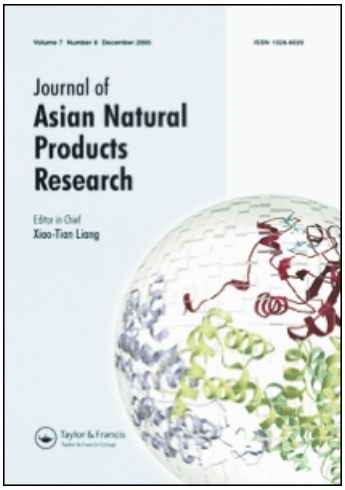

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CHEMICAL CONSTITUENTS OF THE RHIZOMES OF *COELOGLOSSUM VIRIDE* VAR. *BRACTEATUM*

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This paper is dedicated to Professor Xiao-Tian Liang on the occasion of his 80th birthday.

Seven new compounds, named coelovirins A–G (**1**–**7**), along with fourteen known constituents were isolated from the rhizomes of *Coeloglossom viride* var. *bracteatum* (Orchidaceae). On the basis of chemical and spectroscopic methods, including 2D-NMR techniques, the structures of new compounds were elucidated as 1-(4-β-D-glucopyranosyloxybenzyl)-(2*R*,3*S*)-2-isobutyltartrate (**1**), 4-(4-β-D-glucopyranosyloxybenzyl)-(2*R*,3*S*)-2-isobutyltartrate (**2**), 1-(4-β-D-glucopyranosyloxybenzyl)-(2*R*,3*S*)-2-β-D-glucopyranosyl-2-isobutyltartrate (**3**), 4-(4-β-D-glucopyranosyloxybenzyl)-(2*R*,3*S*)-2-β-D-glucopyranosyl-2-isobutyltartrate (**4**), (2*R*,3*S*)-2-β-D-glucopyranosyl-2-isobutyltartaric acid (**5**), bis(4-β-D-glucopyranosyloxybenzyl)-(2*R*,3*S*)-2-[β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl]-2-isobutyltartrate (**6**) and bis(4-β-D-glucopyranosyloxybenzyl)-(2*R*)-2-[β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl]-2-isobutylmalate (**7**). The known compounds are 4-hydroxybenzaldehyde, 4-hydroxybenzyl alcohol, 4,4′-dihydroxydibenzyl ether, 4,4′-dihydroxydiphenylmethane, 4-(4-hydroxybenzyloxy)benzyl alcohol, gastrodin, quercetin-3,7-diglucoside, thymidine, loroglossin, militarine, dactylorhin A, dactylorhin B, β-sitosterol and daucosterol.

Keywords: *Coeloglossom viride* var. *bracteatum*; Coelovirins A–G; Mono- and bis-(4-*O*-β-D-glucopyranosylbenzyl) tartrate and malate derivatives

INTRODUCTION

Coeloglossum viride (L) Hartm. var. *bracteatum* (Willd.) Richter is a plant belonging to the Orchidaceae family and is distributed in Xinjiang, Inner Mongolia, Gansu and Qinghai provinces of China [1]. The dried rhizomes of this plant, known as "Wangla", have long been used as a traditional Tibetan remedy to treat coughs, asthma and syndromes, and as a tonic in Chinese folk medicine [2]. With respect to its various usages nothing is known about the chemical constituents of this plant. Previous chemical investigations [3–12] have indicated that some species of orchidaceous plants contain characteristic components of mono- and bis(4-*O*-β-D-glucopyranosylbenzyl) tartrate and

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malate derivatives as well as tri-(4-*O*-β-D-glucopyranosylbenzyl) citrates. During our search for potent bioactive compounds with structural diversity from the minority medicines of China, a systematic phytochemical study of the roots of *C. viride* var. *bracteatum* has led to the isolation and structural elucidation of six new mono- and bis(4-*O*-β-D-glucopyranosylbenzyl) tartrate and malate derivatives (**1**–**4**, **6**, **7**) together with the new (2*R*,3*S*)-2-*O*-β-D-glucopyranosyl-2-isobutyltartaric acid (**5**). They are designated as coelovirins A–G (**1**–**7**, respectively) (Fig. 1). Although compound **3** is identical to dactylorhin D reported by Kizu *et al.*, the reported FABMS and NMR data did not match the structure [12]. In addition, fourteen known compounds were obtained. By extensive comparison of their measured physical properties with those reported in the literature, the known compounds were identified as 4-hydroxybenzaldehyde, 4-hydroxybenzyl alcohol, 4,4′-dihydroxydibenzyl ether, 4,4′-dihydroxydiphenylmethane, 4-(4-hydroxybenzyloxy)-benzyl alcohol, gastrodin [6,13,14], quercetin-3,7-diglucoside [15], thymidine [16], loroglossin, militarine, dactylorhin A, dactylorhin B [3,5,12], β-sitosterol and daucosterol. We have previously described the isolation and structural elucidation of the known compounds [17] and the preliminary structural determination of compounds **1** and **2** [18]. Here we report in detail the isolation and structural elucidation of the new compounds.

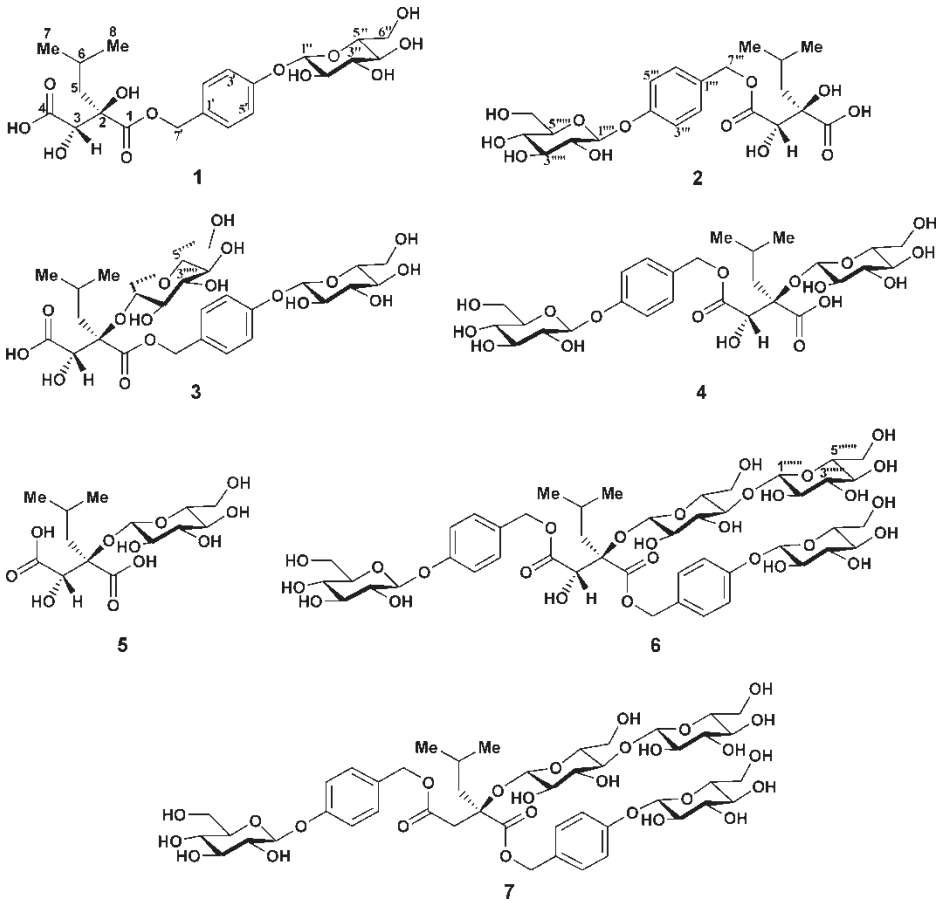

FIGURE 1 Structures of compounds **1**–**7**.

RESULTS AND DISCUSSION

The ethanolic extract of the dried rhizomes of *C. viride* var. *bracteatum* was suspended in water and then partitioned with EtOAc. The aqueous solution was chromatographed successively over macroporous resin, silica gel and reversed-phase silica gel RP-18 and purified by preparative reversed-phase HPLC to yield gastrodin, thymidine, quercetin-3,7-diglucoside, loroglossin, militarine, dactylorhin A, dactylorhin B and the new compounds **1**–**7**. Subsequent separation of the EtOAc fraction by column chromatography over silica gel gave the remaining known compounds.

Coelovirin A (**1**) was obtained as a white amorphous powder (MeOH), $[\alpha]_D^{25}$ −27.8 (*c* 0.12, MeOH). Its IR spectrum showed a strong, broadened absorption band for hydroxyl groups (3425 cm^{-1}), characteristic bands for carbonyl (1732 cm^{-1}), aromatic rings (1614, 1514, 829 cm^{-1}) and *gem*-dimethyl groups (1386 and 1369 cm^{-1}). The negative ion FABMS of **1** exhibited a quasi-molecular ion peak at *m/z* 473 $[M - H]^-$ and the molecular formula was determined as $C_{21}H_{30}O_{12}$ by negative ion HR-FABMS at *m/z* 473.1668 $[M - H]^-$ (calcd. for $C_{21}H_{29}O_{12}$ 473.1659). The ^1H NMR spectrum showed the diagnostic signals attributed to an isobutyl group at δ 0.72 and 0.87 (each 3H, d, $J = 6.5$ Hz, H_3-7 and H_3-8), 1.59 (1H, m, H-6), 1.65 (1H, dd, $J = 14.0, 6.0$ Hz, H-5a) and 1.83 (1H, dd, $J = 14.0, 6.5$ Hz, H-5b) and a *para*-substituted benzyloxyl moiety at δ 7.03 and 7.28 (each 2H, br d, $J =$ 8.5 Hz, H-3′,5′ and H-2′,6′), 5.03 and 5.08 (each 1H, d, $J = 12.0$ Hz, H-7′a and H-7′b). In addition, the ^1H NMR data (Table I) indicated the presence of an isolated oxymethine and a glycosyl unit with a β configuration at the anomeric carbon in **1**. The ^1H–^1H COSY spectrum of **1** confirmed the presence of the above units. The ^{13}C NMR and DEPT spectra revealed the presence of five quaternary carbons in **1,** including a pair of carbonyl carbons at δ 174.9 (C-1) and 174.5 (C-4), two aromatic carbons at δ 130.8 (C-1′) and 159.3 (C-4′) and an oxygenated aliphatic carbon at δ 80.8 (C-2). The protonated carbon signals (Table II) were assigned by the HMQC experiment. The chemical shifts and coupling patterns of proton and carbon signals for the glycosyl unit were in good agreement with those found in gastrodin [8], indicating that the sugar unit is a β-D-glucopyranosyl group. In the HMBC spectrum, two- and three-bond correlations between H-3 and C-1, C-2, C-4 and C-5, and correlations between both H-5a and H-5b and C-1, C-2, C-3, C-6, C-7 and C-8 established the structural residue of 2-isobutyltartrate in **1**. Furthermore, the correlations between two geminal protons H-7′a and H-7′b and C-1, C-1′, C-2′ and C-6′ demonstrated that the *para*-substituted benzyloxyl moiety is esterified at C-1 of the 2-isobutyltartrate residue in **1**, while the correlations between the anomeric proton of the glucose moiety and C-4′ unambiguously indicated that the glucosidic position is at C-4′ of the *para*-substituted benzyloxyl moiety in **1**. Consequently, the structure of **1** was assigned as 1-(4-β-D-glucopyranosyloxybenzyl)-2-isobutyltartrate. To determine the absolute configurations, **1** was subjected to basic hydrolysis, from which two main products were obtained. One was identified as gastrodin by comparison of TLC and its ^1H NMR spectral data with those of an authentic sample obtained from this plant, and the other was assigned as (2*R*,3*S*)-2-isobutyltartric acid by comparing its optical specific rotation and NMR data with those reported in the literature [3,12]. Consequently, the structure of **1** was determined as 1-(4-β-D-glucopyranosyloxybenzyl)-(2*R*,3*S*) -2-isobutyltartrate.

Coelovirin B (**2**), a white amorphous powder, $[\alpha]_D^{25}$ −23.6 (*c* 0.14, MeOH), exhibited a quasi-molecular ion peak at *m/z* 497 $[M + Na]^+$ in the positive ESIMS, and its molecular formula, which is identical to that of **1**, was established as $C_{21}H_{30}O_{12}$ by HR-ESIMS at *m/z* 497.1641 (calcd. for $C_{21}H_{30}O_{12}Na$ 497.1635). The UV, IR and NMR spectral data (Experimental Section and Tables I and II) were similar to those of **1**, except for the carbonyl signals of C-1 and C-4 which shifted from δ 174.9 and 174.5 in **1** to δ 176.7 and 172.8 in **2**,

TABLE I ^{1}H NMR data for compounds **1**–**7**[a]

	1	**2**	**3**	**4**	**5**	**6**	**7**
3a	4.24 s	4.34 s	4.36 s	4.46 s	4.44 s	4.43 s	2.95 d (11)
3b							3.14 d (11)
5a	1.65 dd (14, 6)	1.66 dd (13.5, 5)	1.63 dd (14, 5.5)	1.68 dd (14, 5.5)	1.68 dd (14, 5.5)	1.64 dd (14, 5.5)	1.63 dd (12, 7)
5b	1.83 dd (14, 6.5)	1.82 dd (13.5, 5.5)	2.04 dd (14, 6)	2.05 dd (14, 6)	2.03 dd (14, 6)	2.05 dd (14, 6)	1.71 dd (12, 5.5)
6	1.59 m	1.70 m	1.69 m	1.79 m	1.81 m	1.68 m	1.73 m
7	0.72 d (6.5)	0.83 d (6.5)	0.71 d (6.5)	0.83 d (6.5)	0.85 d (6.5)	0.70 d (6.5)	0.75 d (6.5)
8	0.87 d (6.5)	0.90 d (6.5)	0.87 d (6.5)	0.88 d (6.5)	0.91 d (6.5)	0.84 d (6.5)	0.84 d (6.5)
1-*O*-(4-β-D-Glucopyranosyloxy)benzyl moiety							
2′	7.28 d (8.5)		7.27 d (8)			7.21 d (8)	7.21 d (8)
3′	7.03 d (8.5)		7.03 d (8)			7.03 d (8)	7.00 d (8)
5′	7.03 d (8.5)		7.03 d (8)			7.03 d (8)	7.00 d (8)
6′	7.28 d (8.5)		7.27 d (8)			7.21 d (8)	7.21 d (8)
7′a	5.03 d (12)		5.00 d (12)			4.85 d (12)	4.86 d (12)
7′b	5.08 d (12)		5.15 d (12)			5.03 d (12)	5.02 d (12)
1″	4.85 d (7.5)		4.85 d (7.5)			4.79 d (7.5)	4.87 d (7.5)
2″	3.39 m		3.40 m			3.40 m	3.42 m
3″	3.38 m		3.41 m			3.41 m	2.89 m
4″	3.36 m		3.42 m			3.44 m	3.23 m
5″	3.34 m		3.02 m			3.12 m	3.66 m
6″a	3.64 dd (12, 5.5)		3.64 dd (12, 5.5)			3.65 dd (12, 5.5)	3.64 dd (11, 5.5)
6″b	3.83 dd (12, 2)		3.75 dd (12, 2)			3.78 dd (12, 2)	3.81 dd (11, 2)
4-*O*-(4-β-D-Glucopyranosyloxy)benzyl moiety							
2″		7.27 d (9)		7.30 d (8.5)		7.22 d (8.5)	7.20 d (8)
3″		7.02 d (9)		7.04 d (8.5)		7.04 d (8.5)	7.02 d (8)
5″		7.02 d (9)		7.04 d (8.5)		7.04 d (8.5)	7.02 d (8)
6″		7.27 d (9)		7.30 d (8.5)		7.22 d (8.5)	7.20 d (8)
7″a		4.98 d (12)		4.98 d (12)		4.84 d (12)	4.88 d (12)
7″b		5.10 d (12)		5.19 d (12)		5.07 d (12)	4.95 d (12)
1″		4.84 d (7.5)		4.89 d (6.5)		4.88 d (6.5)	4.90 d (7.5)
2″		3.40 m		3.40 m		3.47 m	3.48 m
3″		3.38 m		3.28 m		3.00 m	2.93 m
4″		3.37 m		3.41 m		3.23 m	3.25 m
5″		3.35 m		3.42 m		3.69 m	3.67 m
6″a		3.64 dd (12, 5.5)		3.64 dd (12, 6)		3.67 dd (12, 6)	3.66 dd (11, 5.5)
6″b		3.83 dd (12, 2)		3.85 dd (12, 2)		3.81 dd (12, 2)	3.78 dd (11, 2)

TABLE I – *continued*

	1	2	3	4	5	6	7
2-*O*-β-D-Glucopyranosyl moiety							
1‴			4.88 d (7.5)	4.47 d (7)	4.90 d (7.5)	4.77 d (8)	4.76 d (8)
2‴			3.19 dd (9,7.5)	3.12 dd (9, 7)	3.20 dd (9, 8)	3.13 m	3.15 m
3‴			3.28 m	3.04 dd (9, 9)	3.29 m	3.25 m	3.26 m
4‴			3.42 m	3.25 m	3.41 m	3.50 m	3.52 m
5‴			3.37 m	2.74 m	3.28 m	3.65 m	3.64 m
6‴a			3.65 dd (12, 5.5)	3.55 dd (12, 4)	3.64 dd (11.5, 5.5)	3.61 dd (11, 4.5)	3.60 dd (11, 4.5)
6‴b			3.83 dd (12, 2)	3.62 dd (12, 2.5)	3.75 br d (11.5)	3.81 dd (11, 2)	3.79 dd (11, 2)
1‴						4.79 d (8)	4.79 d (8)
2‴						3.14 m	3.16 m
3‴						3.28 m	3.28 m
4‴						3.23 m	3.20 m
5‴						3.51 m	3.50 m
6‴a						3.69 dd (11, 4)	3.68 dd (11, 4)
6‴b						3.81 dd (11, 2)	3.83 dd (11, 2)

[a] The data were measured in MeOH-d_4 at 500 MHz. Proton coupling constants (*J*) in Hz are given in parentheses. The assignments were based on ^{1}H–^{1}H DQF-COSY, TOCSY, HMQC, and HMBC experiments.

TABLE II ^{13}C NMR data for compounds **1**–**7**[a]

	1	**2**	**3**	**4**	**5**	**6**	**7**
1	174.9 s	176.7 s	173.8 s	175.7 s	175.7 s	173.8 s	174.9 s
2	80.8 s	80.8 s	85.5 s	86.3 s	85.6 s	86.1 s	81.5 s
3	77.3 d	77.5 d	75.9 d	75.7 d	77.2 d	75.4 d	43.6 t
4	174.5 s	172.8 s	174.4 s	172.3 s	174.4 s	172.1 s	172.0 s
5	45.2 t	45.0 t	47.2 t	46.9 t	46.9 t	46.7 t	49.0 t
6	25.2 d	25.3 d	24.9 d	25.1 d	24.7 d	24.7 d	24.7 d
7	24.0 q	24.1 q	24.2 q	24.1 q	24.1 q	24.9 q	24.9 q
8	24.6 q	24.6 q	24.7 q	24.8 q	24.9 q	24.0 q	24.2 q
1-*O*-(4-β-D-Glucopyranosyloxy)benzyl moiety							
1′	130.8 s		130.5 s			131.6 s	131.1 s
2′	131.3 d		131.4 d			131.9 d	131.4 d
3′	117.7 d		117.7 d			117.9 d	117.9 d
4′	159.3 s		159.3 s			159.3 s	159.2 s
5′	117.7 d		117.7 d			117.9 d	117.9 d
6′	131.3 d		131.4 d			131.9 d	131.4 d
7′	68.0 t		68.3 t			68.4 t	68.3 t
1″	102.5 d		102.2 d			102.2 d	102.3 d
2″	74.9 d		74.9 d			74.8 d	74.9 d
3″	78.0 d		78.1 d			78.1 d	78.4 d
4″	71.4 d		70.0 d			71.3 d	71.4 d
5″	78.2 d		77.2 d			77.8 d	77.6 d
6″	62.5 t		61.3 t			62.4 t	62.5 d
4-*O*-(4-β-D-Glucopyranosyloxy)benzyl moiety							
1″		130.8 s		130.4 s		130.3 s	130.5 s
2″		131.0 d		131.9 d		132.0 d	131.4 d
3″		117.6 d		117.9 d		117.9 d	117.9 d
4″		159.1 s		159.5 s		159.5 s	159.3 s
5″		117.6 d		117.9 d		117.9 d	117.9 d
6″		131.0 d		131.9 d		132.0 d	131.4 d
7″		67.8 t		68.2 t		68.3 t	67.4 t
1″		102.2 d		102.2 d		102.2 d	102.3 d
2″		74.9 d		74.9 d		74.8 d	74.9 d
3″		77.5 d		78.1 d		78.1 d	78.4 d
4″		71.4 d		71.4 d		71.3 d	71.4 d
5″		78.2 d		77.9 d		77.8 d	77.6 d
6″		62.5 t		62.5 t		62.4 t	62.5 d
2-*O*-β-D-Glucopyranosyl moiety							

TABLE II – *continued*

	1	2	3	4	5	6	7
1‴			99.6 d	99.4 d	99.9 d	99.4 d	100.1 d
2‴			75.5 d	75.4 d	75.5 d	75.9 d	75.5 d
3‴			78.5 d	78.1 d	78.6 d	77.2 d	76.8 d
4‴			71.4 d	70.2 d	70.0 d	78.1 d	78.1 d
5‴			78.0 d	77.2 d	78.2 d	70.1 d	70.9 d
6‴			62.5 t	61.7 t	61.4 t	61.5 t	62.3 t
1‴						99.5 d	100.1 d
2‴						75.4 d	74.9 d
3‴						77.9 d	77.9 d
4‴						71.5 d	71.5 d
5‴						77.8 d	77.9 d
6‴						62.5 t	62.7 t

[a] The data were measured in MeOH-d_4 at 125 MHz. The assignments were based on ^{1}H–^{1}H DQF-COSY, TOCSY, HMQC, HMBC and DEPT experiments.

respectively. These observations suggested that the 4-β-D-glucopyranosyloxybenzyloxyl moiety is esterified at C-4 of the 2-isobutyltartrate residue in **2**, which was further confirmed by the long-range correlations between two geminal protons at δ 4.98 and 5.10 (H_a-7″ and H_b-7″) and C-4, C-1″, C-2″ and C-6″ in the HMBC spectrum of **2**. Basic hydrolysis of **2** gave two products that were identical to those obtained from **1**. Accordingly, the structure of **2** was determined as 4-(4-β-D-glucopyranosyloxybenzyl)-(2*R*,3*S*)-2-isobutyltartrate.

Coelovirin C (**3**) was obtained as white amorphous powder, $[\alpha]_D^{25}$ −33.1 (*c* 0.12, MeOH). Its positive ESIMS spectrum exhibited a quasi-molecular ion peak at *m/z* 659 $[M + Na]^+$, and the molecular formula was determined as $C_{27}H_{40}O_{17}$ by HR-ESIMS at *m/z* 659.2178 $[M + Na]^+$ (calcd. for $C_{27}H_{40}O_{17}Na$ 659.2163). The NMR spectral data of **3** (Tables I and II) showed that it is a derivative of **1** with one more β-D-glucopyranosyl unit. After acidic hydrolysis of **3** with 2M HCl, TLC and PC with authentic sugar samples confirmed that only glucose was released from **3**. Comparing the ^{13}C NMR spectral data of **3** with those of **1** showed that the chemical shifts of C-1 and C-3 in **3** were shifted 1.1 and 1.4 ppm upfield from those of **1**, respectively, while the chemical shifts of C-2 and C-5 in **3** were shifted 4.7 and 2.0 ppm downfield from those of **1**, respectively. This evidence indicated that the additional β-D-glucopyranosyl unit was located at C-2 in **3**, which was further confirmed by the long-range correlation between H-1‴ and C-2 in the HMBC experiment for **3**. Basic hydrolysis of **3** gave gastrodin and **5**, which were identified by comparing their physical properties with those of authentic samples obtained from the same plant material. Therefore, the structure of **3** was determined as 1-(4-β-D-glucopyranosyloxybenzyl)-(2*R*,3*S*)-2-β-D-glucopyranosyl-2-isobutyltartrate. Dactylorin D was reported to possess the same structure, but the reported FABMS and NMR data did not match the structure [12].

Coelovirin D (**4**) was obtained as a white amorphous powder, $[\alpha]_D^{25}$ −57.1 (*c* 0.12, MeOH). The positive ESIMS and the HR-ESIMS of **4** revealed that the molecular weight and the molecular formula of **4** are identical to those of **3**. The ^1H, ^{13}C and DEPT spectral data of **4** (Tables I and II) were also similar to those of **3**. These suggested that **4** is an isomer of **3**. An extensive comparison of their NMR data demonstrated that the difference between **4** and **3** is that the 4-β-D-glucopyranosyloxybenzyloxyl moiety is transferred from C-1 of the isobutyltartrate residue in **3** to C-4 in **4**. This suggestion was confirmed by the basic hydrolysis of **4**, which released identical products to those from **3**, and by the HMBC experiment for **4** which showed the clear long-range correlations between H_2-7″ and C-4, and between H_2-5 and C-1. Thus, the structure of **4** was determined as 4-(4-β-D-glucopyranosyloxybenzyl)-(2*R*,3*S*)-2-β-D-glucopyranosyl-2-isobutyltartrate.

Coelovirin E (**5**) was isolated as an amorphous powder, $[\alpha]_D^{25}$ −1.3 (*c* 0.23, MeOH). The positive FABMS of **5** gave a quasi-molecular ion peaks at *m/z* 391 $[M + Na]^+$, and the HR-FABMS at *m/z* 391.1208 (calcd. for $C_{14}H_{24}O_{11}Na$ 391.1216) established the molecular formula $C_{14}H_{24}O_{11}$. Direct comparison of the ^1H, ^{13}C and DEPT spectral data of **5** with those of **3** and **4** (Tables I and II) clearly indicated the presence of 2-isobutyltartrate and β-D-glucopyranosyl units and the absence of a 4-β-D-glucopyranosyloxybenzyl moiety in the structure of **5**. In the HMBC spectrum of **5**, the long-range correlations between both H-5 and H-1‴ and C-2 demonstrated that the structure of **5** is 2-β-D-glucopyranosyl-2-isobutyltartric acid. Basic hydrolysis of both **3** and **4** yielded **5**. Therefore, the structure of **5** was determined as (2*R*, 3*S*)-2-β-D-glucopyranosyl-2-isobutyltartaric acid.

Coelovirin F (**6**), a colorless gum, $[\alpha]_D^{25}$ −29.0 (*c* 0.10, MeOH), displayed a quasi-molecular ion peak at *m/z* 1089 $[M + Na]^+$ in the positive ESIMS. The molecular formula, $C_{46}H_{66}O_{28}$, was determined by the HR-ESIMS at *m/z* 1089.3689 (calcd. for $C_{46}H_{66}O_{28}Na$ 1089.3638). The ^1H, ^{13}C and DEPT spectral data of **6** (Table I) were similar to those of loroglossin [17], except for the data assignable to an additional β-D-glucosyl unit. Acidic hydrolysis of **6** followed by TLC and paper chromatography with authentic sugar samples

confirmed that only glucose was released from **6**. In the HMBC experiment, the long-range correlation between H-1‴ and C-4‴ unambiguously indicated that the additional β-D-glucosyl unit is at C-4‴. Thus, the structure of **6** was determined as bis(4-β-D-glucopyranosyloxybenzyl)-(2*R*,3*S*)-2-[β-D-glucopyranosyl(1 → 4)β-D-glucopyranosyl]-2-isobutyltartrate.

Coelovirin G (**7**) was obtained as a colorless gum, $[\alpha]_D^{25}$ −15.8 (*c* 0.10, MeOH). The positive FABMS gave a quasi-molecular ion peak at *m/z* 1073 $[M + Na]^+$, and the molecular formula was established as $C_{46}H_{66}O_{27}$ by the HR-FABMS at *m/z* 1073.3679 (calcd. for $C_{46}H_{66}O_{27}Na$ 1073.3689), which was one oxygen less than that of **6**. The ^1H, ^{13}C and DEPT spectral data of **7** (Tables I and II) were similar to those of **6**, except that the data attributed to the hydroxymethine of the 2-isobutyltartrate moiety of **6** were replaced by data assignable to a methylene at δ_H 2.95 (1H, d, $J = 11.0$ Hz, H-3a) and 3.14 (1H, d, $J = 11.0$ Hz, H-3b) and δ_C 43.6 (t, C-3), and that the signal of C-2 in **7** was shifted 4.6 ppm upfield from that of **6**. These differences suggested the presence of the 2-isobutylmalate moiety in **7** instead of the 2-isobutyltartrate moiety in **6**. Although basic hydrolysis of **7** followed by enzymatic hydrolysis using cellulase was unsuccessful in obtaining 2-isobutylmaleic acid, due to the limited amount of the sample, from the biogenetic point of view the absolute configuration at C-2 of the 2-isobutylmalate moiety should be identical to that of dactylorhin A and militarine [12] which coexist in the same plant [17]. Accordingly, the structure of **7** was determined as bis(4-β-D-glucopyranosyloxybenzyl)-(2*R*)-2-[β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl]-2-isobutylmalate.

EXPERIMENTAL

General Experimental Procedures

Melting points were determined on an XT-4 micro melting point apparatus and are uncorrected. Optical rotations were measured on a Rudolph Research Autopol III automatic polarimeter. IR spectra were recorded as KBr disks on a Nicolet Impact 400 FT-IR Spectrophotometer. 1D- and 2D-NMR spectra were obtained on an Inova 500 MHz spectrometer in MeOH-d_4 with TMS as internal standard. FABMS, HR-FABMS, ESIMS and HRESIMS data were measured with a Micromass Autospec-Ultima ETOF spectrometer. Column chromatography was performed with RA macroporous resin (Beijing Seventh Chemical Inc., China), silica gel (200–300 mesh, Qingdao Marine Chemical Inc. China), RP-18 reversed-phase silica gel (43–60 μm) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala Sweden). TLC was carried out with glass precoated silica gel GF_{254} plates. Spots were visualized under UV light and by spraying with 7% H_2SO_4 in EtOH followed by heating. All solvents used were either spectral grade or were distilled prior to use.

Plant Material

The rhizomes of *C. viride* (L.) Hartm. var. *bracteatum* (Willd.) Richter were collected at Huzhu north mountain of Qinghai province, China in September 1999. The plant identification was verified by Professor Guoliang Zhang (Department of Biology, Lanzhou University, Lanzhou 730000, China). A voucher specimen (no. 998204) is deposited at the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica.

Extraction and Isolation

Air-dried and grounded rhizomes of *Coeloglossum. viride* (L.) Hartm. var. *bracteatum* (Willd.) Richter (5 kg) were extracted with EtOH at room temperature for 3 × 48 h, and

the solvent was removed under reduced pressure at <40°C to give a residue (220 g). The residue was suspended in water and then partitioned with EtOAc. The EtOAc fraction (140 g) was chromatographed over silica gel, eluting with a gradient increasing MeOH in $CHCl_3$ and separated into ten fractions (I–X) on the basis of TLC analyses. Fraction I was further purified by chromatography over silica gel using light petroleum (60–90°C)–EtOAc (8:1) as the eluent to yield β-sitosterol (68 mg), 4-hydroxybenzaldehyde (62 mg) and 4,4′-dihydroxydibenzyl ether (48 mg). Fraction V was chromatographed over Sephadex LH-20 with light petroleum–$CHCl_3$–MeOH (5:5:1) as mobile phase to yield 4,4′-dihydroxydiphenyl methane (23 mg), 4-(4-hydroxybenzyloxy)benzyl alcohol (18 mg) and 4-hydroxybenzyl alcohol (42 mg). Fraction VIII was purified by chromatography over silica gel, eluting with $CHCl_3$–MeOH (10:1) to yield daucosterol (132 mg). The water phase was subjected to column chromatography over RA resin, successively eluting with H_2O, and 20%, 40% and 80% EtOH in H_2O. The 40% EtOH-eluted solution was concentrated to give a residue (29 g) that was chromatographed over reversed-phase silica gel RP-18, eluting with a gradient increasing MeOH in H_2O, to yield loroglossin (4.8 g), militarine (1.2 g), dactylorhin A (3.4 g), dactylorhin B (4.2 g). The 40% EtOH-eluted portion (14 g) was chromatographed over reversed-phase silica gel, eluting with a gradient increasing MeOH in H_2O, to yield gastrodin (86 mg), thymidine (7 mg), quercetin-3,7-diglucoside (6 mg), and a mixture that was further purified by preparative HPLC with a RP-18 column (250 × 22 mm, particle size 10 μ) and 40% MeCN in H_2O as the mobile phase to yield coelovirins A (**1**, 70 mg), B (**2**, 93 mg), C (**3**, 87 mg), D (**4**, 173 mg), E (**5**, 65 mg), F (**6**, 28 mg) and G (**7**, 17 mg).

Coelovirin a (1)

A white amorphous powder (MeOH), $[\alpha]_D^{25}$ −27.8 (*c* 0.12, MeOH); UV λ_{max} (nm) ($\log \epsilon$) 229 (3.10), 270 (2.93), 277 (2.85); IR (KBr) ν_{max} (cm^{-1}): 3425, 2956, 1732, 1614, 1514, 1369, 1228, 1155, 1074, 1043, 1016, 897, 829; ^{1}H NMR (MeOH-d_4, 500 MHz) see Table I; ^{13}C NMR (MeOH-d_4, 125 MHz) see Table II; negative FABMS *m/z* 473 $[M - H]^-$; HR-FABMS *m/z* 473.1659 (calcd for $C_{21}H_{29}O_{12}$, 743.1659).

Coelovirin B (2)

A white amorphous powder (MeOH), $[\alpha]_D^{25}$ −23.6 (*c* 0.12, MeOH); UV λ_{max} (nm) ($\log \epsilon$) 222 (3.10), 270 (2.87), 277 (2.79); IR (KBr) ν_{max} (cm^{-1}): 3425, 2956, 1734, 1614, 1514, 1456, 1387, 1369, 1234, 1076, 1043, 1017, 897, 858, 831; ^{1}H NMR (MeOH-d_4, 500 MHz) see Table I; ^{13}C NMR (MeOH-d_4, 125 MHz) see Table II; positive ESIMS *m/z* 497 $[M + Na]^+$; HR-ESIMS *m/z* 497.1641 (calcd for $C_{21}H_{30}O_{12}Na$, 497.1635).

Basic Hydrolysis of 1 and 2

A solution of each compound (15 mg) in 2M NaOH (3 ml) was stirred for 20 min at room temperature. The reaction mixture was then neutralized with 1M HCl, and partitioned with EtOAc. The EtOAc phase was concentrated and crystallized in Me_2CO to give (2*R*,3*S*)-2-isobutyltartaric acid, mp 193–194 °C; $[\alpha]_D^{25}$ +3.8° (*c* 0.16, MeOH); ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 4.24 (1H, d, $J = 8.0$ Hz, H-3), 1.77 (1H, dd, $J = 13.5$, 6.0 Hz, H-5a), 1.62 (1H, m, H-6), 1.56 (1H, dd, $J = 13.5$, 6.5 Hz, H-5b), 0.87 (3H, d, $J = 6.5$ Hz, H-7), 0.77 (3H, d, $J = 6.5$ Hz, H-8); ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 178.2 (s, C-1), 176.8 (s, C-4), 81.4 (s, C-2), 76.5 (d, C-3), 43.5 (t, C-5), 24.1 (q, C-7), 23.5 (d, C-6), 23.0 (q, C-8); positive FABMS *m/z* 229 $[M + Na]^+$. The aqueous phase was evaporated under

reduced pressure to give a residue, which was chromatographed over Sephadex LH-20, eluting with $CHCl_3$–MeOH (1:1), to yield gastrodin, mp 159–160 °C; IR (KBr) ν_{max} (cm^{-1}): 3350, 1610, 1590, 1400, 1240, 1230, 1120, 1110, 1075, 1045, 1020, 1000, 995, 860, 830; ^1H NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.19 (2H, d, H-2, H-6), 6.95 (2H, d, $J = 6.5$ Hz, H-3, H-5), 4.80 (1H, d, $J = 7.5$ Hz, H-1′), 4.49 (1H, t, $J = 5.5$ Hz, $ArCH_2OH$), 4.39 (1H, d, $J = 5.5$ Hz, $ArCH_2OH$), 3.05–3.70 (6H, m, glc); ^{13}C NMR (MeOH-d_4, 125 MHz) δ (ppm): 156.1 (s, C-4), 135.7 (s, C-1), 127.5 (d, C-2, C-6), 115.8 (d, C-3, C-5), 100.4 (d, C-1′), 76.9 (d, C-3′), 76.6 (d, C-5′), 73.2 (d, C-2′), 69.7 (d, C-4′), 62.5(t, C-7), 60.7 (t, C-6′); positive FABMS *m/z* 309 $[M + Na]^+$.

Coelovirin C (3)

A white amorphous powder (MeOH), $[\alpha]_D^{25}$ −33.1 (*c* 0.12, MeOH); UV λ_{max} (nm) (log ϵ) 228 (3.17), 270 (2.86), 277 (2.78); IR (KBr) ν_{max} (cm^{-1}): 3425, 2956, 2929, 2873, 1732, 1658, 1614, 1514, 1456, 1390, 1369, 1306, 1230, 1165, 1074, 1041, 1016, 924, 899, 831; ^1H NMR (MeOH-d_4, 500 MHz) see Table I; ^{13}C NMR (MeOH-d_4, 125 MHz) see Table II; positive ESIMS *m/z* 659 $[M + Na]^+$; HR-ESIMS *m/z* 659.2178 (calcd for $C_{27}H_{40}O_{17}Na$, 659.2163).

Coelovirin D (4)

A white amorphous powder (MeOH), $[\alpha]_D^{25}$ −57.1 (*c* 0.12, MeOH); UV λ_{max} (nm) (log ϵ) 228 (3.14), 270 (2.90), 277 (2.82); IR (KBr) ν_{max} (cm^{-1}): 3425, 2958, 2927, 2875, 1732, 1612, 1514, 1388, 1371, 1302, 1232, 1163, 1097, 1074, 1043, 1018, 899, 856, 835; ^1H NMR (MeOH-d_4, 500 MHz) see Table I; ^{13}C NMR (MeOH-d_4, 125 MHz) see Table II; positive ESIMS *m/z* 637 $[M + H]^+$; HR-ESIMS *m/z* 637.2310 (calcd for $C_{27}H_{41}O_{17}$, 637.2344).

Acidic Hydrolysis of 3 And 4

A solution of each compound (10 mg) in 2 M HCl (3 ml) was boiled under reflux for 4 h at 94°C. The reaction mixture was neutralized with 1M NaOH and dried by blowing with nitrogen gas. The residue was dissolved in MeOH (0.5 ml) and analyzed by TLC and PC together with authentic glucose, galactose and arabinose samples. The developing solvent systems were $CHCl_3$–MeOH (2.5:1) for TLC and the upper layer of n-BuOH–AcOH–H_2O (4:1:5) for PC; the spots were colored by spraying with aniline hydrogen phthalate followed by heating at 105 °C. The TLC and PC analyses indicated the presence of glucose (R_f 0.26 for TLC and 0.32 for PC).

Basic Hydrolysis of 3 And 4

A solution of each compound (25 mg) in 2M NaOH (3 ml) was stirred for 10 min at room temperature. The reaction solution was neutralized with 1M HCl, and then desalted by chromatography over Sephadex LH-20 using MeOH as the eluent to give a mixture. The mixture was separated by HPLC to give two main products, their properties were identical to those of gastrodin and **5**, respectively.

Coelovirin E (5)

A white amorphous powder (MeOH), $[\alpha]_D^{25}$ −1.3 (*c* 0.23, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3402, 2958, 1728, 1608, 1512, 1369, 1267, 1232, 1161, 1076, 1024, 827, 769; ^1H NMR

(MeOH-d_4, 500 MHz) see Table I; ^{13}C NMR (MeOH-d_4, 125 MHz) see Table II; positive ESIMS *m/z* 391 $[M + Na]^+$, 369 $[M + H]^+$; HR-ESIMS *m/z* 391.1208 (calcd. for $C_{14}H_{24}O_{11}Na$ 391.1216).

Coelovirin F (6)

A colorless gum, $[\alpha]_D^{25}$ −29.0 (*c* 0.10, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3400, 2925, 1732, 1612, 1514, 1232, 1074, 1038, 899, 831; ^{1}HNMR (MeOH-d_4, 500 MHz) see Table I; ^{13}C NMR (MeOH-d_4, 125 MHz) see Table II; positive ESIMS *m/z* 1089 $[M + Na]^+$; HR-ESIMS *m/z* 1089.3689 (calcd. for $C_{46}H_{66}O_{28}Na$ 1089.3638).

Acidic Hydrolysis of 6

A solution of **6** (10 mg) in 2M HCl (2 ml) was boiled under reflux for 3 h at 94 °C, and the reaction mixture was processed in the same way as for **3** and **4**. TLC and PC revealed that glucose was the only sugar released from **6**.

Coelovirin G (7)

A colorless gum, $[\alpha]_D^{25}$ −15.8 (*c* 0.10, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3408, 2925, 1738, 1734, 1612, 1514, 1232, 1074, 1041, 926, 897, 831; ^{1}H NMR (MeOH-d_4, 500 MHz) see Table I; ^{13}C NMR (MeOH-d_4, 125 MHz) see Table II; positive ESIMS *m/z* 1073 $[M + Na]^+$; HR-FABMS *m/z* 1073.3679 (calcd. for $C_{46}H_{66}O_{27}Na$ 1073.3689).

Acidic Hydrolysis of 7

A solution of **7** (6 mg) in 2M HCl (2 ml) was treated in the same way as for **3** and **4**. Glucose was found by TLC and PC to be the only sugar released from **7**.

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