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Glucosyloxybenzyl 2-Isobutylmalates from the Tubers of *Gymnadenia conopsea*[1]

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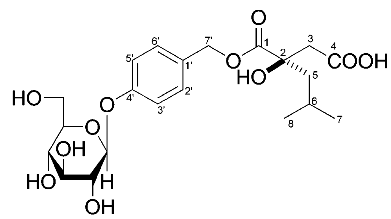
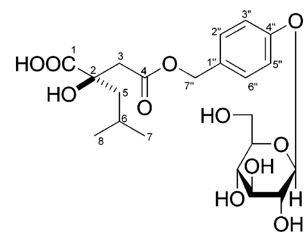
Seven new glucosyloxybenzyl 2-isobutylmalates, gymnosides I−VII (**1**−**7**), were isolated from the tubers of *Gymnadenia conopsea*. The structures of **1**−**7** were determined on the basis of chemical and physicochemical evidence.

Gymnadenia conopsea R. Br., an Orchidaceae perennial herb, is widely distributed in the northern parts of China such as Hebei, Liaoning, and Gansu Provinces. The tubers of this herb have been used in traditional Chinese medicine for the treatment of asthma, neurasthenia, and chronic hepatitis.[2] Recently, we found that the MeOH extract of this natural medicine showed antiallergic activity in the passive cutaneous anaphylaxis (PCA) reactions in mice.[3] In the course of our characterization studies on bioactive constituents from Chinese natural medicines,[1,4−10] we previously reported the structures of three dihydrophenanthrenes, gymconopins A−C, and a dihydrostilbene, gymconopin D, as the less polar constituents from the tubers of *G. conoposa*, together with 10 known constituents and their inhibitory activities on antigen-induced degranulation in RBL-2H3 cells.[3] As a continuing study of this natural medicine, we additionally isolated seven new glycosyloxybenzyl 2-isobutylmalates named gymnosides I−VII (**1**−**7**). This paper deals with the isolation and elucidation of absolute configuration of the glycosides **1**−**7**.

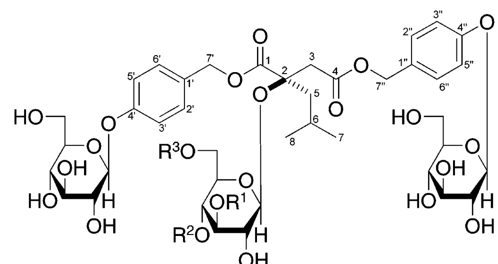
Chart 1



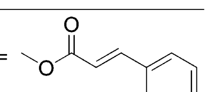
gymnoside I (**1**)



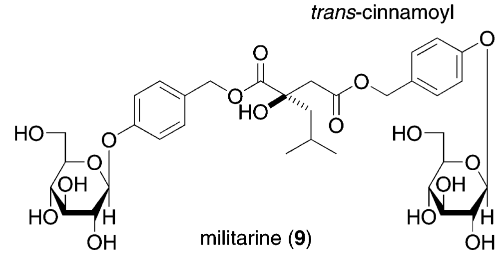
gymnoside II (**2**)



	R1	R2	R3
gymnoside III (**3**):	H	H	Ac
gymnoside IV (**4**):	Cin.	H	H
gymnoside V (**5**):	H	Cin.	H
gymnoside VI (**6**):	H	H	Cin.
gymnoside VII (**7**):	Cin.	H	Ac
dactylorhin A (**8**):	H	H	H

Cin. = 

trans-cinnamoyl



militarine (**9**)

Results and Discussion

The tubers of *G. conoposa* were extracted with MeOH under reflux. The MeOH extract was subjected to Diaion HP-20 column chromatography to give H_2O-, MeOH-, and acetone-eluted fractions. The MeOH-eluted fraction was subjected to ordinary and reversed-phase silica gel column chromatography and finally HPLC to furnish gymnosides I (**1**, 0.0024% from the natural medicine), II (**2**, 0.0009%), III (**3**, 0.017%), IV (**4**, 0.0006%), V (**5**, 0.0014%), VI (**6**, 0.0021%), and VII (**7**, 0.0025%), together with dactylorhin A[11] (**8**, 0.12%) and militarine[11] (**9**, 0.072%). Gymnoside I (**1**) was obtained as a white powder and exhibited a negative specific rotation ($[\alpha]^{24}_{D}$ −28.0 in MeOH). In the UV spectrum of **1**, absorption maxima were observed at 223 (log ϵ 4.10) and 256 (3.20) nm. The IR spectrum of **1** showed absorption bands at 1736, 1710, 1615, 1590, 1514, and 1233 cm^{-1} assignable to ester carbonyl and carboxyl functions and an aromatic ring in addition to strong absorption bands at 3410 and 1075 cm^{-1} suggestive of a glycoside moiety. In the positive- and negative-ion FABMS of **1**, quasimolecular ions were observed at m/z 481 $[M + Na]^+$ and 457 $[M - H]^-$, and HRFABMS analysis revealed the molecular formula of **1** to be $C_{21}H_{30}O_{11}$. The acid hydrolysis of **1** with 1.0 M HCl liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[1,4,6−8,10] The ^{1}H (pyridine-d_5, Table 1) and ^{13}C NMR (Table 2) spectra of **1**, which were assigned by various NMR experiments,[12] showed signals assignable to two methyls [δ 0.95, 1.03 (3H each, both d, J = 6.7 Hz, H_3-7, 8)], three methylenes [δ 1.90, 1.93 (1H each, both dd, J = 6.1, 14.0 Hz, H_2-5), 3.11, 3.37 (1H each, both d, J = 15.9 Hz, H_2-3), 5.36, 5.39 (1H each, both d, J = 12.2 Hz, H_2-7′)], a methine [δ 2.12 (1H, m, H-6)], and an A_2B_2 type aromatic pattern [δ 7.32, 7.47 (2H each, both d, J = 8.5 Hz, H-3′,5′, 2′,6′)], together with a β-glucopyranosyl moiety [δ 5.61

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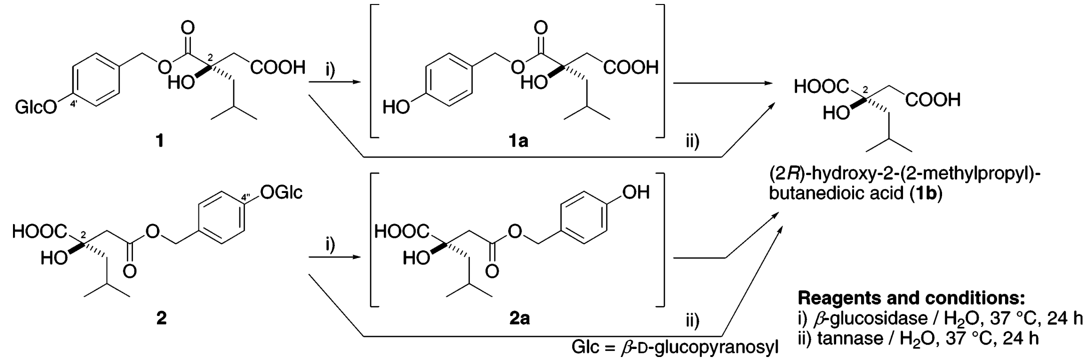

Figure 1.

Table 1. ^{1}H NMR (500 MHz, pyridine-d_5) Data of Gymnosides I (**1**) and II (**2**)

	1		**2**	
H	δ (J Hz)	HMBC	δ (J Hz)	HMBC
3	3.11 (d, 15.9)	C-1,4,5	3.10 (d, 15.0)	C-1,4,5
	3.37 (d, 15.9)		3.40 (d, 15.0)	
5	1.90 (dd, 6.1,14.0)	C-1,3,7,8	1.94 (dd, 6.1,14.0)	C-1,3,7,8
	1.93 (dd, 6.1,14.0)		2.05 (dd, 6.1,14.0)	
6	2.12 (m)	C-2,5,7,8	2.21 (m)	C-2
7	0.95 (d, 6.7)^a	C-5,8	1.05 (d, 6.7)^a	C-5,8
8	1.03 (d, 6.7)^a	C-5,7	1.08 (d, 6.7)^a	C-5,7
2′,6′	7.47 (d, 8.5)	C-4′,7′		
3′,5′	7.32 (d, 8.5)	C-1′		
7′	5.36 (d, 12.2)	C-1,2′,6′		
	5.39 (d, 12.2)			
2″,6″			7.35 (d, 8.9)	C-4″,7″
3″,5″			7.27 (d, 8.9)	C-1″
7″			5.13 (d, 12.2)	C-4,2″,6″
			5.18 (d, 12.2)	
4′-*O*-Glc-1	5.61 (d, 7.6)	C-4′		
2	4.31 (dd, 7.6,8.9)			
3	4.37 (m)			
4	4.36 (m)			
5	4.10 (m)			
6	4.40 (dd, 5.2,11.9)			
	4.52 (dd, 2.1,12.2)			
4″-*O*-Glc-1			5.60 (d, 7.6)	C-4″
2			4.31 (dd, 7.6,9.5)	
3			4.37 (m)	
4			4.36 (m)	
5			4.11 (m)	
6			4.41 (dd, 5.2,12.2)	
			4.54 (dd, 2.5,12.2)	

^{a} May be interchanged within the same column.

Table 2. ^{13}C NMR (125 MHz, pyridine-d_5) Data of Gymnosides I (**1**) and II (**2**)

C	**1**	**2**
1	176.0 (s)	178.5 (s)
2	76.2 (s)	75.9 (s)
3	46.1 (t)	45.9 (t)
4	173.8 (s)	170.9 (s)
5	48.5 (t)	48.6 (t)
6	24.4 (d)	24.7 (d)
7	23.8 (q)^a	23.0 (q)^a
8	24.7 (q)^a	24.8 (q)^a
1′	130.0 (s)	
2′,6′	130.4 (d)	
3′,5′	116.8 (d)	
4′	158.5 (s)	
7′	66.9 (t)	
1″		130.4 (s)
2″,6″		130.2 (d)
3″,5″		116.8 (d)
4″		158.4 (s)
7″		66.1 (t)
4′-*O*-Glc-1	102.0 (d)	
2	74.9 (d)	
3	78.5 (d)	
4	71.2 (d)	
5	78.8 (d)	
6	62.3 (t)	
4″-*O*-Glc-1		102.0 (d)
2		74.9 (d)
3		78.5 (d)
4		71.2 (d)
5		78.9 (d)
6		62.3 (t)

^{a} May be interchanged within the same column.

(1H, d, $J = 7.6$ Hz, H-4′-*O*-Glc-1)]. The enzymatic hydrolysis of **1** with β-glucosidase gave (2*R*)-hydroxy-2-(2-methylpropyl)butanedioic acid[11] [**1b** = (2*R*)-2-isobutylmalic acid], which was deduced to be formed via the *p*-hydroxybenzyl ester (**1a**). Compound **1b** was also obtained by the enzymatic hydrolysis of **1** with tannase, which is known to hydrolyze the ester bond of aromatic compounds such as tannins, flavonoids, and chromones.[13,14] In the HMBC experiment of **1**, long-range correlations were observed between the following proton and carbon pairs: H-3 and C-1, 4, 5; H_2-5 and C-1, 3, 7, 8; H-6 and C-2, 5, 7, 8; H_3-7 and C-5, 8; H_3-8 and C-5, 7; H-2′,6′ and C-4′, 7′; H-3′,5′ and C-1′; H_2-7′ and C-1, 1′, 2′,6′; H-4′-*O*-Glc-1 and C-4′ (Table 1, Figure S1). Thus the positions of the β-glucopyranosyl and *p*-hydroxybenzyl alcohol moieties in **1** were clarified. On the basis of those findings, the structure of gymnoside I was determined to be 1-(4-β-D-glucopyranosyloxybenzyl)-(2*R*)-2-isobutylmalate (**1**). Gymnoside II (**2**) was also isolated as a white powder with negative specific rotation ($[\alpha]^{24}_{D}$ −21.8 in MeOH). The UV spectrum of **2** showed absorption maxima at 223 (log ϵ 4.10) and 257 (3.31) nm. The IR spectrum of **2** showed absorption bands at 3432, 1734, 1719, 1617, 1592, 1514, 1235, and 1076 cm^{-1} ascribable to hydroxyl, ester carbonyl, carboxyl, and ether functions and an aromatic ring. The molecular formula $C_{21}H_{30}O_{11}$ of **2**, which was the same as **1**, was determined by the quasimolecular ion peaks in positive- and negative-ion FABMS and by HRFABMS. The acid hydrolysis of **2** with 1.0 M HCl liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[1,4,6−8,10] The proton and carbon signals in the ^{1}H (pyridine-d_5, Table 1) and ^{13}C NMR (Table 2) spectra[12] of **2** were very similar to those of **1** {two methyls [δ 1.05, 1.08 (3H each, both d, $J = 6.7$ Hz, H_3-7, 8)], three methylenes [δ 1.94, 2.05 (1H each, both dd, $J = 6.1$, 14.0 Hz, H_2-5), 3.10, 3.40 (1H each, both d, $J = 15.0$ Hz, H_2-3), 5.13, 5.18 (1H each, both d, $J = 12.2$ Hz, H_2-7″)], a methine [δ 2.21 (1H, m, H-6)], and an A_2B_2 type aromatic pattern [δ 7.27, 7.35 (2H each, both d, $J = 8.9$ Hz, H-3″,5″, 2″,6″)], together with a β-glucopyranosyl moiety [δ 5.60 (1H, d, $J = 7.6$ Hz, H-4″-*O*-Glc-1)]}. The enzymatic hydrolysis of **2** with β-glucosidase or tannase gave **1b** via **2a** (vide ante). The positions of the β-D-glucopyranosyl and *p*-hydroxybenzyl alcohol moieties in **2** were confirmed by the HMBC experiment, which showed long-range correlations between the following proton and carbon pairs: H-3 and C-1, 4, 5; H_2-5 and C-1, 3, 7, 8; H-6 and C-2, 5, 7, 8; H_3-7 and C-5, 8; H_3-8 and C-5, 7; H-2″,6″ and C-4″, 7″; H-3″,5″ and C-1″; H_2-7″ and C-4, 2″,6″; H-4″-*O*-Glc-1 and C-4″ (Table 1, Figure S1). Consequently, the structure of gymnoside II was determined as 4-(4-β-D-glucopyranosyloxybenzyl)-(2*R*)-2-isobutylmalate (**2**).

Gymnoside III (**3**) was isolated as a white powder with negative specific rotation ($[\alpha]^{22}_{D}$ −47.6 in MeOH). The positive- and negative-ion FABMS of **3** showed quasimolecular ions at *m*/*z* 953 $[M + Na]^+$ and *m*/*z* 929 $[M - H]^-$. The molecular formula

Table 3. ^{1}H NMR (500 MHz, pyridine-d_5) Data of Gymnosides III−VII (**3**−**7**)

	3		4		5		6		7	
H	δ (*J* Hz)	HMBC	δ (*J* Hz)	HMBC	δ (*J* Hz)	HMBC	δ (*J* Hz)	HMBC	δ (*J* Hz)	HMBC
3	3.37 (d, 17.7)	C-1,4,5	3.30 (d, 17.7)	C-1,4,5	3.31 (d, 17.7)	C-1,4,5	3.39 (d, 17.5)	C-1,4,5	3.38 (d, 17.4)	C-1,4,5
	3.56 (d, 17.7)		3.46 (d, 17.7)		3.52 (d, 17.7)		3.48 (d, 17.5)		3.54 (d, 17.4)	
5	1.94 (m)	C-1,3,7,8	1.87 (m)	C-1,3,7,8	1.90 (2H, m)	C-1,3,7,8	1.94 (m)	C-1,3,7,8	1.96 (m)	C-1,3,7,8
	2.02 (m)		2.03 (m)				2.01 (m)		2.06 (m)	
6	2.02 (m)	C-2,5,7,8	2.03 (m)	C-2,5,7,8	2.00 (m)	C-2,5,7,8	2.01 (m)	C-2,5,7,8	2.05 (m)	C-2,5,7,8
7	0.87 (d, 6.1)[a]	C-5,8	0.85 (d, 6.7)[a]	C-5,8	0.86 (d, 6.7)[a]	C-5,8	0.86 (d, 5.8)[a]	C-5,8	0.89 (d, 6.4)[a]	C-5,8
8	0.91 (d, 6.1)[a]	C-5,7	0.93 (d, 6.7)[a]	C-5,7	0.91 (d, 6.7)[a]	C-5,7	0.93 (d, 5.8)[a]	C-5,7	0.93 (d, 6.4)[a]	C-5,7
2′,6′	7.43 (d, 8.9)	C-4′,7′	7.40 (d, 8.9)	C-4′,7′	7.45 (d, 8.9)	C-4′,7′	7.44 (d, 7.9)	C-4′,7′	7.44 (d, 8.9)	C-4′,7′
3′,5′	7.38 (d, 8.9)	C-1′	7.38 (d, 8.9)	C-1′	7.39 (d, 8.9)	C-1′	7.36 (d, 7.9)	C-1′	7.40 (d, 8.9)	C-1′
7′	5.27 (d, 11.9)	C-1,2′,6′	5.30 (d, 11.9)	C-1,2′,6′	5.33 (d, 12.2)	C-1,2′,6′	5.28 (d, 11.9)	C-1,2′,6′	5.28 (d, 12.2)	C-1,2′,6′
	5.33 (d, 11.9)		5.35 (d, 11.9)		5.39 (d, 12.2)		5.35 (d, 11.9)		5.33 (d, 12.2)	
2″,6″	7.45 (d, 8.9)	C-4″,7″	7.46 (d, 8.9)	C-4″,7″	7.47 (d, 8.9)	C-4″,7″	7.41 (d, 7.9)	C-4″,7″	7.48 (d, 8.9)	C-4″,7″
3″,5″	7.37 (d, 8.9)	C-1″	7.38 (d, 8.9)	C-1″	7.39 (d, 8.9)	C-1″	7.36 (d, 7.9)	C-1″	7.41 (d, 8.9)	C-1″
7″	5.13 (d, 11.9)	C-4,2″,6″	5.14 (d, 11.9)	C-4,2″,6″	5.22 (d, 12.2)	C-4,2″,6″	5.12 (d, 11.9)	C-4,2″,6″	5.15 (d, 12.2)	C-4,2″,6″
	5.25 (d, 11.9)		5.22 (d, 11.9)		5.34 (d, 12.2)		5.26 (d, 11.9)		5.21 (d, 12.2)	
2-*O*-Glc-1	5.47 (d, 7.6)	C-2	5.62 (d, 7.6)	C-2	5.69 (d, 7.9)	C-2	5.55 (d, 7.6)	C-2	5.56 (d, 7.6)	C-2
2	4.03 (dd, 7.6,8.9)		4.16 (m)		4.11 (m)		4.09 (dd, 7.6,7.9)		4.15 (d, 7.6,9.2)	
3	4.19 (dd, 8.9,9.2)		6.05 (dd, 9.2,9.5)	Cin.-9	4.38 (m)		4.24 (dd, 7.6,8.8)		5.98 (dd, 9.2,9.5)	Cin.-9
4	4.08 (dd, 8.2,9.2)		4.48 (dd, 9.5,9.5)		5.80 (dd, 9.5,9.8)	Cin.-9	4.20 (dd, 7.9,8.8)		4.23 (dd, 9.5,9.8)	
5	3.83 (m)		3.89 (m)		3.90 (m)		3.91 (m)		3.89 (m)	
6	4.72 (dd, 5.5,11.9)	−O*C*OCH$_3$	4.38 (2H, m)		4.08 (dd, 5.2,12.2)		4.94 (2H, m)	Cin.-9	4.71 (dd, 5.2,12.2)	−O*C*OCH$_3$
	4.78 (dd, 1.8,11.9)				4.15 (dd, 2.0,12.2)				4.80 (dd, 1.8,12.2)	
6-Ac	1.94 (s)	−O*C*OCH$_3$							2.00 (s)	−O*C*OCH$_3$
Cin.-2,6			7.47 (dd, 2.1,7.9)	Cin.-4,7	7.56 (dd, 2.4,7.9)	Cin.-4,7	7.49 (dd, 2.4,7.9)	Cin.-4,7	7.50 (dd, 2.4,7.9)	Cin.-4,7
3,5			7.32 (dd, 7.9,7.9)	Cin.-1	7.36 (dd, 7.9,7.9)	Cin.-1	7.36 (dd, 7.9,7.9)	Cin.-1	7.33 (dd, 7.9,7.9)	Cin.-1
4			7.32 (tt, 2.1,7.9)	Cin.-2,6	7.36 (tt, 2.4,7.9)	Cin.-2,6	7.36 (tt, 2.4,7.9)	Cin.-2,6	7.33 (tt, 2.4,7.9)	Cin.-2,6
7			7.89 (d, 15.9)	Cin.-9	7.91 (d, 16.2)	Cin.-9	7.90 (d, 15.9)	Cin.-9	7.91 (d, 15.9)	Cin.-9
8			6.69 (d, 15.9)	Cin.-1,9	6.72 (d, 16.2)	Cin.-1,9	6.70 (d, 15.9)	Cin.-1,9	6.71 (d, 15.9)	Cin.-1,9
4′-*O*-Glc-1	5.67 (d, 7.6)	C-4′	5.69 (d, 7.6)	C-4′	5.64 (d, 7.9)	C-4′	5.65 (d, 7.6)	C-4′	5.70 (d, 7.6)	C-4′
2	4.32 (dd, 7.6,8.5)		4.33 (m)		4.32 (dd, 7.9,7.9)		4.32 (dd, 7.6,9.1)		4.33 (dd, 7.6,8.9)	
3	4.40 (m)		4.39 (m)		4.39 (m)		4.39 (m)		4.39 (m)	
4	4.37 (m)		4.38 (m)		4.36 (m)		4.37 (m)		4.38 (m)	
5	4.12 (m)		4.16 (m)		4.13 (m)		4.12 (m)		4.18 (m)	
6	4.40 (dd, 5.2,11.9)		4.40 (dd, 5.5,12.0)		4.41 (dd, 4.8,12.2)		4.41 (dd, 4.9,11.9)		4.41 (dd, 5.0,12.1)	
	4.55 (dd, 1.8,11.9)		4.56 (dd, 2.1,12.0)		4.55 (dd, 2.1,12.2)		4.55 (dd, 2.1,11.9)		4.56 (dd, 2.1,12.1)	
4″-*O*-Glc-1	5.62 (d, 7.6)	C-4″	5.68 (d, 7.6)	C-4″	5.63 (d, 7.9)	C-4″	5.62 (d, 7.6)	C-4″	5.66 (d, 7.6)	C-4″
2	4.32 (dd, 7.6,8.5)		4.33 (m)		4.32 (dd, 7.9,7.9)		4.32 (dd, 7.6,9.1)		4.33 (dd, 7.6,8.9)	
3	4.40 (m)		4.39 (m)		4.39 (m)		4.39 (m)		4.39 (m)	
4	4.37 (m)		4.38 (m)		4.36 (m)		4.37 (m)		4.38 (m)	
5	4.12 (m)		4.16 (m)		4.13 (m)		4.12 (m)		4.18 (m)	
6	4.41 (dd, 5.2,12.2)		4.40 (dd, 5.5,12.0)		4.41 (dd, 4.8,12.2)		4.41 (dd, 4.9,11.9)		4.41 (dd, 5.0,12.1)	
	4.53 (dd, 1.8,12.2)		4.54 (dd, 2.1,12.0)		4.55 (dd, 2.1,12.2)		4.53 (dd, 2.1,11.9)		4.54 (dd, 2.1,12.1)	

[a] May be interchanged within the same column.

$C_{42}H_{58}O_{23}$ of **3** was determined by HRFABMS. In the UV spectrum of **3**, absorption maxima were observed at 224 (log ϵ 4.36) and 271 (3.28) nm. The IR spectrum of **3** showed absorption bands at 1736, 1613, 1592, 1514, and 1287 cm^{-1}, assignable to an ester carbonyl function and aromatic ring, and strong absorption bands at 3432 and 1075 cm^{-1} suggestive of a glycoside moiety. Acid hydrolysis of **3** with 1.0 M HCl liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.[1,4,6−8,10] The ^{1}H (pyridine-d_5, Table 3) and ^{13}C NMR (Table 4) spectra[12] of **3** showed signals assignable to two methyls [δ 0.87, 0.91 (3H each, both d, $J = 6.1$ Hz, H$_3$-7, 8)], four methylenes [δ 1.94, 2.02 (1H each, both m, H$_2$-5), 3.37, 3.56 (1H each, both d, $J = 17.7$ Hz, H$_2$-3), 5.13, 5.25 (1H each, both d, $J = 11.9$ Hz, H$_2$-7″), 5.27, 5.33 (1H each, both d, $J = 11.9$ Hz, H$_2$-7′)], a methine [δ 2.02 (1H, m, H-6)], an acetyl group [δ 1.94 (3H, s)], and two A_2B_2 type aromatic spin systems [δ 7.37, 7.45 (2H each, both d, $J = 8.9$ Hz, H-3″,5″, 2″,6″), 7.38, 7.43 (2H each, both d, $J = 8.9$ Hz, H-3′,5′, 2′,6′)], together with three β-glucopyranosyl signals [δ 5.47 (1H, d, $J = 7.6$ Hz, H-2-*O*-Glc-1), 5.62 (1H, d, $J = 7.6$ Hz, H-4″-*O*-Glc-1), 5.67 (1H, d, $J = 7.6$ Hz, H-4′-*O*-Glc-1)]. In the HMBC experiment of **3**, long-range correlations were observed between the following proton and carbon pairs: H-3 and C-1, 4, 5; H$_2$-5 and C-1, 3, 7, 8; H-6 and C-2, 5, 7, 8; H$_3$-7 and C-5, 8; H$_3$-8 and C-5, 7; H-2′,6′ and C-4′, 7′; H-3′,5′ and C-1′; H$_2$-7′ and C-1, 2′,6′; H-2″,6″ and C-4″, 7″; H-3″,5″ and C-1″; H-7″ and C-4, 2″,6″; H-4′-*O*-Glc-1 and C-4′; H-2-*O*-Glc-1 and C-2; H-2-*O*-Glc-6 and −O*C*OCH$_3$; H-4″-*O*-Glc-1 and C-4″ (Table 3, Figure S1). The enzymatic hydrolysis of **3** with β-glucosidase or tannase gave the same product (**3a**), whose ^{1}H NMR (pyridine-d_5, Table 5) spectrum showed signals assignable to a 2-isobutylmalic acid moiety {two methyls [δ 1.02, 1.08 (3H each, both d, $J = 6.4$ Hz, H$_3$-7, 8)], two methylenes [δ 2.07 (2H, br d, $J = 6$ Hz, H$_2$-5), 3.62, 3.69 (1H each, both d, $J = 17.4$ Hz, H$_2$-3)], and a methine [δ 2.30 (1H, m, H-6)]} and a β-glucopyranosyl [δ 6.01 (1H, d, $J = 7.6$ Hz, H-2-*O*-Glc-1)] together with an acetyl group [δ 2.00 (3H, s)]. Comparison of the ^{1}H and ^{13}C NMR spectra of **3a** with those of dactyrohin C[11] (**4b**, vide infra) showed an acetylation shift around the 6-position of the 2-*O*-glucosyl part in **3a**. Acetylation of **3a** with Ac_2O and pyridine gave the tetra-*O*-acetyl derivative (**3b**), which was also obtained by the similar acetylation of **4b**. The absolute configuration at C-2 in **3** is thus *R*.Gymnosides IV (**4**), V (**5**), and VI (**6**) were also isolated as white powders with negative specific rotations (**4**, $[\alpha]^{25}_{D}$ −20.2; **5**, $[\alpha]^{27}_{D}$ −11.6; **6**, $[\alpha]^{27}_{D}$ −45.1 all in MeOH), respectively. In the positive- and negative-ion FABMS of **4**−**6**, the same quasimolecular ions were observed at *m/z* 1041 $[M + Na]^+$ and *m/z* 1017 $[M - H]^-$, and the molecular formula $C_{49}H_{62}O_{23}$ was determined by HRFABMS measurements. The acid hydrolysis of **4**−**6** liberated D-glucose.[1,4,6−8,10] The proton and carbon signals in the ^{1}H (pyridine-d_5, Table 3) and ^{13}C NMR (Table 4) spectra[12] of **4** showed signals assignable to a 1,4-bis(*p*-hydroxybenzyl)-2-isobutylmalate moiety {two methyls [δ 0.85, 0.93 (3H each, both d, $J = 6.7$ Hz, H$_3$-7, 8)], four methylenes [δ 1.87, 2.03 (1H each, both m, H$_2$-5), 3.30, 3.46 (1H each, both d, $J = 17.7$ Hz, H$_2$-3), 5.14, 5.22 (1H each, both d, $J = 11.9$ Hz, H$_2$-7″), 5.30, 5.35 (1H each, both d, $J = 11.9$ Hz, H$_2$-7′)], a methine [δ 2.03 (1H, m, H-6)], and two A_2B_2 type aromatic spin systems [δ 7.38, 7.40 (2H each, both d, $J = 8.9$ Hz, H-3′,5′, 2′,6′), 7.38, 7.46 (2H each, both d, $J = 8.9$ Hz, H-3″,5″, 2″,6″)]} and three β-glucopyranosyl signals [δ 5.62 (1H, d, $J = 7.6$ Hz, H-2-*O*-Glc-1), 5.68 (1H, d, $J = 7.6$ Hz, H-4″-*O*-Glc-1), 5.69 (1H, d, $J = 7.6$

Table 4. ^{13}C NMR (125 MHz, pyridine-d_5) Data of Gymnosides III−VII (**3**−**7**)

C	3	4	5	6	7
1	173.1 (s)	173.1 (s)	173.3 (s)	173.2 (s)	172.7 (s)
2	80.9 (s)	80.9 (s)	80.7 (s)	80.9 (s)	81.3 (s)
3	42.2 (t)	42.7 (t)	42.8 (t)	42.4 (t)	42.0 (t)
4	170.9 (s)	170.9 (s)	171.1 (s)	170.9 (s)	170.8 (s)
5	46.9 (t)	47.6 (t)	47.8 (t)	47.2 (t)	46.6 (t)
6	24.1 (d)	24.1 (d)	24.0 (d)	24.1 (d)	24.1 (d)
7	23.9 (q)[a]	24.0 (q)[a]	23.9 (q)[a]	23.9 (q)[a]	23.9 (q)[a]
8	24.5 (q)[a]	24.6 (q)[a]	24.6 (q)[a]	24.5 (q)[a]	24.5 (q)[a]
1′	129.5 (s)	129.5 (s)	129.5 (s)	129.5 (s)	129.5 (s)
2′,6′	130.7 (d)	130.7 (d)	130.6 (d)	130.7 (d)	130.7 (d)
3′,5′	117.0 (d)	117.0 (d)	117.0 (d)	117.0 (d)	117.0 (d)
4′	158.7 (s)	158.7 (s)	158.7 (s)	158.7 (s)	158.7 (s)
7′	67.3 (t)	67.4 (t)	67.3 (t)	67.3 (t)	67.3 (t)
1″	129.7 (s)	129.7 (s)	129.8 (s)	129.7 (s)	129.7 (s)
2″,6″	130.6 (d)	130.6 (d)	130.6 (d)	130.6 (d)	130.6 (d)
3″,5″	117.0 (d)	117.0 (d)	117.0 (d)	117.0 (d)	117.0 (d)
4″	158.7 (s)	158.7 (s)	158.6 (s)	158.7 (s)	158.7 (s)
7″	66.5 (t)	66.6 (t)	66.7 (t)	66.5 (t)	66.5 (t)
2-*O*-Glc-1	100.0 (d)	100.0 (d)	100.1 (d)	100.1 (d)	99.8 (d)
2	75.4 (d)	73.5 (d)	75.8 (d)	75.5 (d)	73.2 (d)
3	78.4 (d)	80.0 (d)	76.0 (d)	78.4 (d)	79.4 (d)
4	70.9 (d)	69.0 (d)	72.5 (d)	70.9 (d)	69.2 (d)
5	74.9 (d)	78.0 (d)	75.9 (d)	75.0 (d)	74.7 (d)
6	64.3 (t)	61.9 (t)	62.0 (t)	64.4 (t)	63.9 (t)
6-Ac	20.7 (q)				20.8 (q)
	170.8 (s)				170.6 (s)
Cin.-1		135.0 (s)	134.9 (s)	134.8 (s)	135.0 (s)
Cin.-2,6		128.4 (d)	128.6 (d)	128.6 (d)	128.5 (d)
Cin.-3,5		129.2 (d)	129.3 (d)	129.3 (d)	129.2 (d)
Cin.-4		130.7 (d)	130.7 (d)	130.7 (d)	130.5 (d)
Cin.-7		144.6 (d)	145.2 (d)	145.0 (d)	144.8 (d)
Cin.-8		119.5 (d)	118.9 (d)	118.8 (d)	119.3 (d)
Cin.-9		166.9 (s)	166.6 (s)	167.0 (s)	166.8 (s)
4′-*O*-Glc-1	102.1 (d)	102.1 (d)	102.1 (d)	102.1 (d)	102.1 (d)
2	74.9 (d)	74.9 (d)	74.9 (d)	74.9 (d)	74.9 (d)
3	78.4 (d)	78.5 (d)	78.4 (d)	78.4 (d)	78.5 (d)
4	71.2 (d)	71.3 (d)	71.2 (d)	71.2 (d)	71.3 (d)
5	78.8 (d)	78.8 (d)	78.8 (d)	78.8 (d)	78.8 (d)
6	62.3 (t)	62.3 (t)	62.3 (t)	62.3 (t)	62.3 (t)
4″-*O*-Glc-1	102.1 (d)	102.0 (d)	102.0 (d)	102.1 (d)	102.1 (d)
2	74.9 (d)	74.9 (d)	74.9 (d)	74.9 (d)	75.0 (d)
3	78.4 (d)	78.5 (d)	78.4 (d)	78.4 (d)	78.5 (d)
4	71.2 (d)	71.2 (d)	71.2 (d)	71.2 (d)	71.2 (d)
5	78.8 (d)	78.8 (d)	78.8 (d)	78.8 (d)	78.8 (d)
6	62.3 (t)	62.3 (t)	62.3 (t)	62.3 (t)	62.3 (t)

[a] May be interchanged within the same column.

Hz, H-4′-*O*-Glc-1)], together with a *trans*-cinnamoyl ester moiety [δ 6.69, 7.89 (1H each, both d, J = 15.9 Hz), 7.32 (2H, dd, J = 7.9, 7.9 Hz), 7.32 (1H, tt, J = 2.1, 7.9 Hz), 7.47 (2H, dd, J = 2.1, 7.9 Hz)]. The enzymatic hydrolysis of **4** with β-glucosidase gave **4a**, whose ^1H (pyridine-d_5, Table 5) spectrum showed signals assignable to a 2-isobutylmalic acid moiety {two methyls [δ 1.00, 1.14 (3H each, both d, J = 6.4 Hz, H_3-7, 8)], two methylenes [δ 2.15 (2H, br d, J = 6 Hz, H_2-5), 3.63 (2H, br s, H_2-3)], and a methine [δ 2.30 (1H, m, H-6)]} and a β-glucopyranosyl [δ 5.98 (1H, d, J = 7.6 Hz, H-2-*O*-Glc-1)] together with a *trans*-cinnamoyl ester moiety [δ 6.73, 7.89 (1H each, both d, J = 16.2 Hz), 7.31 (2H, dd, J = 7.9, 7.9 Hz), 7.31 (1H, tt, J = 2.4, 7.9 Hz), 7.48 (2H, dd, J = 2.4, 7.9 Hz)]. Enzymatic hydrolysis of **4** with tannase gave *trans*-cinnamic acid[15] and **4b**. The connectivity of the *trans*-cinnamoyl ester moiety in **4** was clarified by an HMBC experiment, in which a long-range correlation was observed between H-3 of the 2-*O*-glucosyl unit [δ 6.05 (1H, dd, J = 9.2, 9.5 Hz)] and the *trans*-cinnamoyl ester carbonyl carbon (δ_C 166.9), as shown in Table 3 and Figure S1. Consequently, the structure of **4** was determined as shown.

The proton and carbon signals in the ^1H (pyridine-d_5, Table 3) and ^{13}C NMR (Table 4) spectra[12] of **5** and **6** were very similar to those of **4**. Enzymatic hydrolysis of **5** and **6** with tannase gave **4b** together with *trans*-cinnamic acid. Treatment of **5** and **6** with β-glucosidase afforded **5a** and **6a**, respectively. Comparison of the proton signals in the ^1H NMR spectrum (pyridine-d_5, Table 5) of **5a** and **6a** with those of **4b** showed acylation shifts around the 4- and 6-positions of the 2-*O*-glucosyl part, respectively. On the basis of this evidence and the long-range correlations in the HMBC experiment for **5** and **6**, the structures of **5** and **6** were determined as shown.

Gymnoside VII (**7**), $[\alpha]^{19}_D$ −12.0 (in MeOH), had the molecular formula $C_{51}H_{64}O_{24}$ as shown by HRFABMS. Treatment of **7** with β-glucosidase gave **7a**, while treatment of **7** with tannase furnished **3a** and *trans*-cinnamic acid. Comparison of the ^1H NMR (pyridine-d_5, Table 5) spectrum of **7a** with those of **3a** showed an acylation shift around the 3-position of the 2-*O*-glucosyl unit in **7a**. The position of the *trans*-cinnamoyl ester moiety of **7** was elucidated on the basis of an HMBC experiment as shown in Table 3 and Figure S1. Consequently, the structure of **7** was constructed as shown.

Experimental Section

General Experimental Procedures. The following instruments were used to obtain physical data: optical rotations, Horiba SEPA-300 digital polarimeter (l = 5 cm); UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; ^1H NMR spectra, JEOL JNM-LA500 (500 MHz) spectrometer; ^{13}C NMR spectra, JEOL JNM-LA500 (125 MHz) spectrometer with TMS as an internal standard; FABMS and HRFABMS, JEOL JMS-SX 102A mass spectrometer; HPLC detector, Shimadzu RID-6A refractive index and SPD-10A UV−vis detectors. An HPLC column and 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: normal-phase silica gel column chromatography, silica gel BW-200 (Fuji Silysia Chemical, Ltd., Aichi, Japan, 150−350 mesh); reversed-phase silica gel column chromatography, Diaion HP-20

Table 5. ^1H NMR (500 MHz, pyridine-d_5) Data of **3a**−**7a**

	3a	4a	5a	6a	7a
H	δ (J Hz)	δ (J Hz)	δ (J Hz)	δ (J Hz)	δ (J Hz)
3	3.62 (d, 17.4)	3.63 (2H, br s)	3.63 (2H, br s)	3.63 (d, 17.4)	3.65 (2H, br s)
	3.69 (d, 17.4)			3.75 (d, 17.4)	
5	2.07 (2H, br d, 6)	2.15 (2H, br d, 6)	2.12 (2H, br d, 6)	2.10 (2H, br d, 6)	2.08 (2H, br s)
6	2.30 (m)	2.30 (m)	2.30 (m)	2.31 (m)	2.29 (m)
7	1.02 (d, 6.4)[a]	1.00 (d, 6.4)[a]	1.03 (d, 6.4)[a]	1.02 (d, 6.4)[a]	1.01 (d, 6.4)[a]
8	1.08 (d, 6.4)[a]	1.14 (d, 6.4)[a]	1.11 (d, 6.4)[a]	1.09 (d, 6.4)[a]	1.10 (d, 6.4)[a]
2-*O*-Glc-1	6.01 (d, 7.6)	5.98 (d, 7.6)	5.99 (d, 7.9)	6.04 (d, 7.9)	6.02 (d, 7.6)
2	4.12 (dd, 7.6,8.9)	4.26 (m)	4.23 (m)	4.22 (m)	4.25 (dd, 7.6,9.2)
3	4.23 (dd, 8.9,9.2)	6.08 (dd, 8.9,9.5)	4.42 (dd, 9.2,9.2)	4.29 (dd, 8.2,8.2)	6.05 (dd, 9.2,9.5)
4	4.16 (dd, 8.2,9.2)	4.53 (dd, 9.2,9.5)	5.87 (dd, 9.2,9.8)	4.22 (m)	4.26 (dd, 9.5,9.8)
5	4.02 (m)	3.92 (m)	4.02 (m)	4.09 (m)	4.08 (m)
6	4.76 (dd, 5.2,11.3)	4.37 (dd, 4.6,11.6)	4.23 (dd, 5.8,11.2)	4.90 (dd, 5.5,11.6)	4.74 (dd, 5.5,11.6)
	4.92 (dd, 1.8,11.3)	4.50 (dd, 1.5,11.6)	4.27 (dd, 2.2,11.2)	5.12 (dd, 1.8,11.6)	4.92 (dd, 2.1,11.6)
6-Ac	2.00 (s)				2.03 (s)
Cin.-2,6		7.48 (dd, 2.4,7.9)	7.49 (dd, 2.1,7.9)	7.55 (dd, 2.4,7.9)	7.50 (dd, 2.4,7.9)
3,5		7.31 (dd, 7.9,7.9)	7.33 (dd, 7.9,7.9)	7.33 (dd, 7.9,7.9)	7.32 (dd, 7.9,7.9)
4		7.31 (tt, 2.4,7.9)	7.33 (tt, 2.1,7.9)	7.33 (tt, 2.4,7.9)	7.32 (tt, 2.4,7.9)
7		7.89 (d, 16.2)	7.84 (d, 16.2)	7.88 (d, 15.9)	7.91 (d, 15.9)
8		6.73 (d, 16.2)	6.61 (d, 16.2)	6.77 (d, 15.9)	6.74 (d, 15.9)

[a] May be interchanged within the same column.

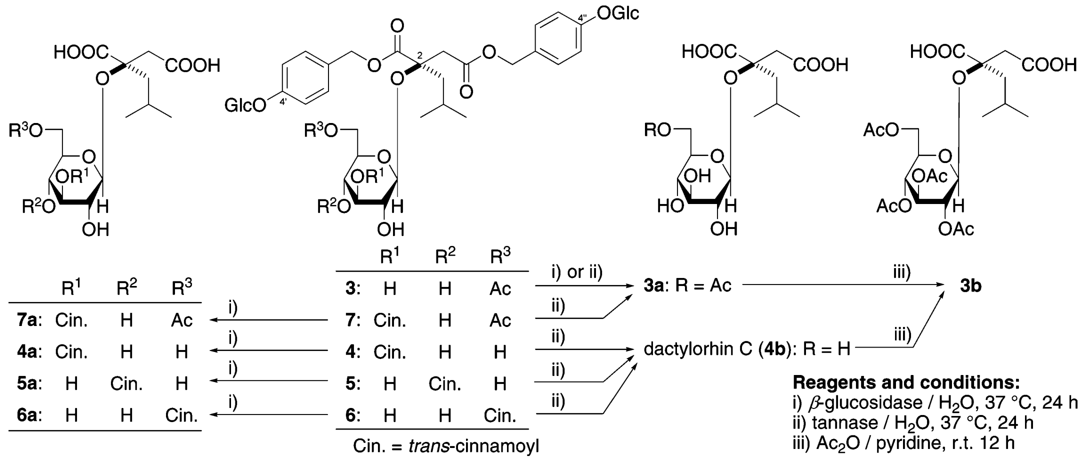

Figure 2.

(Nippon Rensui Co., Tokyo, Japan), Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., Aichi, Japan, 100−200 mesh); TLC, precoated TLC plates with silica gel 60F_{254} (Merck, 0.25 mm) (normal-phase) and silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed-phase); reversed-phase HPTLC, precoated 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.

Plant Material. Reported before.[3]

Extraction and Isolation. The dried tubers of *G. conopsea* (12.0 kg) were powdered and extracted three times with MeOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a MeOH extract (930 g, 7.8% from the dried tubers). The MeOH extract (307 g) was subjected to Diaion HP-20 column chromatography [3.0 kg, H_2O−MeOH−acetone] to give H_2O-, MeOH-, and acetone-eluted fractions (251, 51, and 5 g, respectively). Normal-phase silica gel column chromatography [1.4 kg, *n*-hexane−EtOAc (5:1−1:1, v/v) to $CHCl_3$−MeOH−H_2O (10:3:1−7:3:1, lower layer 6:4:1)−MeOH] of the MeOH-eluted fraction (45.5 g) gave 10 fractions [1 (1.82 g), 2 (1.03 g), 3 (0.40 g), 4 (0.95 g), 5 (1.35 g), 6 (3.34 g), 7 (6.59 g), 8 (21.36 g), 9 (6.79 g), and 10 (1.87 g)]. From fractions 3−5, phenanthrenes and stilbenes were isolated.[3] 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) to MeOH] to give six fractions [6-1 (920 mg), 6-2 (384 mg), 6-3 (352 mg), 6-4 (772 mg), 6-5 (280 mg), and 6-6 (632 mg)]. Fraction 6-4 (772 mg) was further purified by HPLC [YMC-Pack ODS-A (20 × 250 mm), MeOH−H_2O (40:60, v/v)] to give gymnosides I (**1**, 85 mg, 0.0024%) and II (**2**, 33 mg, 0.0009%). Fraction 7 (6.59 g) was subjected to reversed-phase silica gel column chromatography [210 g, MeOH−H_2O (10:90−30:70−50:50−70:30, v/v) to MeOH] to give seven fractions [7-1 (1070 mg), 7-2 (370 mg), 7-3 (3260 mg), 7-4 (190 mg), 7-5 (380 mg), 7-6 (840 mg), and 7-7 (480 mg)]. Fraction 7-3 (710 mg) was subjected to HPLC [MeOH−H_2O (50:50, v/v)] to give militarine (**9**, 557 mg, 0.072%). Fraction 7-6 (0.84 g) was further purified by HPLC [MeOH−H_2O (65:35, v/v)] to give gymnosides IV (**4**, 23 mg, 0.0006%), V (**5**, 47 mg, 0.0014%), VI (**6**, 14 mg, 0.0004%), and VII (**7**, 89 mg, 0.0025%). Fraction 8 (21.36 g) was subjected to reversed-phase silica gel column chromatography [630 g, MeOH−H_2O (10:90−20:80−30:70−60:40−50:50−70:30, v/v) to MeOH] to give nine fractions [8-1 (4.24 g), 8-2 (3.26 g), 8-3 (4.17 g), 8-4 (4.71 g), 8-5 (1.05 g), 8-6 (0.29 g), 8-7 (0.44 g), 8-8 (0.13 g), and 8-9 (3.07 g)]. Fraction 8-3 (0.27 g) was subjected to HPLC [MeOH−H_2O (45:55, v/v)] to give dactylorhin A (**8**, 229 mg, 0.11%). Fraction 8-5 (1.05 g) was subjected to HPLC [MeOH−H_2O (50:50, v/v)] to give gymnoside III (**3**, 595 mg, 0.017%) and **8** (78 mg, 0.0022%). Fraction 8-8 (0.13 g) was further purified by HPLC [MeOH−H_2O (65:35, v/v)] to give **6** (62 mg, 0.0017%). Dactylorhin A (**8**) and militarine (**9**) were identified by comparison of the physical data ([α]$_D$, UV, IR, ^{1}H and ^{13}C NMR, MS) with reported values.[11]

Gymnoside I (1): white powder, $[\alpha]^{24}_D$ −28.0 (*c* 3.52, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (4.10), 256 (3.20); IR (KBr) ν_{max} 3410, 2957, 1736, 1710, 1615, 1590, 1514, 1233, 1075 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; positive-ion FABMS *m/z* 481 [M + Na]$^+$; negative-ion FABMS *m/z* 457 [M − H]$^-$; HRFABMS *m/z* 481.1694 (calcd for $C_{21}H_{30}O_{11}Na$ [M + Na]$^+$, 481.1686).

Gymnoside II (2): white powder, $[\alpha]^{24}_D$ −21.8 (*c* 0.98, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (4.10), 257 (3.31); IR (KBr) ν_{max} 3432, 2957, 1734, 1719, 1617, 1592, 1514, 1235, 1076 cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; positive-ion FABMS *m/z* 481 [M + Na]$^+$; negative-ion FABMS *m/z* 457 [M − H]$^-$; HRFABMS *m/z* 481.1691 (calcd for $C_{21}H_{30}O_{11}Na$ [M + Na]$^+$, 481.1686).

Gymnoside III (3): white powder, $[\alpha]^{22}_D$ −47.6 (*c* 2.19, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (4.36), 271 (3.28); IR (KBr) ν_{max} 3432, 2957, 1736, 1613, 1592, 1514, 1287, 1075 cm^{-1}; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 4; positive-ion FABMS *m/z* 953 [M + Na]$^+$; negative-ion FABMS *m/z* 929 [M − H]$^-$; HRFABMS *m/z* 953.3258 (calcd for $C_{42}H_{58}O_{23}Na$ [M + Na]$^+$, 953.3267).

Gymnoside IV (4): white powder, $[\alpha]^{25}_D$ −20.2 (*c* 1.32, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (4.57), 278 (4.37); IR (KBr) ν_{max} 3432, 2928, 1734, 1636, 1613, 1514, 1233, 1075 cm^{-1}; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 4; positive-ion FABMS *m/z* 1041 [M + Na]$^+$; negative-ion FABMS *m/z* 1017 [M − H]$^-$; HRFABMS *m/z* 1041.3574 (calcd for $C_{49}H_{62}O_{23}Na$ [M + Na]$^+$, 1041.3580).

Gymnoside V (5): white powder, $[\alpha]^{27}_D$ −11.6 (*c* 1.61, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (4.56), 278 (4.36); IR (KBr) ν_{max} 3432, 2955, 1736, 1638, 1613, 1514, 1458, 1232, 1075 cm^{-1}; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 4; positive-ion FABMS *m/z* 1041 [M + Na]$^+$; negative-ion FABMS *m/z* 1017 [M − H]$^-$; HRFABMS *m/z* 1041.3582 (calcd for $C_{49}H_{62}O_{23}Na$ [M + Na]$^+$, 1041.3580).

Gymnoside VI (6): white powder, $[\alpha]^{25}_D$ −45.1 (*c* 2.74, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (4.51), 278 (4.29); IR (KBr) ν_{max} 3432, 2957, 1736, 1638, 1514, 1451, 1233, 1075 cm^{-1}; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 4; positive-ion FABMS *m/z* 1041 [M + Na]$^+$; negative-ion FABMS *m/z* 1017 [M − H]$^-$; HRFABMS *m/z* 1041.3574 (calcd for $C_{49}H_{62}O_{23}Na$ [M + Na]$^+$, 1041.3580).

Gymnoside VII (7): white powder, $[\alpha]^{19}_D$ −12.0 (*c* 0.78, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (4.49), 278 (4.28); IR (KBr) ν_{max} 3432, 2926, 1736, 1638, 1613, 1514, 1235, 1075 cm^{-1}; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 4; positive-ion FABMS *m/z* 1083 [M + Na]$^+$; negative-ion FABMS *m/z* 1059 [M − H]$^-$; HRFABMS *m/z* 1083.3676 (calcd for $C_{51}H_{64}O_{24}Na$ [M + Na]$^+$, 1083.3685).

Acid Hydrolysis of 1−7. A solution of **1**−**7** (each 1.5 mg) in 1 M HCl (2.0 mL) was heated under reflux for 3 h. After cooling, the reaction mixture was extracted with EtOAc. The aqueous layer was subjected to HPLC analysis under the following conditions, respectively: 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]. Identification of D-glucose in the aqueous layer was carried out by comparison of its retention time and specific rotation with those of an authentic sample, t_R: 12.3 min (D-glucose, positive specific rotation).

Enzymatic Hydrolysis of 1−7 with β-Glucosidase. A solution of **1** or **2** (5.5 mg each, both 0.012 mmol) in H_2O (2.0 mL) was treated with β-glucosidase (5.0 mg, from Almond, Oriental Yeast Co., Ltd., Tokyo, Japan), and the solution was stirred at 37 °C for 24 h. After EtOH was added, the solvent was removed under reduced pressure and the residue was purified by HPLC [MeOH−1% aqueous HOAc (45:

55, v/v)] to furnish (2*R*)-hydroxy-2-(2-methylpropyl)butanedioic acid[11] (**1b**, 1.8 mg, 79% from **1**; 1.7 mg, 71% from **2**), respectively. Through a similar procedure, a solution of **3** (7.5 mg, 0.008 mmol), **4** (7.0 mg, 0.007 mmol), **5** (7.0 mg, 0.007 mmol), **6** (9.4 mg, 0.009 mmol), or **7** (8.8 mg, 0.008 mmol) in H_2O (2.0 mL) was treated with β-glucosidase (5.0 mg), and the solution was stirred at 37 °C for 24 h. Workup as above gave a residue, which was purified by HPLC [MeOH−1% aqueous HOAc (45:55, v/v)] to give **3a** (1.8 mg, 57% from **3**), **4a** (2.6 mg, 79% from **4**), **5a** (2.3 mg, 70% from **5**), **6a** (2.7 mg, 61% from **6**), and **7a** (2.4 mg, 55% from **7**), respectively.

Compound 3a: white powder, $[\alpha]^{15}_{D}$ −17.8 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 256 (4.14); IR (KBr) ν_{max} 3423, 1736, 1718, 1619, 1509, 1458, 1248, 1090 cm^{-1}; ^{1}H NMR data, see Table 5; ^{13}C NMR (125 MHz, pyridine-d_5) δ_C 177.6 (C-1), 81.4 (C-2), 44.2 (C-3), 173.8 (C-4), 49.4 (C-5), 24.6 (C-6), 24.2 (C-7), 24.8 (C-8), 100.4 (Glc-1), 76.0 (Glc-2), 79.2 (Glc-3), 71.0 (Glc-4), 75.1 (Glc-5), 64.5 (Glc-6), 20.8 (−OCOCH_3), 171.0 (−O*C*OCH$_3$); positive-ion FABMS *m*/*z* 417 [M + Na]$^+$; HRFABMS *m*/*z* 417.1367 (calcd for $C_{16}H_{26}O_{11}Na$ [M + Na]$^+$, 417.1373).

Compound 4a: white powder, $[\alpha]^{24}_{D}$ −22.6 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (4.35), 276 (4.28); IR (KBr) ν_{max} 3423, 2962, 1719, 1636, 1509, 1456, 1076 cm^{-1}; ^{1}H NMR data, see Table 5; ^{13}C NMR (125 MHz, pyridine-d_5) δ_C 177.6 (C-1), 80.7 (C-2), 44.2 (C-3), 173.8 (C-4), 48.8 (C-5), 24.6 (C-6), 24.4 (C-7), 24.8 (C-8), 100.5 (Glc-1), 73.8 (Glc-2), 80.7 (Glc-3), 68.8 (Glc-4), 78.2 (Glc-5), 62.0 (Glc-6), 135.1 (Cin.-1), 128.4 (Cin.-2,6), 129.2 (Cin.-3,5), 130.6 (Cin.-4), 144.5 (Cin.-7), 119.7 (Cin.-8), 167.2 (Cin.-9); positive-ion FABMS *m*/*z* 505 [M + Na]$^+$; HRFABMS *m*/*z* 505.1692 (calcd for $C_{23}H_{30}O_{11}Na$ [M + Na]$^+$, 505.1686).

Compound 5a: white powder, $[\alpha]^{23}_{D}$ −22.4 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (4.42), 278 (4.35); IR (KBr) ν_{max} 3432, 2962, 1718, 1638, 1509, 1455, 1078 cm^{-1}; ^{1}H NMR data, see Table 5; ^{13}C NMR (125 MHz, pyridine-d_5) δ_C 177.7 (C-1), 81.3 (C-2), 44.3 (C-3), 173.9 (C-4), 49.0 (C-5), 24.6 (C-6), 24.3 (C-7), 24.8 (C-8), 100.4 (Glc-1), 76.1 (Glc-2), 76.8 (Glc-3), 72.4 (Glc-4), 76.2 (Glc-5), 62.1 (Glc-6), 134.9 (Cin.-1), 128.6 (Cin.-2,6), 129.2 (Cin.-3,5), 130.7 (Cin.-4), 145.1 (Cin.-7), 119.0 (Cin.-8), 166.5 (Cin.-9); positive-ion FABMS *m*/*z* 505 [M + Na]$^+$; HRFABMS *m*/*z* 505.1692 (calcd for $C_{23}H_{30}O_{11}Na$ [M + Na]$^+$, 505.1686).

Compound 6a: white powder, $[\alpha]^{27}_{D}$ −24.8 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (4.20), 277 (4.30); IR (KBr) ν_{max} 3432, 2962, 1719, 1638, 1509, 1458, 1078 cm^{-1}; ^{1}H NMR data, see Table 5; ^{13}C NMR (125 MHz, pyridine-d_5) δ_C 177.6 (C-1), 81.4 (C-2), 44.2 (C-3), 173.9 (C-4), 49.3 (C-5), 24.6 (C-6), 24.2 (C-7), 24.8 (C-8), 100.5 (Glc-1), 76.0 (Glc-2), 79.3 (Glc-3), 71.1 (Glc-4), 75.2 (Glc-5), 64.7 (Glc-6), 135.0 (Cin.-1), 128.6 (Cin.-2,6), 129.2 (Cin.-3,5), 130.5 (Cin.-4), 144.8 (Cin.-7), 119.1 (Cin.-8), 167.1 (Cin.-9); positive-ion FABMS *m*/*z* 505 [M + Na]$^+$; HRFABMS *m*/*z* 505.1682 (calcd for $C_{23}H_{30}O_{11}Na$ [M + Na]$^+$, 505.1686).

Compound 7a: white powder, $[\alpha]^{27}_{D}$ −21.5 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (4.21), 276 (4.31); IR (KBr) ν_{max} 3432, 2957, 1731, 1638, 1509, 1451, 1246, 1086 cm^{-1}; ^{1}H NMR data, see Table 5; ^{13}C NMR (125 MHz, pyridine-d_5) δ_C 177.4 (C-1), 81.8 (C-2), 44.2 (C-3), 173.7 (C-4), 49.2 (C-5), 24.7 (C-6), 24.3 (C-7), 24.7 (C-8), 100.3 (Glc-1), 73.9 (Glc-2), 80.3 (Glc-3), 69.4 (Glc-4), 74.9 (Glc-5), 64.2 (Glc-6), 20.8 (−OCOCH_3), 170.1 (−O*C*OCH$_3$), 135.0 (Cin.-1), 128.5 (Cin.-2,6), 129.2 (Cin.-3,5), 130.5 (Cin.-4), 144.7 (Cin.-7), 119.5 (Cin.-8), 166.8 (Cin.-9); positive-ion FABMS *m*/*z* 547 [M + Na]$^+$; HRFABMS *m*/*z* 547.1796 (calcd for $C_{25}H_{32}O_{12}Na$ [M + Na]$^+$, 547.1791).

Enzymatic Hydrolysis of 1−7 with Tannase. A solution of **1** (5.7 mg, 0.012 mmol) or **2** (5.1 mg, 0.011 mmol) in H_2O (2.0 mL) was treated with tannase (3.5 mg, from *Aspergillus oryzae*, Wako Pure Chemical Ind., Ltd., Osaka, Japan), and the solution was stirred at 37 °C for 24 h. After EtOH was added, the solvent was removed under reduced pressure and the residue was purified by HPLC [MeOH−1% aqueous HOAc (45:55, v/v)] to furnish **1b**[11] (2.0 mg, 85% from **1**, 1.6 mg, 76% from **2**). Through a similar procedure, a solution of **3** (10.1 mg, 0.011 mmol), **4** (6.1 mg, 0.006 mmol), **5** (7.0 mg, 0.007 mmol), **6** (9.8 mg, 0.010 mmol), or **7** (10.0 mg, 0.009 mmol) in H_2O (2.0 mL) was treated with tannase (5.0 mg), and the solution was stirred at 37 °C for 24 h. Workup as above gave a residue, which was purified by HPLC [MeOH−1% aqueous HOAc (45:55 or 50:50 v/v)] to furnish **3a** (2.8 mg, 66% from **3** or 2.4 mg, 65% from **7**), dactylirhin C (**4b**, 1.5 mg, 72% from **4**, 1.6 mg, 66% from **5**, or 2.4 mg, 71% from **6**), and *trans*-cinnamic acid (0.7 mg, 80% from **4**, 0.7 mg, 70% from **5**, or 1.0 mg, 72% from **6** or 1.0 mg, 73% from **7**).

Acetylation of 3a and 4b. A solution of **3a** (2.3 mg, 0.006 mmol) in pyridine (1.0 mL) was treated with Ac_2O (0.8 mL), and the mixture was stirred at room temperature for 12 h. The reaction mixture was poured into ice−water and extracted with EtOAc. The EtOAc extract was successively washed with 5% HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent under reduced pressure furnished a residue, which was purified by HPLC [MeOH−1% aqueous HOAc (60:40, v/v)] to give **3b** (2.5 mg, 83%). Through a similar procedure, **3b** (2.7 mg, 87%) was also prepared from **4b** (2.1 mg, 0.006 mmol).

Compound 3b: white powder, $[\alpha]^{23}_{D}$ −6.7 (*c* 0.14, MeOH); IR (KBr) ν_{max} 2957, 1748, 1368, 1235, 1038 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) δ 0.91, 1.00 (3H each, both d, J = 6.4 Hz, H$_3$-7, 8), 1.97 (2H, m, H$_2$-5), 1.99 (1H, m, H-6), 1.91, 2.03, 2.04, 2.04 (3H each, all s, −OCOC*H*$_3$), 3.22, 3.60 (1H each, both d, J = 17.4 Hz, H$_2$-3), 4.23 (1H, m, H-5′), 4.93 (1H, dd, J = 8.2, 9.2 Hz, H-2′), [4.38 (1H, dd, J = 2.0, 12.2 Hz), 4.53 (1H, dd, J = 5.2, 12.2 Hz), H$_2$-6′], 5.58 (1H, dd, J = 9.2, 9.8 Hz, H-4′), 6.00 (1H, dd, J = 9.8, 9.8 Hz, H-3′), 6.06 (1H, d, J = 7.6 Hz, H-1′); ^{13}C NMR (125 MHz, pyridine-d_5) δ_C 177.6 (C-1), 80.4 (C-2), 45.4 (C-3), 173.7 (C-4), 48.6 (C-5), 24.4 (C-6), 23.7 (C-7), 24.7 (C-8), 94.4 (Glc-1), 77.8 (Glc-2), 72.6 (Glc-3), 69.3 (Glc-4), 73.6 (Glc-5), 62.4 (Glc-6), 20.4, 20.4, 20.5, 20.5 (−OCO*C*H$_3$), 170.0, 170.0, 170.4, 170.5 (−O*C*OCH$_3$); positive-ion FABMS *m*/*z* 543 [M + Na]$^+$; HRFABMS *m*/*z* 543.1682 (calcd for $C_{22}H_{32}O_{14}Na$ [M + Na]$^+$, 543.1690).

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Supporting Information Available: H−H COSY, H−H HOHAHA, and HMBC correlations of gemnosides I−VII (**1**−**7**) (Figure S1). This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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