **2a**[1] and *cis*-cinnamic acid.[40,41] The proton and carbon signals in the ^{1}H- and ^{13}C-NMR data of **3** were very similar to those of **2**, except for the signals due to the *cis*-cinnamoyl group in the 2-*O*-glucosyl part. Namely, the ^{1}H- (pyridine-d_5, Table 2) and ^{13}C-NMR (Table 3) spectra[39] of **3** showed signals assignable to two methyls [δ 0.86, 0.91 (3H each, both d, J=6.4 Hz, 7,8-H$_3$)], four methylenes and a methine [δ 1.93, 2.01 (1H each, both m, 5-H$_2$), 2.01 (1H, m, 6-H), 3.35, 3.52 (1H each, both d, J=17.2 Hz, 3-H$_2$), 5.12, 5.20 (1H each, both d, J=12.2 Hz, 7″-H$_2$), 5.26, 5.30 (1H each, both d, J=12.2 Hz, 7′-H$_2$)], an acetyl group [δ 1.96 (3H, s, Ac-H)], two *ortho*-coupled A_2B_2 type aromatic protons [δ 7.37, 7.40 (2H each, both d, J=8.5 Hz, 3′,5′, 2′,6′-H), 7.41, 7.44 (2H each, both d, J=8.5 Hz, 3″,5″, 2″,6″-H)], and a *cis*-cinnamoyl ester moiety [δ 6.17, 6.90 (1H each, both d, J=12.5 Hz, Cin.-8, 7-H), 7.25 (1H, tt, J=1.9, 7.3 Hz, Cin.-4H), 7.30 (2H, ddd, J=1.9, 7.3, 7.6 Hz, Cin.-3,5-H), 7.90 (2H, dd, J=1.9, 7.6 Hz, Cin.-2,6-H)] together with three β-glucopyranosyl signals [δ 5.54 (1H, d, J=7.6 Hz, 2-*O*-Glc-1-H), 5.63 (1H, d, J=7.6 Hz, 4″-*O*-Glc-1-H), 5.67 (1H, d,

Table 3. ^{13}C-NMR (125 MHz, Pyridine-d_5) Data of Gymnosides VIII (**1**), IX (**2**), and X (**3**)

C-	1	2	3
1	172.3 (s)	172.8 (s)	172.7 (s)
2	80.3 (s)	81.0 (s)	81.2 (s)
3	43.7 (t)	42.3 (t)	42.0 (t)
4	170.8 (s)	170.8 (s)	170.8 (s)
5	48.6 (t)	46.9 (t)	46.7 (t)
6	23.9 (d)	24.0 (d)	24.1 (d)
7	23.3 (q)	23.9 (q)	23.9 (q)
8	24.7 (q)	24.5 (q)	24.5 (q)
1′	129.6 (s)	129.5 (s)	129.5 (s)
2′,6′	130.7 (d)	130.6 (d)	130.7 (d)
3′,5′	117.1 (d)	117.1 (d)	117.1 (d)
4′	158.7 (s)	158.7 (s)	158.7 (s)
7′	67.0 (t)	67.3 (t)	67.3 (t)
1″	129.7 (s)	129.7 (s)	129.7 (s)
2″,6″	130.6 (d)	130.6 (d)	130.6 (d)
3″,5″	117.1 (d)	117.0 (d)	117.0 (d)
4″	158.7 (s)	158.7 (s)	158.7 (s)
7″	66.6 (t)	66.5 (t)	66.5 (t)
2-*O*-Glc-1‴	98.4 (d)	100.0 (d)	99.9 (d)
2‴	75.7 (d)	75.5 (d)	73.1 (d)
3‴	74.9 (d)	75.6 (d)	79.2 (d)
4‴	71.0 (d)	72.0 (d)	69.0 (d)
5‴	74.6 (d)	72.3 (d)	74.7 (d)
6‴	63.7 (t)	63.3 (t)	63.8 (t)
3‴-Ac	21.3 (q)		
	170.4 (s)		
6‴-Ac	20.7 (q)	20.8 (q)	20.7 (q)
	171.1 (s)	170.7 (s)	170.7 (s)
3‴-Cin.-1			135.1 (s)
2,6			128.4 (d)
3,5			129.4 (d)
4			130.7 (d)
7			142.4 (d)
8			120.4 (d)
9			166.4 (s)
4‴-Cin.-1		134.8 (s)	
2,6		128.6 (d)	
3,5		129.3 (d)	
4		130.7 (d)	
7		145.5 (d)	
8		118.5 (d)	
9		166.4 (s)	
4′-*O*-Glc-1⁗	102.2 (d)	102.1 (d)	102.1 (d)
2⁗	74.9 (d)	74.9 (d)	75.0 (d)
3⁗	78.5 (d)	78.4 (d)	78.5 (d)
4⁗	71.3 (d)	71.2 (d)	71.3 (d)
5⁗	78.9 (d)	78.8 (d)	78.8 (d)
6⁗	62.4 (t)	62.3 (t)	62.3 (t)
4″-*O*-Glc-1⁗′	102.1 (d)	102.1 (d)	102.1 (d)
2⁗′	74.9 (d)	74.9 (d)	74.9 (d)
3⁗′	78.5 (d)	78.4 (d)	78.5 (d)
4⁗′	71.3 (d)	71.2 (d)	71.3 (d)
5⁗′	78.9 (d)	78.8 (d)	78.8 (d)
6⁗′	62.4 (t)	62.3 (t)	62.3 (t)

J=7.6 Hz, 4′-*O*-Glc-1-H)]. Finally, the positions of acyl moieties of **3** were elucidated on the basis of acylation shifts and HMBC experiment as shown in Fig. 1. Consequently, the structure of **3** was constructed to be 3‴-*O*-*cis*-cinnamoyl-6‴-*O*-acetyldactylorhin A.

DPPH Radical and $\cdot O_2^-$ Scavenging Activities 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.[42,43] When this compound accepts an electron or hydrogen radical to become 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 disulfate sodium salt (WST-1) in the present study.[44,45] As a part of our studies to characterize the bioactive components of natural medicines, we have investigated several antioxidants such as stilbenes,[46] anthraquinones,[46] and flavonoids.[45,47] As shown in Table 4, 1-(4-hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene-2,7-diol (**9**, SC_{50}=8.2 μM) and blestriarene A (**11**, 5.8 μM) exhibited scavenging activity on DPPH radical, whose activities were equipotent to that of (+)-catechin (6.0 μM). However, dihydrostilbene constituents from *G. conopsea* did not show DPPH radical scavenging activity (SC_{50}>40 μM). On the other hand, 4-methoxy-9,10-dihydrophenanthrene-2,7-diol (**8**, IC_{50}=0.95 μM), **9** (0.19 μM), and **11** (0.27 μM) showed potent $\cdot O_2^-$ scavenging activity and their activities were stronger than that of (+)-catechin (1.5 μM). These compounds (**8**, **9**, **11**) showed weak inhibition against the xanthine oxidase activity in the present conditions (IC_{50}=44.0, 30.5, and 4.5 μM, respectively). Furthermore, phenanthrene and dihydrostilbene constituents showed radical scavenging activities and suggested the following structural requirements on $\cdot O_2^-$ scavenging activity; a) phenanthrenes: 1) dihydrogenation at the 9,10-positions enhances the activities, 2) the 1 or 3-*p*-hydroxybenzyl groups enhances the activities; b) dihydrostilbenes: 1) methylation of the 3′-position reduces the activities, 2) the 2- and/or 6-*p*-hydroxybenzyl groups enhance the activities.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu UV-1600 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) and JNM-LA500 (500 MHz) spectrometers; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) and JNM-LA500 (125 MHz) spectrometers with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV–VIS detectors. HPLC column, YMC-Pack ODS-A (250×4.6 mm i.d.) and (250×20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

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); reverse-phase silica gel column chromatography, Diaion HP-20 (Nippon Rensui), Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); TLC, precoated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reverse phase); reverse-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Plant Material This item is described as that of previous report.[1,13]

Extraction and Isolation The methanolic extract (7.8%) from the tubers of *G. conopsea* was subjected to Diaion HP-20 column chromatography (H_2O→MeOH→acetone) to give H_2O-, MeOH-, and acetone-eluted fractions (6.4%, 1.3%, and 0.1%, respectively). Normal-phase silica gel column chromatography [*n*-hexane–EtOAc (5 : 1→1 : 1, v/v)→$CHCl_3$–MeOH–H_2O (10 : 3 : 1→7 : 3 : 1, lower layer→6 : 4 : 1, v/v/v)→MeOH] of the MeOH-eluted fraction (45.5 g) gave ten fractions [Fr. 1 (1.82 g), Fr. 2 (1.03 g), Fr. 3 (0.40 g), Fr. 4 (0.95 g), Fr. 5 (1.35 g), Fr. 6 (3.34 g), Fr. 7 (6.59 g), Fr. 8 (21.36 g), Fr. 9 (6.79 g), and Fr. 10 (1.87 g)], as described previously.[1,13] From fraction 3—5, phenanthrenes and stilbenes were isolated.[13] Fraction 2 (1.03 g) was subjected to reversed-phase silica gel column chromatography [30 g, MeOH–H_2O (50 : 50→70 : 30, v/v)→MeOH] to give three fractions [Fr. 2-1 (627 mg), Fr. 2-2 (63 mg), and Fr. 2-3 (324 mg)]. Fraction 2-1 (627 mg) was separated by HPLC [MeOH–H_2O (40 : 60, v/v)] to give

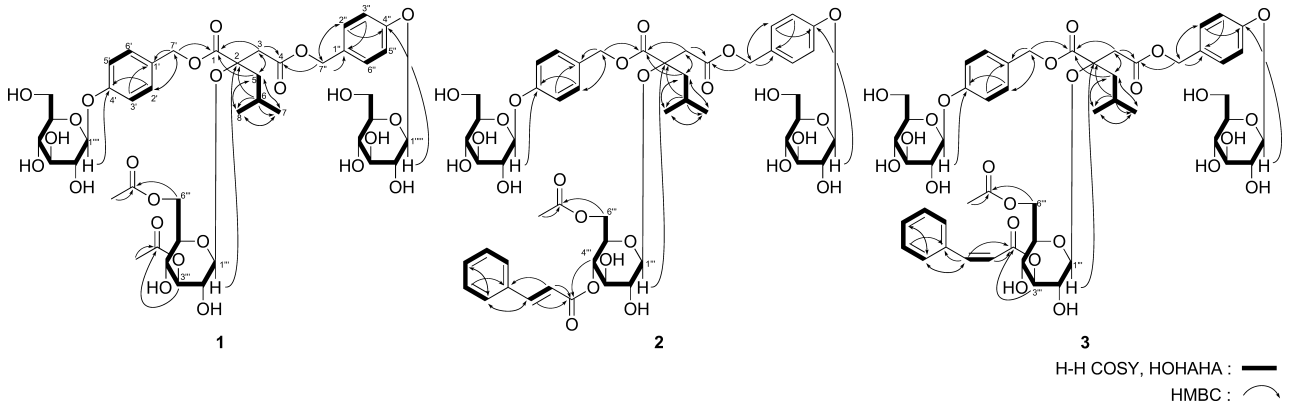

Fig. 1

Table 4. DPPH Radical and $^{\cdot}O_2^-$ Scavenging Activities and Xanthine Oxidase Inhibitory Activity of the Phenolic Constituents from the Tubers of *G. conopsea*

	DPPH radical SC_{50} (μM)[a]	$^{\cdot}O_2^-$	
		Formozan formation IC_{50} (μM)	Xanthine oxidase IC_{50} (μM)
Phenanthrenes			
Gymconopin A (**5**)	29.2	45.8	>100 (23.1%)[b]
Gymconopin B (**6**)	33.4	21.5	>100 (40.5%)[b]
2-Methoxy-9,10-dihydrophenanthrene-4,5-diol (**7**)	31.2	>100 (26.9%)[b]	—
4-Methoxy-9,10-dihydrophenanthrene-2,7-diol (**8**)	12.7	0.95	44.0
1-(4-Hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene-2,7-diol (**9**)	8.2	0.19	30.5
1-(4-Hydroxybenzyl)-4-methoxyphenanthrene-2,7-diol (**10**)	15.7	9.4	>100 (44.4%)[b]
Blestriarene A (**11**)	5.8	0.27	4.5
Dihydrostilbenes			
Batatacin III (**12**)	>40 (9.8%)[c]	82.8	>100 (13.2%)[b]
3′,5-Dihydroxy-2-(4-hydroxybenzyl)-3-methoxybibenzyl (**13**)	>40 (23.0%)[c]	9.3	72.9
3,3′-Dihydroxy-2-(4-hydroxybenzyl)-5-methoxybibenzyl (**14**)	>40 (43.0%)[c]	13.4	45.1
3,3′-Dihydroxy-2,6-bis(4-hydroxybenzyl)-5-methoxybibenzyl (**15**)	>40 (29.6%)[c]	13.4	65.2
α-Tocopherol	11.0	—	—
(+)-Catechin	6.0	1.5	>10 (−7%)[d]

a) Concentration required for 50% reduction of 40 μM DPPH radical. *b*) Values in parentheses represent the inhibition (%) at 100 μM. *c*) Values in parentheses represent the inhibition (%) at 40 μM. *d*) Values in parentheses represent the inhibition (%) at 10 μM.

syringol (10 mg, 0.0003%), 4-hydroxybenzaldehyde (78 mg, 0.0022%), 4-hydroxybenzyl methyl ether (279 mg, 0.0078%), and 4-hydroxybenzyl ethyl ether (85 mg, 0.0024%). Fraction 4 (0.95 g) was subjected to reversed-phase silica gel column chromatography [30 g, MeOH–H_2O (30 : 70→50 : 50→70 : 30, v/v)→MeOH] to give seven fractions [Fr. 4-1 (263 mg), Fr. 4-2 (49 mg), Fr. 4-3 (81 mg), Fr. 4-4 (169 mg), Fr. 4-5 (38 mg), Fr. 4-6=gymconopin A (**5**, 24 mg, 0.0007%), and Fr. 4-7 (326 mg)].[13] Fraction 4-1 (263 mg) was separated by HPLC [MeOH–H_2O (30 : 70, v/v)] to yield 5-hydroxymethylfuraldehyde (16 mg, 0.0004%), pyrocatechol (3 mg, 0.0001%), 4-hydroxybenzyl alcohol (95 mg, 0.0027%), 4-hydroxybenzoic acid (33 mg, 0.0009%), and vanillic acid (38 mg, 0.0011%). Fraction 4-2 (49 mg) was separated by HPLC [MeOH–H_2O (40 : 60, v/v)] to provide *p*-coumaric acid (20 mg, 0.0006%). Fraction 4-4 (169 mg) was separated by HPLC [MeOH–H_2O (55 : 45, v/v)] to give blestriarenes B (4 mg, 0.0001%) and C (2 mg, 0.0001%) together with 1-(4-hydroxybenzyl)-4-methoxyphenanthrene-2,7-diol (**10**, 12 mg, 0.0003%), blestriarene A (**11**, 15 mg, 0.0004%), 3′,5-dihydroxy-2-(4-hydroxybenzyl)-3-methoxybibenzyl (**13**, 33 mg, 0.0009%), 3,3′-dihydroxy-2-(4-hydroxybenzyl)-5-methoxybibenzyl (**14**, 28 mg, 0.0008%), and 3,3′-dihydroxy-2,6-bis(4-hydroxybenzyl)-5-methoxybibenzyl (**15**, 9 mg, 0.0004%).[13] Fraction 4-7 (326 mg) was separated by HPLC [MeOH–H_2O (60 : 40, v/v)] to provide 3,3′-dihydyoxy-4-(4-hydroxybenzyl)-5-methoxybibenzyl (9 mg, 0.0003%) and bulbocodins C (4 mg, 0.0001%) and D (4 mg, 0.0001%). Fraction 5 (1.35 g) was subjected to reversed-phase silica gel column chromatography [40 g, MeOH–H_2O (50 : 50→70 : 30, v/v)→MeOH] to furnish six fractions [Fr. 5-1 (441 mg), Fr. 5-2 (149 mg), Fr. 5-3 (117 mg), Fr. 5-4 (90 mg), Fr. 5-5 (237 mg), and Fr. 5-6 (316 mg)].[1,13] Fraction 5-1 (440 mg) was separated by HPLC [MeOH–H_2O (30 : 70, v/v)] to give (−)-pinoresinol (8 mg, 0.0002%), syringaldehyde (7 mg, 0.0002%), 4-hydroxybenzyl methyl ether (4 mg, 0.0001%), bis(4-hydroxybenzyl) ether (9 mg, 0.0003%), and ferulic acid (6 mg, 0.0002%). Fraction 5-5 (237 mg) was separated by HPLC [MeOH–H_2O (65 : 35, v/v)] to yield 5-*O*-methylbatatacin III (5 mg, 0.0001%) and bulbocol (8 mg, 0.0003%) together with gymconopin B (**6**, 10 mg, 0.0003%).[1] Fraction 6 (3.34 g) was subjected to reversed-phase silica gel column chromatography [100 g, MeOH–H_2O (20 : 80→30 : 70→50 : 50, v/v)→MeOH] to furnish six fractions [Fr. 6-1 (920 mg), Fr. 6-2 (384 mg), Fr. 6-3 (352 mg), Fr. 6-4 (772 mg), Fr. 6-5 (280 mg), and Fr. 6-6 (598 mg)].[1] Fraction 6-1 (920 mg) was separated by HPLC [MeOH–H_2O (25 : 75, v/v)] to give 2-*C*-(4-hydroxybenzyl)-α-L-*xylo*-3-ketohexulofuranosono-1,4-lactone (27 mg, 0.0008%), 2,4-dihydroxybenzaldehyde (12 mg, 0.0003%), 4-formylphenyl β-D-glucopyranoside (14 mg, 0.0004%), benzyl β-D-glucopyranoside (23 mg, 0.0007%), phenyl β-D-glucopyranoside (7 mg, 0.0002%), 4-methoxyphenyl β-D-glucopyranoside (12 mg, 0.0003%), 4-hydroxylmethylphenyl β-D-glucopyranoside (17 mg, 0.0005%), dactyloses A (20 mg, 0.0006%) and B (11 mg, 0.0003%), and thymidine (5 mg, 0.0005%). Fraction 6-2 (384 mg) was subjected to HPLC [MeOH–H_2O (25 : 75, v/v)] to afford 4-methylphenyl β-D-glucopyranoside (26 mg, 0.0007%). Fraction 6-3 (352 mg) was separated by HPLC [MeOH–H_2O (30 : 70, v/v)] to give 4-hydroxybenzyl methyl ether (11 mg, 0.0003%). Fraction 7 (6.59 g) was subjected to reversed-phase silica gel column chromatography [200 g, MeOH–H_2O (10 : 90→30 : 70→50 : 50→70 : 30, v/v)→MeOH] to give seven fractions [Fr. 7-1 (1390 mg), Fr. 7-2 (370 mg), Fr. 7-3 (3260 mg), Fr. 7-4 (190 mg), Fr. 7-5 (380 mg), Fr. 7-6 (840 mg), and Fr. 7-7 (480 mg)]. Fraction 7-1 (530 mg) was separated by HPLC [MeOH–H_2O (10 : 90, v/v)] to give 4-hydroxybenzyl β-D-glucopyranoside (12 mg, 0.0009%). Fr. 7-3 (710 mg) was separated by HPLC [MeOH–H_2O (50 : 50, v/v)] to give militarine

(557 mg, 0.072%). Fr. 7-6 (840 mg) was separated by HPLC [MeOH–H_2O (65 : 35, v/v)] to give gymnosides IX (**2**, 450 mg, 0.013%) and X (**3**, 17 mg, 0.0005%) together with gymnosides IV (23 mg, 0.0006%), V (47 mg, 0.0014%), VI (14 mg, 0.0004%), and VII (89 mg, 0.0025%).[1)] Fraction 8 (21.36 g) was subjected to reversed-phase silica gel column chromatography [640 g, MeOH–H_2O (10 : 90→30 : 70→50 : 50→70 : 30, v/v)→MeOH] to give nine fractions [Fr. 8-1 (4240 mg), Fr. 8-2 (3260 mg), Fr. 8-3 (4170 mg), Fr. 8-4 (4710 mg), Fr. 8-5 (1050 mg), Fr. 8-6 (290 mg), Fr. 8-7 (440 mg), Fr. 8-8 (130 mg), and Fr. 8-9 (3140 mg)]. Fraction 8-2 (502 mg) was separated by HPLC [MeOH–H_2O (40 : 50, v/v)] to give loroglossin (60 mg, 0.0110%) and dactylorhin B (112 mg, 0.021%). Fraction 8-6 (290 mg) was separated by HPLC [MeOH–H_2O (52 : 48, v/v)] to give gymnoside VIII (**1**, 9 mg, 0.0003%).

The known compounds were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-, ^{13}C-NMR, MS) with reported values[14—19,21—38)] or commercial samples.[20)]

Gymnoside VIII (**1**): A white powder, $[\alpha]_D^{24}$ −37.3° (c=0.39, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{44}H_{60}O_{24}Na$ $(M+Na)^+$ 995.3372; Found 995.3369. UV [MeOH, nm (log ε)]: 224 (4.36), 271 (3.41). IR (KBr): 3432, 2962, 1734, 1617, 1516, 1458, 1237, 1076 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: given in Table 2. ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 3. Positive-ion FAB-MS: m/z 995 $(M+Na)^+$. Negative-ion FAB-MS: m/z 971 $(M-H)^-$.

Gymnoside IX (**2**): A white powder, $[\alpha]_D^{24}$ −26.5° (c=1.61, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{51}H_{64}O_{24}Na$ $(M+Na)^+$ 1083.3685; Found 1083.3690. UV [MeOH, nm (log ε)]: 224 (4.56), 278 (4.36). IR (KBr): 3432, 2962, 1734, 1636, 1516, 1235, 1075 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: given in Table 2. ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 3. Positive-ion FAB-MS: m/z 1083 $(M+Na)^+$. Negative-ion FAB-MS: m/z 1059 $(M-H)^+$.

Gymnoside X (**3**): A white powder, $[\alpha]_D^{24}$ −11.2° (c=1.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{51}H_{64}O_{24}Na$ $(M+Na)^+$ 1083.3685; Found 1083.3690. UV [MeOH, nm (log ε)]: 223 (4.47), 262 (4.12), 271 (4.09). IR (KBr): 3432, 2928, 1736, 1638, 1514, 1235, 1076 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: given in Table 2. ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 3. Positive-ion FAB-MS: m/z 1083 $(M+Na)^+$. Negative-ion FAB-MS: m/z 1059 $(M-H)^-$.

Acid Hydrolysis of Gymnosides VIII (1), IX (2), and X (3) A solution of **1**—**3** (each 1.5 mg) in 1 M HCl (0.5 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was poured into ice-water and neutralized with Amberlite IRA-400 (OH^- form), and the resin was removed by filtration. Then, the filtrate was extracted with EtOAc. The aqueous layer was subjected to HPLC analysis under the following conditions: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d.×250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; mobile phase, CH_3CN–H_2O (75 : 25, v/v); flow rate 0.8 ml/min; column temperature, room temperature. Identification of D-glucose present in the aqueous layer was carried out by comparison of its retention time and optical rotation with those of an authentic sample, t_R: 12.3 min (positive optical rotation).

Acetylation of Gymnoside VIII (1) and Dactylorhin A (4) A solution of **1** (2.5 mg, 0.0026 mmol) in pyridine (1.0 ml) was treated with acetic anhydride (Ac_2O, 0.5 ml) and the mixture was stirred at room temperature for 12 h. The reaction mixture was poured into ice-water and the whole was extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder and filtrated. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by silica gel column chromatography [3 g, $CHCl_3$–MeOH–H_2O (6 : 4 : 1, v/v)] to give **1a** (2.7 mg, 75%). Through the similar procedure, **1a** (8.4 mg, 82%) was obtained from **4** (6.5 mg, 0.0073 mmol).

1a: A white powder, $[\alpha]_D^{23}$ −12.2° (c=0.31, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{64}H_{80}O_{34}Na$ $(M+Na)^+$ 1415.4429; Found 1415.4435. UV [MeOH, nm (log ε)]: 222 (4.43), 270 (3.47), 276 (3.43). IR (KBr): 2959, 1755, 1613, 1514, 1368, 1227, 1040 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.86, 0.87 (3H each, d, J=6.7 Hz, 7,8-H_3), 1.77, 1.81 (1H each, m, 5-H_2), 1.98 (1H, m, 6-H), 2.03—2.13 (36H, Ac), 3.27, 3.35 (1H each, d, J=17.8 Hz, 3-H_2), 5.16, 5.27 (1H each, d, J=11.9 Hz, 7″-H_2), 5.29, 5.38 (1H each, d, J=11.9 Hz, 7′-H_2), 5.77, 5.78, 5.78 (1H each, all d, J=7.6 Hz, 1‴,1⁗,1⁗′-H), 7.30, 7.33 (2H each, d, J=8.6 Hz, 3′,5′, 3″,5″-H), 7.49, 7.52 (2H each, d, J=8.6 Hz, 2′,6′, 2″,6″-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: 20.5—20.7 (all q, $\underline{C}H_3CO$), 23.2 (q, 7-C), 23.9 (d, 6-C), 24.6 (q, 8-C), 43.8 (3-C), 48.8 (5-C), 61.9, 62.3, 62.4 (all t, 6‴,6⁗,6⁗′-C), 66.4, 66.9 (both t, 7′,7″-C), 68.8, 68.9, 69.0 (all d, 4‴,4⁗,4⁗′-C), 71.6, 71.9, 71.9 (all d, 2‴,2⁗,2⁗′-C), 72.5 (all d, 5‴,5⁗,5⁗′-C), 73.3 (all d, 3‴,3⁗,3⁗′-C), 80.6 (s, 2-C), 97.9, 99.0, 99.2 (all d, 1‴,1⁗,1⁗′-C), 117.3, 117.4 (both d, 3′,5′,3″,5″-C), 130.8, 1310. (both d, 2′,6′,2″,6″-C), 130.9, 130.9 (both s, 1′,1″-C), 157.6 (both s, 4′,4″-C), 169.7—170.5 (all s, $CH_3\underline{C}O$), 171.1 (s, 1-C), 173.8 (4-C). Positive-ion FAB-MS: m/z 1415 $(M+Na)^+$.

Enzymatic Hydrolysis of Gymnosides IX (2) and X (3) with Tannase A solution of **2** (15.3 mg, 0.0144 mmol) or **3** (5.7 mg, 0.0054 mmol) in H_2O (2.0 ml) was treated with tannase (10 or 4 mg, from *Aspergillus oryzae*, Wako Pure Chemical Ind., Ltd., Japan) and the solution was stirred at 37 °C for 24 h. After EtOH was added to the reaction mixture, the solvent was removed under reduced pressure and the residue was purified by HPLC [MeOH–1% aqueous AcOH (45 : 55, v/v)] to furnish **2a**[1)] (4.5 mg, 79% from **2**, 1.6 mg, 75% from **3**), *trans*-cinnamic acid[20)] (1.3 mg, 61% from **2**), and *cis*-cinnamic acid[40,41)] (0.4 mg, 50% from **3**).

Bioassay. DPPH Radical Scavenging Activity The free radical scavenging activity was assessed using the DPPH radical.[42,43)] An ethanol solution of DPPH (100 μM, 1.0 ml) was mixed with different concentrations of each test compound (0—200 μM, 0.5 ml) 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 The assay method for superoxide dismutase described by Ukeda *et al.*[44)] was used with a slight modification.[45)] Briefly, a reaction mixture containing 100 μM xanthine, 100 μM EDTA, 25 μM WST-1, and *ca*. 1.9 mU/ml xanthine oxidase in 50 mM sodium carbonate buffer (pH 10.2) was incubated with or without each test sample for 20 min at 37 °C (total volume: 3.0 ml). After incubation, the solution was mixed with 0.1 ml of 2 M HCl to stop the reaction. The formazan formation was monitored at 450 nm. Measurements were performed in duplicate, and the concentration required for a 50% inhibition (IC_{50}) of the WST-1 formazan formation was determined graphically.

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References and Notes

1) Part XVI: Morikawa T., Xie H., Matsuda H., Yoshikawa M., *J. Nat. Prod.*, **69** (2006), in press.
2) Xie H., Wang T., Matsuda H., Morikawa T., Yoshikawa M., Tani T., *Chem. Pharm. Bull.*, **53**, 1416—1422 (2005).
3) Morikawa T., Sun B., Matsuda H., Wu L. J., Harima S., Yoshikawa M., *Chem. Pharm. Bull.*, **52**, 1194—1199 (2004).
4) Sun B., Morikawa T., Matsuda H., Tewtrakul S., Harima S., Yoshikawa M., *J. Nat. Prod.*, **67**, 1464—1469 (2004).
5) Yoshikawa M., Morikawa T., Kashima Y., Ninomiya K., Matsuda H., *J. Nat. Prod.*, **66**, 922—927 (2003).
6) Tao J., Morikawa T., Ando S., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **51**, 654—662 (2003).
7) Morikawa T., Tao J., Ando S., Matsuda H., Yoshikawa M., *J. Nat. Prod.*, **66**, 638—645 (2003).
8) Matsuda H., Pongpiriyadacha Y., Morikawa T., Kishi A., Kataoka S., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **13**, 1101—1106 (2003).
9) Tao J., Morikawa T., Toguchida I., Ando S., Matsuda H., Yoshikawa M., *Bioorg. Med. Chem.*, **10**, 4005—4012 (2002).
10) Morikawa T., Matsuda H., Ueda K., Yoshikawa M., *J. Nat. Prod.*, **65**, 1468—1474 (2002).
11) Matsuda H., Morikawa T., Tao J., Ueda K., Yoshikawa M., *Chem. Pharm. Bull.*, **50**, 208—215 (2002).
12) Muraoka O., Fujimoto M., Tanabe G., Kubo M., Minematsu T., Matsuda H., Morikawa T., Toguchida I., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **11**, 2217—2220 (2001).
13) Matsuda H., Morikawa T., Xie H., Yoshikawa M., *Planta Med.*, **70**, 847—855 (2004).
14) Kizu H., Kaneko E., Tomimori T., *Chem. Pharm. Bull.*, **47**, 1618—1625 (1999).
15) Yamaki M., Bai L., Inoue K., Takagi S., *Phytochemistry*, **28**, 3503—3505 (1989).
16) 5-*O*-Methylbatatacin III: A white powder. ^{1}H-NMR (270 MHz, CD_3OD) δ: 2.80 (4H, s, α, β-H_2), 3.71 (6H, s, 3,5-OCH_3), 6.27 (1H, dd, J=2.2, 2.2 Hz, 6-H), 6.32 (2H, dd, J=2.2, 2.2 Hz, 2,4-H), 6.58

(1H, ddd, J=2.4, 2.4, 7.6 Hz, 4′-H), 6.60 (1H, dd, J=2.4, 2.4 Hz, 2′-H), 6.64 (1H, ddd, J=2.4, 2.4, 7.6 Hz, 6′-H), 7.05 (1H, dd, J=7.6, 7.6 Hz, 5′-H). ^{13}C-NMR (68 MHz, CD_3OD) δ_C: 38.8, 39.2 (both d, α, β-C), 55.5 (q, 3,5-OCH_3), 98.8 (d, 4-C), 107.3 (d, 2,6-C), 113.6 (d, 4′-C), 116.2 (d, 2′-C), 120.7 (d, 6′-C), 130.0 (d, 5′-C), 144.4 (s, 1′-C), 145.2 (s, 1-C), 158.1 (s, 3,5-C), 161.9 (s, 3′-C). EI-MS: m/z (%): 244 (M^+, 28), 137 (100), 107 (59).

17) Bai L., Masukawa N., Yamaki M., Takagi S., *Phytochemistry*, **48**, 327—331 (1998).
18) Bai L., Masukawa N., Yamaki M., Takagi S., *Phytochemistry*, **47**, 1637—1640 (1998).
19) Bai L., Kato T., Inoue K., Yamaki M., Takagi S., *Phytochemistry*, **33**, 1481—1483 (1993).
20) The known compounds were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR, MS] with commercial samples.
21) Ternai B., Markham K. R., *Tetrahedron*, **32**, 565—569 (1976).
22) Casabuono A. C., Pomilio A. B., *Phytochemistry*, **35**, 479—483 (1994).
23) (−)-Pinoresinol: A white powder. $[\alpha]_D^{29}$ −13.8° (c=0.38, MeOH). ^{1}H-NMR (500 MHz, CD_3OD) δ: 3.14 (2H, m, 1,5-H), 3.84 (2H, dd, J=4.0, 9.2 Hz, 4,8-H), 3.85 (6H, s, 3′,3″-OCH_3), 4.22 (2H, dd, J=7.0, 9.2 Hz, 4,8-H), 4.70 (2H, d, J=4.6 Hz, 2,6-H), 6.77 (2H, d, J=8.2 Hz, 5′,5″-H), 6.81 (2H, dd, J=1.8, 8.2 Hz, 6′,6″-H), 6.95 (2H, d, J=1.8 Hz, 2′,2″-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: 55.4 (d, 1,5-C), 56.4 (q, 3′,3″-OCH_3), 72.6 (t, 4,8-C), 87.6 (d, 2,6-C), 111.0 (d, 2′,2″-C), 116.1 (d, 5′,5″-C), 120.1 (d, 6′,6″-C), 133.8 (s, 1′,1″-C), 147.4 (s, 4′,4″-C), 149.3 (s, 3′,3″-C). EI-MS: m/z (%): 358 (M^+, 44), 151 (100).
24) Fujita M., Nagai M., Inoue T., *Chem. Pharm. Bull.*, **30**, 1151—1156 (1982).
25) Fujita M., Yamada M., Nakajima S., Kawai K., Nagai M., *Chem. Pharm. Bull.*, **32**, 2622—2627 (1984).
26) Taguchi H., Yosioka I., Yamasaki K., Kim I. H., *Chem. Pharm. Bull.*, **29**, 55—62 (1981).
27) Galetto W. G., Hoffman P. G., *J. Agric. Food Chem.*, **26**, 195—197 (1978).
28) 2,4-Dihydroxybenzaldehyde: A white powder. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 7.31 (1H, br d, J=7.1 Hz, 5-H), 7.52 (1H, d, J=7.1 Hz, 6-H), 7.90 (1H, br s, 3-H), 10.01 (1H, s, CHO). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: 115.4 (d, 3-C), 116.4 (d, 5-C), 125.3 (d, 6-C), 130.2 (s, 1-C), 148.0 (s, 2-C), 154.3 (s, 4-C), 191.0 (d, CHO). EI-MS: m/z (%): 138 (M^+, 90), 137 (100).
29) Nagata C., Hasegawa T., Tanaka S., *J. Chem. Soc. Jpn.*, **1977**, 1087—1090 (1977).
30) Morgan K. R., Newman R. H., *J. Am. Chem. Soc.*, **112**, 4—7 (1990).
31) Phenyl β-D-glucopyranoside: A white powder. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 4.14 (1H, m, 5′-H), 4.34 (1H, m, 2′-H), 4.37 (1H, m, 4′-H), 4.39 (1H, m, 3′-H), [4.42 (1H, dd, J=5.2, 11.9 Hz), 4.56 (1H, dd, J=2.4, 11.9 Hz), 6′-H_2], 5.65 (1H, d, J=7.3 Hz, 1′-H), 7.01 (1H, t, J=7.3 Hz, 4-H), 7.30 (2H, dd, J=7.3, 7.6 Hz, 3,5-H), 7.39 (2H, d, J=7.6 Hz, 2,6-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: 62.4 (t, 6′-C), 71.3 (d, 4′-C), 75.0 (d, 2′-C), 78.6 (d, 3′-C), 78.9 (d, 5′-C), 102.2 (d, 1′-C), 117.0 (d, 2,6-C), 122.2 (d, 4-C), 129.9 (d, 3,5-C), 158.7 (s, 1-C). Positive-ion FAB-MS: m/z 279 $(M+Na)^+$.
32) 4-Methylphenyl β-D-glucopyranoside: A white powder. ^{1}H-NMR (270 MHz, pyridine-d_5) δ: 2.16 (3H, s, 4-CH_3), 4.11 (1H, m, 5′-H), 4.33 (1H, m, 2′-H), 4.35 (1H, m, 4′-H), 4.37 (1H, m, 3′-H), [4.42 (1H, dd, J=4.9, 11.9 Hz), 4.56 (1H, dd, J=2.1, 11.9 Hz), 6′-H_2], 5.62 (1H, d, J=7.1 Hz, 1′-H), 7.08 (2H, d, J=8.2 Hz, 3,5-H), 7.31 (2H, d, J=8.2 Hz, 2,6-H). ^{13}C-NMR (68 MHz, pyridine-d_5) δ_C: 20.4 (q, 4-CH_3), 62.3 (t, 6′-C), 71.2 (d, 4′-C), 74.9 (d, 2′-C), 78.4 (d, 3′-C), 78.7 (d, 5′-C), 102.2 (d, 1′-C), 116.7 (d, 2,6-C), 130.1 (d, 3,5-C), 131.0 (s, 4-C), 156.4 (s, 1-C). Positive-ion FAB-MS: m/z 293 $(M+Na)^+$.
33) 4-Formylphenyl β-D-glucopyranoside: A white powder. ^{1}H-NMR (270 MHz, pyridine-d_5) δ: 4.20 (1H, m, 5′-H), 4.35 (1H, m, 2′-H), 4.37 (1H, m, 4′-H), 4.38 (1H, m, 3′-H), [4.41 (1H, dd, J=5.4, 11.9 Hz), 4.57 (1H, dd, J=2.2, 11.9 Hz), 6′-H_2], 5.75 (1H, d, J=6.8 Hz, 1′-H), 7.40 (2H, d, J=8.6 Hz, 3,5-H), 7.88 (2H, d, J=8.6 Hz, 2,6-H), 9.97 (1H, s, CHO). ^{13}C-NMR (68 MHz, pyridine-d_5) δ_C: 62.2 (t, 6′-C), 71.0 (d, 4′-C), 74.7 (d, 2′-C), 78.3 (d, 3′-C), 79.1 (d, 5′-C), 101.4 (d, 1′-C), 116.8 (d, 2,6-C), 131.2 (s, 4-C), 131.8 (d, 3,5-C), 162.9 (s, 1-C), 190.5 (d, CHO). Positive-ion FAB-MS: m/z 307 $(M+Na)^+$.
34) 4-Methoxyphenyl β-D-glucopyranoside: A white powder. ^{1}H-NMR (270 MHz, pyridine-d_5) δ: 3.64 (3H, s, OCH_3), 4.12 (1H, m, 5′-H), 4.35 (1H, m, 2′-H), 4.37 (1H, m, 4′-H), 4.38 (1H, m, 3′-H), [4.40 (1H, dd, J=5.1, 11.9 Hz), 4.57 (1H, dd, J=2.0, 11.9 Hz), 6′-H_2], 5.56 (1H, d, J=6.9 Hz, 1′-H), 6.92 (2H, d, J=9.1 Hz, 3,5-H), 7.38 (2H, d, J=8.6 Hz, 2,6-H). ^{13}C-NMR (68 MHz, pyridine-d_5) δ_C: 55.4 (q, OCH_3), 62.3 (t, 6′-C), 71.2 (d, 4′-C), 74.9 (d, 2′-C), 78.4 (d, 3′-C), 78.8 (d, 5′-C), 103.1 (d, 1′-C), 114.8 (d, 3,5-C), 118.1 (d, 2,6-C), 152.5 (s, 1-C), 154.6 (s, 4-C). Positive-ion FAB-MS: m/z 309 $(M+Na)^+$.
35) Miyase T., Ueno A., Takizawa N., Kobayashi H., Karasawa H., *Chem. Pharm. Bull.*, **35**, 1109—1117 (1987).
36) 4-Hydroxybenzyl β-D-glucopyranoside: A white powder. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 3.99 (1H, m, 5′-H), 4.12 (1H, dd, J=7.9, 8.6 Hz, 2′-H), 4.27 (1H, m, 4′-H), 4.29 (1H, m, 3′-H), [4.44 (1H, dd, J=5.5, 11.9 Hz), 4.61 (1H, dd, J=2.4, 11.9 Hz), 6′-H_2], 4.82, 5.15 (1H each, both d, J=11.3 Hz, 7-H_2), 5.02 (1H, d, J=7.9 Hz, 1′-H), 7.16, 7.49 (2H each, both d, J=8.5 Hz, 3,5- and 2,6-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: 62.9 (t, 6′-C), 71.0 (t, 7-C), 71.8 (d, 4′-C), 75.3 (d, 2′-C), 78.6 (d, 3′-C), 78.6 (d, 5′-C), 103.7 (d, 1′-C), 116.1 (d, 3,5-C), 129.2 (s, 1-C), 130.5 (d, 2,6-C), 158.6 (s, 4-C). Positive-ion FAB-MS: m/z 309 $(M+Na)^+$.
37) Thymidine: Colorless needles. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 1.88 (1H, d, J=0.9 Hz, 5-CH_3), 2.66 (2H, m, 2′-H), 4.14, 4.24 (1H each, both dd, J=3.1, 11.6 Hz, 5′-H_2), 4.48 (1H, m, 4′-H), 7.05 (1H, t, J=6.7 Hz, 1′-H), 8.16 (1H, d, J=1.2 Hz, 6-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: 12.8 (q, 5-CH_3), 41.4 (t, 2′-C), 62.3 (t, 5′-C), 71.5 (d, 3′-C), 85.3 (d, 1′-C), 88.9 (d, 4′-C), 110.5 (s, 5-C), 136.7 (d, 6-C), 152.0 (s, 2-C), 165.0 (s, 4-C). EI-MS: m/z (%): 242 (M^+, 1), 126 (100).
38) Hearn M. T. W., *Aust. J. Chem.*, **29**, 107—113 (1976).
39) The ^{1}H- and ^{13}C-NMR spectra of **1**—**3** were assigned with the aid of distortionless enhancement by polarization transfer (DEPT), double quantum filter correlation spectroscopy (DQF COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond connectivity (HMBC), and homo- and heteronuclear Hartmann-Hahn spectroscopy (^{1}H–^{1}H, ^{13}C–^{1}H HOHAHA) experiments.
40) Chalonar P. A., *J. Chem. Soc. Perkin Trans. II*, **1980**, 1028—1032 (1980).
41) *cis*-Cinnamic acid: A white powder. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 6.32 (1H, d, J=12.5 Hz, 8-H), 7.28 (1H, tt, J=1.9, 7.3 Hz, 4-H), 7.35 (2H, br dd, J= *ca.* 7, 7 Hz, 3,5-H), 7.60 (2H, dd, J=1.9, 7.3 Hz, 2,6-H), 7.91 (1H, d, J=12.5 Hz, 7-H). EI-MS: m/z (%): 148 (M^+, 50), 147 (100), 131 (28), 103 (65), 91 (12), 77 (40).
42) Uchiyama M., Suzuki Y., Fukuzawa K., *Yakugaku Zasshi*, **88**, 678—683 (1968).
43) Blois M. S., *Nature* (London), **181**, 1199—1200 (1958).
44) Ukeda H., Kawana D., Maeda S., Sawamura M., *Biosci. Biotechnol. Biochem.*, **63**, 485—488 (1999).
45) Matsuda H., Morikawa T., Ishiwada T., Managi H., Kagawa M., Higashi Y., Yoshikawa M., *Chem. Pharm. Bull.*, **51**, 440—443 (2003).
46) Matsuda H., Morikawa T., Toguchida I., Park J.-Y., Harima S., Yoshikawa M., *Bioorg. Med. Chem.*, **9**, 41—50 (2001).
47) Matsuda H., Wang T., Managi H., Yoshikawa M., *Bioorg. Med. Chem.*, **11**, 5317—5323 (2003).