Glycosidic Constituents of the Tubers of *Gymnadenia conopsea*

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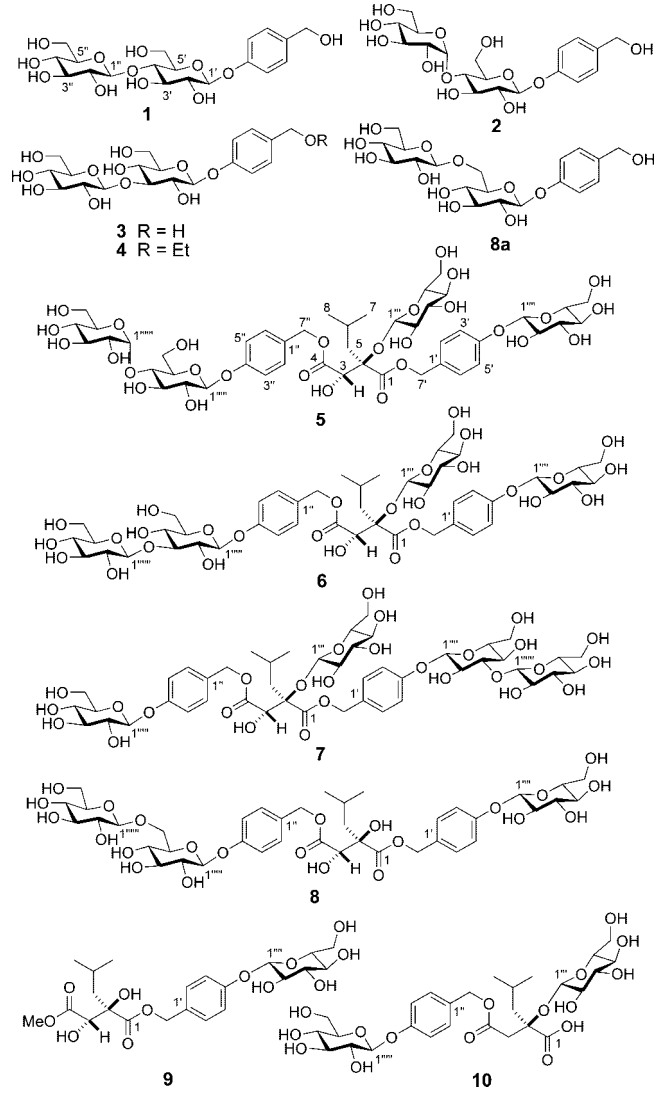
Ten minor new glycosidic constituents (**1**−**10**), together with 10 known compounds, have been isolated from a neuroprotective fraction of an ethanolic extract of the tubers of *Gymnadenia conopsea*. The structures of **1**−**10** were determined using spectroscopic and chemical methods. The compounds isolated were evaluated for activity in in vitro assays for acetylcholine esterase and monoamine oxidase inhibitory activities.

Gymnadenia conopsea R. Br. is a plant belonging to the Orchidaceae family and is distributed in the provinces of Tibet, Xinjiang, Inner Mongolia, Sichuan, Qinghai, and Gansu of mainland China. The dried tubers of this plant and other species of this genus and the genus *Coeloglossum*, known as "Wangla" (Chinese), have been used as a traditional Tibetan remedy to treat cough, asthma, and other syndromes, and as a tonic in Chinese folk medicine, with multiple indications such as invigorating vital energy, promoting the production of body fluids, having a tranquilizing effect, and enhancing intelligence.[1−3] Antiallergic phenanthrenes and stilbenes,[4] as well as nine glycosyloxybenzyl 2-isobutylmalate derivatives,[5] were recently reported from a methanol extract of the tubers of *G. conopsea*.

As part of a program to assess the chemical and biological diversity of traditional Chinese medicines, we have reported chemical and pharmacological investigations of the "Wangla" source species *Coeloglossum viride* (L.) Hartm. var. *bracteatum* (Willd.) Richter, collected from Qinghai Province. A fraction (CE) from an ethanolic extract of *C. viride* var. *bracteatum*, containing mainly mono- and bis(4-β-D-glucopyranosyloxybenzyl) 2-isobutyltartrate and 2-isobutylmalate derivatives,[6] showed neuroprotective effects on memory deficits and pathological changes in senescent mice.[7−9] Meanwhile, a main component of the fraction, dactylorhin B, reduced toxic effects of β-amyloid fragment (25–35) on neuron cells and isolated rat brain mitochondria.[9] By using the same protocol for the tubers of *C. viride* var. *bracteatum*,[10] a fraction was obtained in the present investigation from an ethanolic extract of the tubers of *G. conopsea* collected in Sichuan Province. HPLC analysis indicated that the fraction contained constituents similar to those of the active fraction from the tubers of *C. viride* var. *bracteatum*. An in vivo preliminary pharmacological test indicated it was also active in improving the impaired memory in mice caused by scopolamine and cycloheximide at the same dosage (5.0 mg kg^{-1}).[10] A further chemical investigation of the active fraction has led to the isolation and structural elucidation of 20 glycosidic constituents including 10 minor new compounds (**1**−**10**). By comparing with corresponding literature data, the known compounds were identified as gastrodin,[11] loroglossin,[12,13] militarine,[13] dactylorhins A, B, and E,[14] and coelovirins A−D.[6] This paper deals with the structural elucidation of the new compounds (**1**−**10**) and their preliminary biological evaluation.



Results and Discussion

The ethanolic extract of the dried tubers of *G. conopsea* was suspended in water and then partitioned with EtOAc. The aqueous solution was chromatographed successively over macroporous resin, silica gel, and reversed-phase preparative HPLC to yield 10 minor new glycosidic constituents, **1**−**10**.

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Compound **1** was obtained as a colorless gum, $[\alpha]_D^{20}$ −36.6 (*c* 0.08, MeOH), and showed the presence of hydroxy (3325 cm^{-1}) and aromatic ring (1611 and 1511 cm^{-1}) functional groups in its IR spectrum. The positive ESIMS gave a quasimolecular ion peak at *m/z* 471 $[M + Na]^+$, and the HRESIMS at *m/z* 471.1476 $[M + Na]^+$ indicated the molecular formula of **1** as $C_{19}H_{28}O_{12}$ (calcd for $C_{19}H_{28}O_{12}Na$, 471.1478). The ^1H NMR spectrum of **1** (CD_3OD) showed characteristic signals due to a 4-substitued benzyl alcohol moiety at δ 7.22 (2H, d, $J = 9.0$ Hz, H-2 and H-6), 7.01 (2H, d, $J = 9.0$ Hz, H-3 and H-5), and 4.48 (2H, s, H_2-7). In addition, two diagnostic doublets attributed to anomeric protons at δ 4.87 (1H, d, $J = 7.5$ Hz, H-1′) and 4.39 (1H, d, $J = 8.0$ Hz, H-1″), together with partially overlapped signals assigned to oxymethylene and oxymethine protons between δ 3.17 and 3.85, indicated the presence of two β-glycopyranosyl units in **1**. Acid hydrolysis of **1** produced glucose as the sole sugar identified on the basis of TLC by comparing with an authentic sugar sample. The glucose isolated from the hydrolysate gave a positive optical rotation, $[\alpha]_D^{20}$ +41.3 (*c* 0.35, H_2O) and indicated that it is D-glucose.[15,16] By comparing the ^1H NMR data of **1** with those of the co-occurring gastrodin, the chemical shift and coupling pattern of H_2-7 of **1** suggested that it was a gastrodin derivative with an additional β-D-glucopyranosyl unit substituted at the sugar moiety. The above deduction was supported by the ^{13}C NMR and DEPT spectra of **1** (Table 1). Analysis of $^1H-^1H$ gCOSY and gHSQC spectra led to the unambiguous assignment of the protons and corresponding carbon signals in the NMR spectra (Table 1). In the gHMBC spectrum of **1**, long-range correlations from H_2-7 to C-1 and C-6, from H-2 and H-6 to C-4, from H-3 and H-5 to C-1, and from H-1′ to C-4, in combination with chemical shifts of these protons and carbons, confirmed the presence of the gastrodin moiety in **1**. Also, HMBC correlations from H-1″ to C-4′ and from H-4′ to C-1″ indicated unequivocally that the additional β-D-glucopyranosyl unit was located at C-4′ of the gastrodin moiety. Therefore, the structure of **1** was determined as (−)-4-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxy]benzyl alcohol.

Compound **2** was obtained as a colorless gum, $[\alpha]_D^{20}$ +17.4 (*c* 0.14, MeOH), and showed IR, ESIMS, and NMR spectroscopic features similar to those of **1**. However, in the ^1H NMR spectrum of **2**, an anomeric proton signal attributed to an α-glycosyl unit at δ 5.14 (1H, d, $J = 3.5$ Hz, H-1″) replaced that of the outer β-D-glucopyranosyl of **1**.[17] Acid hydrolysis of **2** yielded D-glucose with $[\alpha]_D^{20}$ +42.1 (*c* 0.15, H_2O) as the only sugar. These data suggested that **2** is an analogue of **1** with an outer α-glucopyranosyl unit. Unambiguous assignments of the NMR data of **2** (Table 1) were accomplished from the 1D TOCSY and 2D NMR spectra of **2**. Meanwhile, the location of the α-glucopyranosyl in **2** was indicated unambiguously by HMBC correlations from H-1″ to C-4′ and from H-4′ to C-2′, C-3′, C-6′, and C-1″. Therefore, the structure of **2** was determined as (+)-4-[α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxy]benzyl alcohol. Although **2** was synthesized earlier, its spectroscopic data were unavailable in the literature.[18]

Compound **3** was obtained as a colorless gum, $[\alpha]_D^{20}$ −17.3 (*c* 0.10, DMSO), and its spectroscopic data (Table 1 and Experimental Section) indicated that it is another isomer of **1** with a different connectivity between the two β-D-glucopyranosyl units. Acid hydrolysis of **3** released D-glucose, with $[\alpha]_D^{20}$ +39.7 (*c* 0.11, H_2O). In the ^{13}C NMR spectrum of **3**, a characteristic resonance at δ_C 87.7 indicated a (1→3) connection between the two β-D-glucopyranosyl moieties in **3**.[19] The NMR data assignments (Tables 1 and 2) and the structure of **3** were confirmed by 2D NMR experiments. Therefore, the structure of **3** was elucidated as (−)-4-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyloxy]benzyl alcohol.

Compound **4** was obtained as colorless needles, $[\alpha]_D^{20}$ −24.0 (*c* 0.25, MeOH), and its positive-mode ESIMS gave quasimolecular ion peaks at *m/z* 499 $[M + Na]^+$ and 515 $[M + K]^+$. The IR and NMR spectra were similar to those of **3**. However, the NMR spectra of **4** showed a pair of additional signals assignable to an ethoxyl at δ 1.14 (3H, t, $J = 7.2$ Hz, H-2‴) and 3.47 (2H, q, $J = 7.2$ Hz, H-1‴) and δ 15.4 (C-2‴) and 75.5 (C-1‴). In addition, C-1 and C-7 of the benzyl moiety in **4** were shifted −2.3 and +4.1 ppm from those of **3** (Table 1), respectively. These data indicated that **4** is the 7-ethyl ether of **3**. Therefore, the structure of **4** was determined as (−)-4-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyloxy]benzyl ethyl ether.

Compound **5** was obtained as a colorless gum, $[\alpha]_D^{20}$ −9.9 (*c* 0.06, MeOH), and showed IR absorption bands for hydroxy (3330 cm^{-1}), carbonyl (1731 cm^{-1}), and aromatic ring (1612 and 1513 cm^{-1}) functional groups. The positive and negative ESIMS data of **5** displayed quasimolecular ion peaks at *m/z* 1089 $[M + Na]^+$ and 1105 $[M + K]^+$, and 1065 $[M - H]^-$, respectively, and the HRESIMS at *m/z* 1089.3595 $[M + Na]^+$ indicated the molecular formula of **5** as $C_{46}H_{66}O_{28}$ (calcd for $C_{46}H_{66}O_{28}Na$, 1089.3638). The ^1H NMR spectrum of **5** in CD_3OD showed diagnostic signals attributed to an isobutyl group at δ 0.70 and 0.84 (each 3H, d, $J =$ 6.5 Hz, H_3-7 and H_3-8), 1.70 (1H, m, H-6), and 2.06 and 1.65 (each 1H, dd, $J = 14.0$ and 5.5 Hz, H-5a and H-5b), in addition to two pairs of partially overlapped A_2B_2 coupling systems and two pairs of AB systems attributed to two 4-*O*-substituted benzyloxy groups at δ 7.21 (2H, d, $J = 9.0$ Hz, H-2′ and H-6′) and 7.03 (2H, d, $J =$ 9.0 Hz, H-3′ and H-5′), and 7.23 (2H, d, $J = 8.5$ Hz, H-2″ and H-6″) and 7.03 (2H, d, $J = 8.5$ Hz, H-3″ and H-5″), and 5.02 (1H, d, $J = 12.5$ Hz, H-7′a) and 4.86 (1H, d, $J = 12.5$ Hz, H-7′b), and 5.07 (1H, d, $J = 12.5$ Hz, H-7″a) and 4.83 (1H, d, $J = 12.5$ Hz, H-7″b). Moreover, the ^1H NMR spectrum showed signals assignable to three anomeric protons with a β-configuration and an anomeric proton with an α-configuration at δ 4.52 (1H, d, $J = 7.0$ Hz, H-1‴), 4.83 (1H, d, $J = 7.5$ Hz, H-1⁗), and 4.89 (1H, d, $J = 8.0$ Hz, H-1⁗′), and 5.15 (1H, d, $J = 3.0$ Hz, H-1⁗″), together with an isolated oxymethine singlet at δ 4.43 (1H, s, H-3), as well as partially overlapped signals attributed to oxymethylenes and oxymethines of four glucosyl moieties (Tables 2 and 3). These spectroscopic data indicated that **5** is a bis(4-glycosyloxybenzyl) 2-isobutyltartrate derivative similar to the co-occurring dactylorhin B.[14] This was confirmed by the ^{13}C NMR and DEPT spectra of **5** that showed carbon signals corresponding to the above moieties (Table 3), including two ester carbonyls at δ 173.8 (C-1) and 172.1 (C-4) and an oxygenated quaternary carbon at δ 86.1 (C-2). Acid hydrolysis of **5** afforded D-glucose, $[\alpha]_D^{20}$ +37.2 (*c* 0.14, H_2O), as the only sugar, indicating that all of the sugar units in **5** are D-glucopyranosyl substituents. After basic hydrolysis of **5** with 3.0% NaOH, gastrodin, coeloverin E,[6] and **2** were obtained from the hydrolysate by chromatographic techniques, and the spectroscopic data of the products were identical to those of the natural products obtained from the plant material. These experimental results demonstrated that **5** is a derivative of dactylorhin B[13,14] with a 4-[α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxy]benzyl moiety replacing a 4-β-D-glucopyranosyloxybenzyl unit.

The structure of **5** was substantiated using several 2D NMR experiments. An analysis of the gCOSY and gHSQC spectra of **5** led to the unambiguous assignment of the protons and corresponding carbon signals in the NMR spectra (Tables 2 and 3). The presence of a 2-isobutyltartrate, two 4-*O*-substituted benzyloxy units, and four glycosyl moieties was proved by two- and three-bond correlations from their protons to carbons in the gHMBC spectrum of **5**. In addition, HMBC correlations from H-7′a and H-7′b to C-1, and from H-1⁗′ to C-4′, in combination with couplings and chemical shifts of H-1⁗′ to H_2-6⁗′ and C-1⁗′ to C-6⁗′, confirmed that the 4-β-D-glucopyranosyloxybenzyl unit is esterified at C-1 of the 2-isobutyltartrate unit in **5**. Other HMBC correlations from H-7″a and H-7″b to C-4, from H-1⁗″ to C-4″, and from H-1⁗‴ to C-4⁗″, together with couplings and chemical shifts of H-1⁗″ to H_2-6⁗″, H-1⁗‴ to H_2-6⁗‴, C-1⁗″ to C-6⁗″, and C-1⁗‴ to C-6⁗‴, revealed that the 4-[α-D-glucopyranosyl-(1→4)-β-D-glu-

Table 1. NMR Spectroscopic Data (δ) of Compounds **1**−**4** and **8a**[a]

	1		**2**		**3**		**4**[b]		**8a**[c]	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		136.7		136.7		136.0		133.7		136.7
2	7.22 d (9.0)	129.4	7.22 d (8.5)	129.4	7.21 d (8.5)	127.7	7.21 d (8.4)	130.3	7.23 d (8.4)	129.7
3	7.01 d (9.0)	117.7	7.01 d (8.5)	117.7	6.97 d (8.5)	116.0	7.02 d (8.4)	117.7	7.04 d (8.4)	117.4
4		158.4		158.4		156.0		158.6		158.4
5	7.01 d (9.0)	117.7	7.01 d (8.5)	117.7	6.97 d (8.5)	116.0	7.02 d (8.4)	117.7	7.04 d (8.4)	117.4
6	7.22 d (9.0)	129.4	7.22 d (8.5)	129.4	7.21 d (8.5)	127.7	7.21 d (8.4)	130.3	7.23 d (8.4)	129.7
7	4.48 s	64.8	4.48 s	64.8	4.41 s	62.4	4.38 s	66.5	4.48 s	64.6
1′	4.87 d (7.5)	102.2	4.86 d (8.0)	102.3	4.95 d (7.5)	99.8	4.90 d (7.2)	101.9	4.92 d (8.0)	101.4
2′	3.46 dd (7.5, 8.5)	74.7	3.45 dd (8.0, 9.5)	74.6	3.43 dd (7.5, 8.5)	71.9	3.60 t (7.2)	73.2		74.3
3′	3.57 t (8.5)	76.3	3.67 t (9.5)	77.7	3.48 t (8.5)	87.7	3.60 t (7.2)	87.7		76.9
4′	3.63 dd (8.5, 9.0)	80.3	3.59 t (9.5)	80.9	3.28, t (8.5)	68.1	3.47 dd (9.6, 7.2)	69.8		70.8
5′	3.52 dt (9.0, 2.5)	76.6	3.50 ddd (9.5, 4.5, 2.0)	76.7	3.38 m	76.4	3.42 ddd (9.6, 5.4, 3.0)	77.83		77.1
6′a	3.84 d (2.5)	61.7	3.85 dd (12.0, 2.0)	62.0	3.67 brd, (11.0)	60.4	3.84 dd (12.0, 3.0)	62.4	4.09 d (11.2)	68.7
6′b	3.84 d (2.5)		3.80 dd (12.0, 4.5)		3.47 dd (11.0, 2.0)		3.66 dd (12.0, 5.4)		3.75 dd (11.2, 6.0)	
1″	4.39 d (8.0)	104. 6	5.14 d (3.5)	102.9	4.35 d (8.0)	104.0	4.54 d (7.8)	105.3	4.31 d (8.0)	104.4
2″	3.19 dd (8.0, 8.5)	74.9	3.40 dd (9.5, 3.5)	74.2	3.07 dd (8.5, 8.0)	73.8	3.24 m	74.3		74.6
3″	3.32 dd (8.5, 9.0)	77.9	3.57 t (9.5)	74.8	3.20 t (8.5)	76.0	3.34 t (9.0)	77.79		77.4
4″	3.26 t (9.0)	71.4	3.23 t (9.5)	71.5	3.04 t (8.5)	70.1	3.23, m	71.6		71.2
5″	3.29 ddd (9.0, 5.0, 2.0)	78.2	3.65 m	75.1	3.20 m	76.9	3.29 m	78.2		78.0
6″a	3.83 dd (12.0, 2.0)	62.5	3.77 dd (12.0, 1.5)	62.8	3.69 brd (10.5)	61.1	3.84 dd (12.0, 3.0)	62.7	3.77 dd (11.6)	61.8
6″b	3.63 dd (12.0, 5.0)		3.63 m		3.40 m		3.60 dd (12.0, 5.4)		3.55−3.64 (m)	

[a] NMR data (δ) were measured in MeOH-d_4 for **1**, **2**, **4**, and **8a** and in DMSO-d_6 for **3** at 400, 500, or 600 MHz for ^{1}H NMR and at 125 or 150 MHz for ^{13}C NMR. Proton coupling constants (J) in Hz are given in parentheses. The assignments are based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC experiments. [b] Data for ethyl: δ 3.47 (2H, q, 7.2 Hz) and 1.14 (3H, t, 7.2 Hz). [c] The partially overlapped signals between δ 3.07 and 3.64 were assigned to H-2′−H-5′ and H-2″−H-5″, respectively.

Table 2. ^{1}H NMR Spectroscopic Data (δ) of Compounds **5**–**10**[a]

position	**5**	**6**	**7**	**8**	**9**[b]	**10**
3a	4.43 s	4.43 s	4.42 s	4.22 s	4.28 s	3.12 d (16.5)
3b						2.75 d (16.5)
5a	2.06 dd (5.5, 14.0)	2.05 dd (5.5, 14.0)	1.94 dd (14.4, 4.0)	1.72 dd (16.0, 8.0)	1.83 dd (14.0, 6.0)	1.72 d (5.5)
5b	1.65 dd (5.5, 14.0)	1.67 dd (5.5, 14.0)	1.71 dd (14.4, 6.8)	1.52 dd (16.0, 6.4)	1.64 dd (14.0, 6.0)	1.72 d (5.5)
6	1.70 m	1.69 m	1.81 m	1.53 m	1.59 m	1.83 m
7	0.70 d (6.5)	0.70 d (6.5)	0.63 d (6.4)	0.66 d (6.4)	0.73 d (6.8)	0.91 d (6.5)
8	0.84 d (6.5)	0.84 d (6.5)	0.79 d (6.4)	0.80 d (6.4)	0.87 d (6.8)	0.91 d (6.5)
2′	7.21 d (9.0)	7.20 d (8.5)	7.22 d (8.0)	7.17 d (8.8)	7.30 d (8.8)	
3′	7.03 d (9.0)	7.03 d (8.5)	6.98 d (8.0)	6.97 d (8.8)	7.05 d (8.8)	
5′	7.03 d (9.0)	7.03 d (8.5)	6.98 d (8.0)	6.97 d (8.8)	7.05 d (8.8)	
6′	7.21 d (9.0)	7.20 d (8.5)	7.22 d (8.0)	7.17 d (8.8)	7.30 d (8.8)	
7′a	5.02 d (12.5)	5.02.d (12.0)	4.97 d (11.6)	4.84 d (12.0)	5.07 s	
7′b	4.86 d (12.5)	4.87.d (12.0)	4.87 d (11.6)	4.77 d (12.0)	5.07 s	
2″	7.23 d (8.5)	7.23 d (8.5)	7.22 d (8.0)	7.24 d (8.8)		7.27 d (8.0)
3″	7.03 d (8.5)	7.03 d (8.5)	6.98 d (8.0)	7.02 d (8.8)		7.05 d (8.0)
5″	7.03 d (8.5)	7.03d (8.5)	6.98 d (8.0)	7.02 d (8.8)		7.05 d (8.0)
6″	7.23 d (8.5)	7.23 d (8.5)	7.22 d (8.0)	7.24 d (8.8)		7.27 d (8.0)
7″a	5.07 d (12.5)	5.08 d (12.0)	4.97 d (11.6)	4.86 d (12.0)		5.05 d (12.0)
7″b	4.83 d (12.5)	4.83 d (12.0)	4.81 d (11.6)	4.98 d (12.0)		4.99 d (12.0)
1‴	4.52 d (7.0)	4.53 d (7.5)	4.67 d (8.0)			4.78 d (7.5)
2‴	3.14 dd (9.0, 7.0)	3.13 dd (9.0, 7.5)	3.00 dd (8.4, 8.0)			3.46 dd (7.5, 9.0)
3‴	3.11 t (9.0)	3.15 t (9.0)	3.18 t (8.4)			3.91 t (9.0)
4‴	3.32 t (9.0)	3.30 t (9.0)	3.06 t (8.4)			3.32 t (9.0)
5‴	2.76 brd (9.0)	2.77 dt (9.0, 3.5)	2.94 m			3.20 dd (9.0, 6.0)
6‴a	3.62 m	3.66 dd (11.5, 3.5)	3.47 dd (11.6, 4.0)			3.80 d (12.0)
6‴b	3.57 m	3.56 dd (11.5, 3.5)	3.45 m			3.62 dd (12.0, 6.0)
1⁗	4.83 d (7.5)	4.83 d (7.5)	4.95 d (7.2)	4.81 d (7.6)	4.85 d (7.6)	
2⁗	3.41 m	3.40 t (7.5)	3.43 dd (8.4, 7.2)	3.22 m	3.39 dd (8.0, 7.6)	
3⁗	3.41 m	3.40 t (7.5)	3.50 t (8.4)	3.10 t (8.4)	3.39 t (8.0)	
4⁗	3.35 t (9.0)	3.34 t (7.5)	3.28 t (8.4)	3.04 m	3.36 dd (8.0, 7.6)	
5⁗	3.36 dd (9.0, 3.0)	3.38 dd (7.5, 3.5)	3.31 m	3.10 m	3.34 ddd (7.6, 5.6, 2.0)	
6⁗a	3.84 brd (12.0)	3.82 brd (10.0)	3.65 brd (11.6)	3.65 m	3.83 dd (12.0, 2.0)	
6⁗b	3.66 dd (12.0, 3.0)	3.64 dd (10.0, 3.5)	3.42 brd (11.6)	3.38 dd (12, 4.8)	3.64 dd (12.0, 5.6)	
1⁗′	4.89 d (8.0)	4.93 d (7.5)	4.81 d (7.6)	4.80 d (6.5)		4.88 d (6.5)
2⁗′	3.45 dd (9.0, 8.0)	3.63 m	3.21 dd (8.0, 7,6)	3.22 m		3.40 m
3⁗′	3.70 t (9.0)	3.64 m	3.26 dd (8.4, 8.0)	3.26 m		3.40 m
4⁗′	3.59 t (9.0)	3.46 m	3.18 t (8.4)	3.18 t (8.0)		3.32 t (9.0)
5⁗′	3.54 dd (9.0, 4.0)	3.46 m	3.07 m	3.55 m		3.40 m
6⁗′a	3.82 d (12.0)	3.84 brd (11.5)	3.65 brd (11.6)	3.94 d (10.4)		3.84 d (12.0)
6⁗′b	3.64 dd (12.0, 4.0)	3.65 m	3.42 m	3.72 m		3.64 dd (12.0, 6.0)
1⁗″	5.15 d (3.0)	4.55 d (8.0)	4.37 d (7.6)	4.18 d (8.0)		
2⁗″	3.41 dd (9.5, 3.0)	3.24 dd (8.5, 8.0)	3.02 dd (8.4, 7.6)	2.95 dd (8.4, 8.0)		
3⁗″	3.59 dd (9.5, 8.5)	3.36 t (8.5)	3.14 t (8.4)	3.01 t (8.4)		
4⁗″	3.24 t (8.5)	3.24 t (8.5)	3.14 t (8.4)	3.11 t (8.4)		
5⁗″	3.66 m	3.30 dd (8.5, 3.5)	3.11 m	3.28 m		
6⁗″a	3.78 brd (11.0)	3.84 brd (11.5)	3.69 m	3.57 m)		
6⁗″b	3.67 m	3.59 dd (11.5, 3.5)	3.65 m	3.44 dd (12.0, 5.6)		

[a] ^{1}H NMR data (δ) were measured in MeOH-d_4 for **5**, **6**, **9**, and **10** and DMSO-d_6 + D_2O for **7** and **8** at 400 or 500 MHz. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC experiments. [b] Data for O*Me*: δ 3.51 (3H, s).

copyranosyloxy]benzyl residue is esterified at C-4 of the 2-isobutyltartrate unit. Furthermore, a HMBC correlation from H-1‴ to C-2 indicated unequivocally that the remaining β-D-glucopyranosyl is located at C-2 of the 2-isobutyltartrate unit. Therefore, the structure of **5** was determined as (−)-(2*R*,3*S*)-1-(4-β-D-glucopyranosyloxybenzyl)-2-*O*-β-D-glucopyranosyl-4-{4-[α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxy]benzyl}-2-isobutyltartrate.

Compound **6** was obtained as a colorless gum, $[\alpha]_D^{20}$ −33.7 (*c* 0.05, MeOH), and its ESIMS, IR, and NMR spectra were similar to those of **5**. HRESIMS indicated that it is an isomer of **5**. Acid hydrolysis of **6** yielded D-glucose, $[\alpha]_D^{20}$ +41.6 (*c* 0.15, H_2O). However, the ^{1}H NMR spectra of **6** indicated the presence of four anomeric protons with a β-configuration at δ 4.93 (1H, d, J = 7.5 Hz, H-1⁗′), 4.83 (1H, d, J = 7.5 Hz, H-1⁗), 4.55 (1H, d, J = 8.0 Hz, H-1⁗″), and 4.53 (1H, d, J = 7.5 Hz, H-1‴). A comparison of the NMR data between **5** and **6**, and between **3** and **6** (Tables 2 and 3), together with the observation of a diagnostic carbon signal at δ 87.6 (C-3⁗′) in the ^{13}C NMR spectrum of **6**, suggested the presence of a 4-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyloxy]benzyl moiety in **6**. This was confirmed by basic hydrolysis of **6**, which afforded gastrodin, coeloverin E, and **3**. The location of the 4-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyloxy]benzyl moiety was also determined by an extensive analysis of 2D NMR spectra of **6**, which resulted in the unambiguous assignment of NMR data of **6** (Tables 2 and 3). Especially, in the HMBC spectrum of **6**, correlations from both H-7″a and H-7″b to C-4, from H-1⁗″ to C-4″, and from H-1⁗′ to C-3⁗′ indicated that the 4-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyloxy]benzyl unit was located at C-4 of the 2-isobutyltartrate moiety in **6**. Therefore, the structure of **6** was determined as (−)-(2*R*,3*S*)-1-(4-β-D-glucopyranosyloxybenzyl)-2-*O*-β-D-glucopyranosyl-4-{4-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyloxy]benzyl}-2-isobutyltartrate.

Compound **7** was obtained as a colorless gum, $[\alpha]_D^{20}$ −36.1 (*c* 0.03, MeOH), and its spectroscopic data (Tables 2 and 3 and Experimental Section) were very similar to those of **6**. Acid and basic hydrolysis of **7** yielded products identical to those of **6**. This indicated that **7** is an isomer of **6** in which the location of the 4-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyloxy]benzyl and 4-β-D-glucopyranosyloxybenzyl units are transposed. The 2D NMR spectra of **7** confirmed the deduction and led to the assignment of NMR data of **7** (Tables 2 and 3). Therefore, the structure of **7** was determined as (−)-(2*R*,3*S*)-1-{4-[β-D-glucopyranosyl-(1→3)-β-D-

Table 3. ^{13}C NMR Spectroscopic Data (δ) of Compounds **5**−**10**[a]

position	5	6	7	8	9[b]	10
1	173.8	173.8	170.8	173.4	174.8	172.3
2	86.1	86.1	84.1	79.7	81.0	80.9
3	75.8	75.8	74.5	76.3	77.5	49.8
4	172.1	172.1	170.3	171.3	173.1	171.6
5	46.8	46.8	43.6	44.3	45.1	45.3
6	24.9	24.9	22.8	23.8	25.2	25.2
7	24.0	24.0	23.1	23.7	24.0	24.9
8	24.7	24.7	24.4	24.6	24.6	24.0
1′	130.3	130.3	127.7	129.3	130.7	
2′	131.5	131.5	129.7	130.2	131.6	
3′	117.9	117.9	116.1	116.57	117.7	
4′	159.4	159.35	157.1	157.6	159.3	
5′	117.9	117.9	116.1	116.57	117.7	
6′	131.5	131.5	129.7	130.2	131.6	
7′	68.4	68.5	66.1	66.43	68.1	
1″	130.3	130.4	128.7	129.3		130.9
2″	132.1	132.0	130.0	130.5		131.3
3″	118.0	118.0	116.2	116.64		117.9
4″	159.5	159.42	157.4	157.6		159.3
5″	118.0	118.0	116.2	116.64		117.9
6″	132.1	132.0	130.0	130.5		131.3
7″	68.3	68.3	66.1	66.40		67.6
1‴	99.5	99.5	97.3			94.9
2‴	75.5	75.46	73.9			74.98
3‴	78.1	78.1	77.0			81.2
4‴	70.1	70.1	70.1			71.4
5‴	77.2	77.2	76.7			79.6
6‴	61.6	61.6	60.1			62.3
1⁗	102.3	102.3	99.6	100.6	102.2	
2⁗	75.1	74.9	71.9	73.5	74.9	
3⁗	77.9	77.8	87.7	76.6	78.0	
4⁗	71.3	71.3	67.6	70.4	71.4	
5⁗	78.1	77.9	76.5	77.0	78.2	
6⁗	62.5	62.4	60.4	61.3	62.5	
1⁗′	102.1	101.8	100.3	100.6		102.3
2⁗′	74.5	74.2	73.2	73.5		74.92
3⁗′	77.6	87.6	77.0	76.1		78.1
4⁗′	80.9	69.8	69.7	69.9		71.4
5⁗′	76.7	77.7	76.0	76.7		78.0
6⁗′	62.0	62.5	60.7	68.7		62.5
1⁗″	102.9	105.2	104.0	103.6		
2⁗″	74.2	75.52	73.8	73.8		
3⁗″	74.9	78.1	76.6	76.5		
4⁗″	71.5	71.6	68.0	70.0		
5⁗″	74.9	78.1	76.4	77.2		
6⁗″	62.8	62.6	61.1	61.0		

[a] ^{13}C NMR data (δ) were measured in MeOH-d_4 for **5**, **6**, **9**, and **10** and DMSO-d_6 + D_2O for **7** and **8** at 100, 125, or 150 MHz. The assignments were based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC experiments. [b] Data for O*Me*: δ 52.5.

glucopyranosyloxy]benzyl}-2-*O*-β-D-glucopyranosyl-4-(4-β-D-glucopyranosyloxybenzyl)-2-isobutyltartrate.

Compound **8**, obtained as a colorless gum, $[\alpha]_D^{20}$ −29.9 (*c* 0.07, MeOH), showed IR absorption bands similar to those of **6**. The positive- and negative-mode ESIMS data showed quasimolecular ion peaks at *m*/*z* 927 $[M + Na]^+$ and 943 $[M + K]^+$, and 903 $[M - H]^-$, respectively. HRESIMS at *m*/*z* 927.3054 $[M + Na]^+$ indicated the molecular formula of **8** to be $C_{40}H_{56}O_{23}$, one glucopyranosyl unit less than in **6**. A comparison of the NMR spectroscopic data between **6** and **8** (Tables 2 and 3) indicated that the data assigned to the β-D-glucopyranosyl at C-2 in **6** were absent in the NMR spectra of **8**, and the C-2 signal of **8** was shifted 6.4 ppm upfield from that of **6**. In addition, the data assigned to C-3⁗′ and C-6⁗′ of the di-β-D-glucopyranosyl unit were shifted from δ 87.6 and 62.5 of **6** to δ 76.1 and 68.7 of **8**, respectively. This indicated that the β-D-glucopyranosyloxy at C-2 and the β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyloxy at C-4″ in **6** were replaced by a hydroxy and a β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyloxy in **8**, respectively. Acid hydrolysis of **8**, with 2 N HCl at 80 °C for 6 h, produced D-glucose, $[\alpha]_D^{20}$ +40.0 (*c* 0.13, H_2O), while basic hydrolysis of **8** gave gastrodin, **8a**, and (+)-(2*R*,3*S*)-2-isobutyltartric acid.[14] The structure of **8a** was identified as (-)-4-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyloxybenzyl alcohol from its ^{1}H and ^{13}C NMR and ESIMS data (Table 1 and Experimental Section). In the HMBC spectrum of **8**, correlations of C-1 with both H-7′a and H-7′b, C-4 with both H-7″a and H-7″b, C-4′ with H-1‴, C-4″ with H-1⁗, and C-6⁗′ with H-1⁗′, in combination with chemical shifts of these protons and carbons, confirmed the connection among the structural moieties in **8**. Therefore, the structure of **8** was determined as (−)-(2*R*,3*S*)-1-(4-β-D-glucopyranosyloxybenzyl)-4-{4-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyloxy]benzyl}-2-isobutyltartrate.

Compound **9** was obtained as colorless needles, with $[\alpha]_D^{20}$ −13.4 (*c* 0.08, MeOH), and its positive-mode ESIMS gave quasimolecular ion peaks at *m*/*z* 511 $[M + Na]^+$ and 527 $[M + K]^+$. The IR and NMR spectroscopic features of **9** were very similar to those of the co-occurring coelovirin A.[6] However, the NMR spectra of **9** showed additional signals due to a methoxy group at δ_H 3.51 and δ_C 52.5. A comparison of the ^{13}C NMR data between coelovirin A and **9** indicated that C-4 of **9** was shifted upfield by 1.4 ppm. These spectroscopic data revealed **9** to be coelovirin A methyl ester. Therefore, the structure of **9** was determined as (−)-(2*R*,3*S*)-1-(4-β-D-glucopyranosyloxybenzyl)-4-methyl-2-isobutyltartrate.

Compound **10** was obtained as a colorless gum, $[\alpha]_D^{20}$ −13.2 (*c* 0.43, MeOH), and its positive-mode ESIMS gave a quasimolecular ion peak at *m*/*z* 643 $[M + Na]^+$. The UV, IR, and NMR spectroscopic data were similar to those of **9** (Tables 2 and 3 and Experimental Section). However, a comparison of the NMR spectroscopic data of **9** and **10** indicated that the signals assigned to the oxymethine (H-3 and C-3) of the 2-isobutyltartric acid moiety in **9** were replaced by those assignable to an isolated aliphatic methylene of a 2-isobutylmalate moiety at δ 3.12 (1H, d, J = 16.5 Hz, H-3a) and 2.75 (1H, d, J = 16.5 Hz, H-3b) and δ 49.8 (C-3). This indicated that **10** is a 2-isobutylmalate derivative similar to the co-occurring dactylorhin E.[14] Comparison of NMR data between **10** and dactylorhin E showed that the data of C-1 and C-4 of **10** were shifted 3.3 and 2.3 ppm upfield, respectively, from those of dactylorhin E. This indicated that the 4-β-D-glucopyranosyloxybenzyl was esterified with the terminal carboxylic group (C-4) of the 2-isobutylmalic acid unit in **10**. This was confirmed by 2D NMR experiments of **10**. The basic hydrolysis of **10** yielded gastrodin and dactylorhin C, $[\alpha]_D^{20}$ −20.7 (*c* 1.01, H_2O).[14] Consequently, the structure of **10** was determined as (−)-(2*R*)-2-*O*-β-D-glucopyranosyl-4-(4-β-D-glucopyranosyloxybenzyl)-2-isobutylmalate.

Compound **2** may be generated from **5**, and compound **3** from **6** and **7** during the isolation procedure. However, **2** and **3** may also be biosynthetic precursors of **5**−**7**. With this hypothesis, the fresh tubers of this plant were collected in September 2007, from the initial collection location, and an ethanolic extract of the fresh material was analyzed by HPLC/UV/ESIMS. However, none of the new compounds (**1**−**10**) were detectable. In addition, in simulated isolation conditions by heating the aqueous ethanol (50%) solutions of compounds **5**−**10** and the purified known compounds with the ester bond, with or without silica gel at 50 °C for 36 h, they were not hydrolyzed on the basis of TLC and HPLC analysis. In addition, the ethylation of **3** did not occur as a result of heating the ethanol solution of **3** with and without silica gel at 50 °C for 50 h.

Pharmacological studies have shown that the active fraction containing mono- and bis(4-β-D-glucopyranosyloxybenzyl) 2-isobutyltartrate and 2-isobutylmalate derivatives[6] showed neuroprotective effects on memory deficits and pathological changes in senescent mice,[7,8] while the main component, dactylorhin B, reduced toxic effects of β-amyloid fragment (25–35) on neuron cells and isolated rat brain mitochondria.[9] In in vitro assays against acetylcholine esterase (AChE) and monoamine oxidase (MAO), compounds **1**−**10** were inactive at 10^{-5} M.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a PE model 343 polarimeter. UV spectra were measured on a Cary 300 spectrometer. IR spectra were recorded on a Nicolet 5700 FT-IR microscope spectrometer (FT-IR microscope transmission). 1D- and 2D-NMR spectra were obtained at 400, 500, or 600 MHz for ^{1}H and 100, 125, or 150 MHz for ^{13}C, respectively, on INOVA 400, 500, and 600 MHz spectrometers in MeOH-d_4 or DMSO-d_6, with solvent peaks as references. ESIMS data were measured with a Q-Trap LC/MS/MS (Turbo Ionspray source) spectrometer. HRESIMS data were measured using a JMS-T100CS AccuToF CS spectrometer. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc. Qingdao, People's Republic of China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). HPLC separation was performed on an instrument consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector, with an Alltima (250 × 10 mm i.d.) preparative column packed with C_{18} (5 μm). TLC was carried out with glass precoated silica gel GF254 plates. Spots were visualized under UV light or by spraying with 7% H_2SO_4 in 95% EtOH followed by heating.

Plant Material. The tubers of *G. conoposa* were collected at Ganzi, Sichuan Province, People's Republic of China, in September 2005. The plant identification was verified by Associate Professor Lin Ma (Institute of Materia Medica). A voucher specimen (No. 05916) is deposited at the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica.

Extraction and Isolation. The air-dried tubers of *G. conoposa* (16.2 kg) were powdered and extracted with 20.0 L of EtOH at room temperature for 4 × 42 h. The EtOH extract was evaporated under reduced pressure at <40 °C to yield a residue (618.2 g). The residue was suspended in H_2O (2000 mL) and then partitioned with EtOAc (5 × 1200 mL). The aqueous phase was applied to a HP-20 macroporous adsorbent resin (1500 g) column. Successive elution of the column with H_2O, 15% EtOH, 40% EtOH, and 95% EtOH (5000 mL each) yielded four fractions after removing the solvents. The active fraction (100.2 g) eluted by 40% EtOH was suspended in H_2O (500 mL) and then partitioned with *n*-BuOH (5 × 400 mL) to give H_2O and *n*-BuOH fractions. The H_2O (40 g) fraction was separated by medium-pressure liquid chromatography over reversed-phase silica gel eluting with a gradient of increasing MeOH (0–90%) in H_2O to give five fractions (A−E) on the basis of TLC analysis. Fraction B (0.12 g) was subjected to column chromatography over Sephadex LH-20, using MeOH−H_2O (50:50) as the eluting solvent, to afford four subfractions, B_1−B_4. Subfractions B_2 (66 mg) and B_3 (38 mg) were separately purified by reversed-phase preparative HPLC, using the mobile phase MeOH−H_2O (12:88), to afford **1** (15 mg, 0.000093%), **2** (17 mg, 0.00011%), **3** (27 mg, 0.00017%), and **4** (13 mg, 0.000080%). Separation of fraction E (9.72 g) by normal-phase silica gel column chromatography, eluting with a gradient of increasing MeOH (0–100%) in $CHCl_3$, afforded seven subfractions (E_1−E_7). Subfraction E_1 (751 mg) was further separated by silica gel column chromatography, using $CHCl_3$−MeOH−H_2O as the eluting solvent, and then purified by reversed-phase preparative HPLC, using MeOH−H_2O (29:71) as the mobile phase, to yield **5** (43 mg, 0.00026%), **6** (31 mg, 0.00019%), **7** (25 mg, 0.00015%), and **8** (21 mg, 0.00013%). Subfractions E_3 (105 mg) and E_7 (52 mg) were separately purified by preparative reversed-phase HPLC, using MeOH−H_2O (32:68 and 42:58) as the mobile phases, respectively, to afford **9** (91 mg, 0.00056%) and **10** (25 mg, 0.00015%).

(−)-4-[β-D-Glucopyranosyl-(1→4)-β-D-glucopyranosyloxy]benzyl alcohol (1): colorless gum, $[\alpha]_D^{20}$ −36.6 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (3.87), 222 (3.99), 270 (2.97) nm; IR ν_{max} 3325, 2883, 1611, 1511, 1411, 1230, 1164, 1023, 898, 830 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table 1; positive-mode ESIMS *m/z* 471 $[M + Na]^+$; negative-mode ESIMS *m/z* 447 $[M - H]^-$; HRESIMS *m/z* 471.1476 $[M + Na]^+$ (calcd for $C_{19}H_{28}O_{12}Na$, 471.1478).

(+)-4-[α-D-Glucopyranosyl-(1→4)-β-D-glucopyranosyloxy]benzyl alcohol (2): colorless gum, $[\alpha]_D^{20}$ +17.4 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (3.82), 221 (3.96), 270 (2.89) nm; IR ν_{max} 3334, 2924, 2882, 1608, 1589, 1511, 1407, 1230, 1145, 1035, 831, 782 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Table 1; positive-mode ESIMS *m/z* 471 $[M + Na]^+$ and 487 $[M + K]^+$; negative-mode ESIMS *m/z* 447 $[M - H]^-$.

(−)-4-[β-D-Glucopyranosyl-(1→3)-β-D-glucopyranosyloxy]benzyl alcohol (3): colorless gum, $[\alpha]_D^{20}$ −17.3 (*c* 0.10, DMSO); UV (MeOH) λ_{max} (log ϵ) 201 (3.91), 220 (4.02), 270 (2.93) nm; IR ν_{max} 3457, 3333, 2863, 1611, 1509, 1468, 1411, 1367, 1234, 1171, 1118, 1082, 1028, 992, 902, 822 cm^{-1};^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 1; positive-mode ESIMS *m/z* 471 $[M + Na]^+$; negative-mode ESIMS *m/z* 447 $[M - H]^-$; HRESIMS *m/z* 471.1476 $[M + Na]^+$ (calcd for $C_{19}H_{28}O_{12}Na$, 471.1478).

(−)-4-[β-D-Glucopyranosyl-(1→3)-β-D-glucopyranosyloxy]benzyl ethyl ether (4): colorless needles, $[\alpha]_D^{20}$ −24.0 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (3.84), 222 (3.87), 273 (2.99) nm; IR ν_{max} 3364, 2879, 1612, 1513, 1412. 1375, 1237, 1164, 1083, 894, 851, 827 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Table 1; positive-mode ESIMS *m/z* 499 $[M + Na]^+$ and 515 $[M + K]^+$; negative-mode ESIMS *m/z* 475 $[M - H]^-$.

(−)-(2*R*,3*S*)-1-(4-β-D-Glucopyranosyloxybenzyl)-2-*O*-β-D-glucopyranosyl-4-{4-[α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxy]benzyl}-2-isobutyltartrate (5): colorless gum, $[\alpha]_D^{20}$ −9.9 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.33), 224 (4.36), 270 (3.19) nm; IR ν_{max} 3330, 2925, 1731, 1612, 1513, 1413, 1308, 1226, 1164, 1067, 1032, 928, 897, 830 cm^{-1};^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Tables 2 and 3; positive-mode ESIMS *m/z* 1089 $[M + Na]^+$ and 1105 $[M + K]^+$; negative-mode ESIMS *m/z* 1065 $[M - H]^-$; HRESIMS *m/z* 1089.3595 $[M + Na]^+$ (calcd for $C_{46}H_{66}O_{28}Na$, 1089.3638).

(−)-(2*R*,3*S*)-1-(4-β-D-Glucopyranosyloxybenzyl)-2-*O*-β-D-glucopyranosyl-4-{4-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyloxy]benzyl}-2-isobutyltartrate (6): colorless gum, $[\alpha]_D^{20}$ −33.7 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.34), 224 (4.39), 270 (3.23) nm; IR ν_{max} 3321, 2917, 1731, 1612, 1512, 1413, 1308, 1223, 1163, 1066, 1016, 927, 896, 828 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Tables 2 and 3; positive-mode ESIMS *m/z* 1089 $[M + Na]^+$ and 1105 $[M + K]^+$; negative-mode ESIMS *m/z* 1065 $[M - H]^-$; HRESIMS *m/z* 1089.3610 $[M + Na]^+$ (calcd for $C_{46}H_{66}O_{28}Na$, 1089.3638).

(−)-(2*R*,3*S*)-1-{4-[β-D-Glucopyranosyl-(1→3)-β-D-glucopyranosyloxy]benzyl}-2-*O*-β-D-glucopyranosyl-4-(4-β-D-glucopyranosyloxybenzyl)-2-isobutyltartrate (7): colorless gum, $[\alpha]_D^{20}$ −36.1 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.29), 224 (4.31), 270 (3.15) nm; IR ν_{max} 3349, 2920, 1730, 1612, 1512, 1414, 1307, 1224, 1163, 1066, 1015, 927, 897, 830 cm^{-1}; ^{1}H NMR (DMSO-d_6 +D_2O, 400 MHz) and ^{13}C NMR (DMSO-d_6 +D_2O, 150 MHz) data, see Tables 2 and 3; positive-mode ESIMS *m/z* 1089 $[M + Na]^+$ and 1105 $[M + K]^+$; negative-mode ESIMS *m/z* 1065 $[M - H]^-$ and 1101 $[M + Cl]^-$; HRESIMS *m/z* 1089.3682 $[M + Na]^+$ (calcd for $C_{46}H_{66}O_{28}Na$, 1089.3638).

(−)-(2*R*,3*S*)-1-(4-β-D-Glucopyranosyloxybenzyl)-4-{4-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyloxy]benzyl}-2-isobutyltartrate (8): colorless gum; $[\alpha]_D^{20}$ −29.9 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.27), 222 (4.32), 270 (3.12) nm; IR ν_{max} 3297, 2920, 1732, 1612, 1513, 1415, 1308, 1227, 1164, 1071, 1042, 928, 897, 831 cm^{-1}; ^{1}H NMR (DMSO-d_6 + D_2O, 400 MHz) and ^{13}C NMR (DMSO-d_6 + D_2O, 125 MHz) data, see Tables 2 and 3; positive-mode ESIMS *m/z* 927 $[M + Na]^+$ and 943 $[M + K]^+$; negative-mode ESIMS *m/z* 903 $[M - H]^-$; HRESIMS *m/z* 927.3054 $[M + Na]^+$ (calcd for $C_{46}H_{66}O_{28}Na$, 927.3110).

(−)-(2*R*,3*S*)-1-(4-β-D-Glucopyranosyloxybenzyl)-4-methyl-2-isobutyltartrate (9): colorless gum; $[\alpha]_D^{20}$ −13.4 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (4.30), 223 (4.35), 270 (3.17) nm; IR ν_{max} 3345, 2951, 1731, 1611, 1513, 1440, 1399, 1300, 1218, 1188, 1074, 1046, 1018, 979, 900, 839 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data, see Tables 2 and 3; positive-mode ESIMS *m/z* 511 $[M + Na]^+$ and 527 $[M + K]^+$.

(−)-(2*R*)-2-*O*-β-D-Glucopyranosyl-4-(4-β-D-glucopyranosyloxybenzyl)-2-isobutylmalate (10): colorless gum, $[\alpha]_D^{20}$ −13.2 (*c* 0.43, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (4.34), 222 (4.34) 270 (3.20) nm; IR ν_{max} 3389, 2931, 2888, 1760, 1727, 1713, 1612, 1512, 1398, 1382, 1291, 1233, 1190, 1072, 1051, 1014, 975, 827 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data, see Tables 2 and 3; positive-mode ESIMS *m/z* 643 $[M + Na]^+$; negative-mode ESIMS *m/z* 619 $[M - H]^-$.

Acidic Hydrolysis of 1–10. A solution of each compound (5−10 mg) in 2 N HCl (5.0 mL) was individually refluxed at 80 °C for

6 h. The reaction mixture was extracted with EtOAc (3 × 5 mL), and the aqueous phase was neutralized with 1 N NaOH and dried using a stream of N_2. The residue was subjected to column chromatography over silica gel with MeCN−H_2O (8:1) to yield glucose (1.1−2.6 mg) from compounds **1**−**10**. The solvent systems $CHCl_3$−MeOH (2.5:1) and MeCN−H_2O (6:1) were used for TLC identification of glucose. Glucose from compounds **1**−**10** gave positive optical rotation, with $[\alpha]_D^{20}$ values in a range +37.2 to +44.8 (*c* in a range of 0.11 to 0.35, H_2O).

Basic Hydrolysis of 5–10. A solution of each compound in 3% NaOH (5 mL) was stirred at room temperature for 2 h. The reaction mixture was acidified to pH 2 by 2 N HCl and then evaporated under reduced pressure to give a dry residue. The residue was dissolved in 5 mL of H_2O and then subjected to column chromatography over ODS (20 g). The column was successively eluted with H_2O, 5% MeCN, 15% MeCN, and 50% MeCN (100 mL, each). The fraction eluted by 5% MeCN was evaporated under reduced pressure and then subjected to reversed-phase preparative HPLC using 11% MeOH as mobile phase. From the hydrolysates of compounds **5**−**10** (6.1–10.2 mg), gastrodin (1.3–2.4 mg) was obtained with a yield range of 58%−73%. Coeloverin E (2.4, 1.9, and 1.3 mg) was obtained from the hydrolysates of **5** (10.1 mg), **6** (8.1 mg), and **7** (6.2 mg), respectively. 2-Isobutyltartric acid (1.1 and 1.6 mg) was obtained from the hydrolysates of **8** (6.1 mg) and **9** (6.0 mg), respectively. Dactylorhin C (2.3 mg) was obtained from **10** (6.1 mg). In addition, compound **2** (2.8 mg) was produced from **5**, while compound **3** (2.2 and 1.4 mg) was produced from **6** and **7**. The ^{1}H NMR, ESIMS, and optical rotation data of gastrodin, coeloverin E, dactylorhin C, 2-isobutyltartric acid, and compounds **1** and **2**, obtained from the hydrolysates, were identical to those of the natural products isolated from the plant material and reported in the literature.[6,11,13,14] Compound **8a** (1.9 mg) was obtained as a colorless gum from the hydrolysate of **8**: $[\alpha]_D^{20}$ −28.3 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (3.79), 221 (3.92), 270 (2.86) nm; IR ν_{max} 3272, 2923, 2902, 2865, 1611, 1511, 1378, 1244, 1170, 1083, 1049, 1035, 887, 833, 650 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Table 1; positive-mode ESIMS *m*/*z* 471 $[M + Na]^+$.

Enzymatic Inhibitory Assay. The fresh rat brains were quickly prepared into brain homogenate in 9-fold volume ice-cold PBS and incubated with tested compounds, separately.

AChE inhibitory activity was evaluated spectrophotometrically at 25 °C by the method reported by Ellman,[20] and donepezil was used as the positive control. At 10^{-5} M, inhibition rates of the tested compounds were less than 10%, and donepezil gave an inhibition rate of 77.2%.

The activity of monoamine oxidase-B was determined by quantifying the oxidative product of benzyl amines,[21] with pargyline as the positive control. At 10^{-5} M, the tested compounds gave inhibition rates less than 15.2%, and pargyline had an inhibition rate of 94.5%.

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Supporting Information Available: NMR spectra of compounds **1**–**10** and **8a**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Beijing Institute of Botany, The Chinese Academy of Sciences. *Iconographia Cormophytorum Sinicorum*, Tomus V; Science Press: Beijing, 1976; Vol. 5, p 631.
(2) Jiangsu New Medical College. *Dictionary of Traditional Chinese Medicine*; Shanghai Science and Technology Publishing House: Shanghai, 1977; Vol. 1, pp 436–437..
(3) Lang, K-Y.; Chen, X.-Q.; Zhu, G.-H. In *Chinese Flora (Zhongguo ZhiwuZhi)*; Science Press: Beijing, 1999; Vol. 17, pp 389–390.
(4) Matsuda, H.; Morikawa, T.; Xie, H.-H.; Yoshikawa, M. *Planta Med.* **2004**, *70*, 847–855.
(5) Morikawa, T.; Xie, H.-H; Matsuda, H.; Yoshikawa, M. *J. Nat. Prod.* **2006**, *69*, 881–886.
(6) (a) Huang, S.-Y.; Shi, J.-G.; Yang, Y.-C.; Hu, S.-L. *Chin. Chem. Lett.* **2002**, *13*, 551–554. (b) Huang, S.-Y.; Shi, J.-G.; Yang, Y.-C.; Tu, P.-F. *Chin. Chem. Lett.* **2003**, *14*, 814–817. (c) Huang, S.-Y.; Li, G.-Q.; Shi, J.-G.; Mo, S.-Y.; Wang, S.-J.; Yang, Y.-C. *J. Asian Nat. Prod. Res.* **2004**, *6*, 49–61.
(7) (a) Zhang, D.; Wang, Y.-F.; Zhang, J.-J. *Zhong Guo Xin Yao Za Zhi* **2005**, *14*, 1301–1304. (b) Zhang, D.; Zhang, J.-J. *Chin. J. Pharmacol. Toxicol.* **2005**, *19*, 259–262. (c) Zhang, D.; Zhang, J.-J. *Zhong Guo Yi Xue Ke Xue Yuan Xue Bao* **2005**, *27*, 729–733.
(8) (a) Zhang, D.; Liu, G.-T.; Shi, J.-G.; Zhang, J.-J. *J. Ethnopharmacol.* **2006**, *104*, 250–256. (b) Zhang, D.; Liu, G.-T.; Shi, J.-G.; Zhang, J.-J. *Basic Clin. Pharmacol. Toxicol.* **2006**, *98*, 55–60.
(9) Zhang, D.; Zhang, Y.; Liu, G.-T.; Zhang, J.-J. *Naunyn-Schmiedbergs Arch. Pharmacol.* **2006**, *374*, 117–125.
(10) Zhang, J.-J.; Shi, J.-G.; Wang, Y.-F.; Zhang, D.; Gao, M.; Yang, Y.-C.; Huang, S.-Y. PCT patent WO 2004 058244, 2004.
(11) Dahman, J.; Leander, K. *Phytochemistry* **1976**, *15*, 1986–1987.
(12) Gray, R. W.; Guggisberg, A.; Segebarth, K. P.; Hesse, M.; Schmid, H. *Helv. Chim. Acta* **1977**, *60*, 1304–1311.
(13) Aasen, A.; Behr, D.; Leander, K. *Acta Chem. Scand. Ser. B* **1975**, *29*, 1002–1004.
(14) Kizu, H.; Kaneko, E.; Tomimori, T. *Chem. Pharm. Bull.* **1999**, *47*, 1618–1625.
(15) Hudson, C. S.; Dale, J. K. *J. Am. Chem. Soc.* **1917**, *39*, 320–328.
(16) Bednarski, M.; Danishefsky, S. *J. Am. Chem. Soc.* **1986**, *108*, 7060–7067.
(17) Sugimoto, K.; Nishimura, T.; Nomura, K.; Sugimoto, K.; Kuriki, T. *Chem. Pharm. Bull.* **2003**, *51*, 798–801.
(18) Heiferich, B.; Petersen, S. R.; *Abhandlungen***1935**, *68B*, 790–794; *Chem. Abstr.* **1935**, *29*, 5091.
(19) (a) Viladot, J. L.; Moreau, V.; Planas, A.; Driguez, H. *J. Chem. Soc., Perkin Trans. 1* **1997**, *16*, 2383–2388. (b) Karthaus, O.; Jansson, P.-E.; Kenne, L.; Schweda, E. *J. Chem. Soc., Perkin Trans. 1* **1988**, *7*, 2729–2736.
(20) Ellman, G. L.; Courtney, K. D.; Andres, B.; Featherstone, R. M., Jr *Biochem. Pharmacol.* **1961**, *7*, 88–95.
(21) Nag, M.; Nandy, N. *Indian J. Exp. Biol.* **2001**, *39*, 802–806.

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