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Two New Tartrate Derivative Glucosides from *Coeloglossum viride* (L.) Hartm. var. *bracteatum* (Willd.) Richter†

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Abstract: Two new tartrate derivative glucosides, coelovirin C (**1**) and D (**2**), were isolated from rhizomes of *Coeloglossum viride* (L.) Hartm. var. *bracteatum* (Willd.) Richter (Orchidaceae). Their structures were elucidated as (2*R*, 3*S*)-2-β-D-glucopyranosyl-2-isobutyltartrate–1-(4-β-D-glucopyranosyloxybenzyl) ester **1** and (2*R*, 3*S*)-2-β-D-glucopyranosyl-2-isobutyltartrate-4-(4-β-D-glucopyranosyloxybenzyl) ester **2** by means of chemical and spectroscopic methods.

Keywords: *Coeloglossum viride* (L.) Hartm. var. *bracteatum* (Willd.) Richter, Orchidaceae, tartrate derivative glucoside, coelovirin C and D.

Coeloglossum viride (L.) Hartm. var. *bracteatum* (Willd.) Richter is an orchidaceous plant. Its dried rhizomes, named as "Wangla" , have long been used as a traditional Tibetan medicine in China to treat cough, asthma and syndrome[1]. In previous papers, we reported two new isobutyltartrate monoesters coelovirin A and B[2]. The continuation of our study on chemical constituents of this material led to the isolation of two new tartrate derivative glucosides named as coelovirin C **1** and D **2**. We describe here the isolation and structural elucidation of these two compounds.

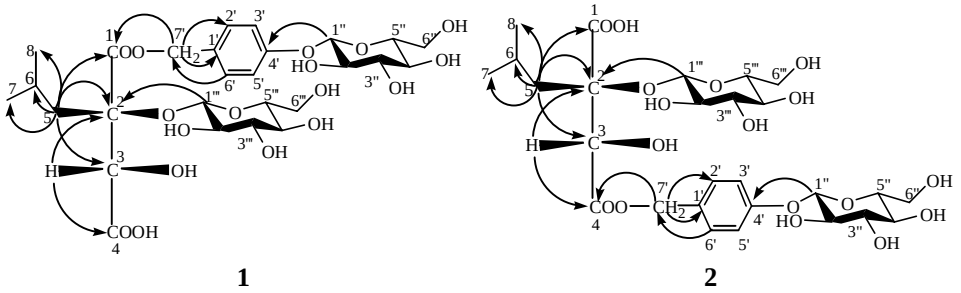
The ethanolic extract of the dried rhizomes of *C. viride* (L.) Hartm. var. *bracteatum* (Willd.) Richter was suspended in water and then extracted sequentially with petroleum ether and EtOAC. The aqueous solution was firstly evaporated under vacuum to remove the remained EtOAC and then chromatographed successively on macroporous resin, silica gel and reverse phase silica gel Rp-18 to yield a mixture, which was further purified by preparative reverse phase HPLC eluting with 40% MeOH in H_2O to give **1** and **2**.

Compound **1** was obtained as white amorphous powder, $[\alpha]_D^{25}$ –33.1 (*c* 0.12, MeOH), UVλ_{max}(MeOH) nm (log ε): 228 (3.17), 270 (2.86), 277 (2.78). Its IR spectrum revealed the presence of hydroxyl group (3425 cm^{-1}), carboxyl group (1732 cm^{-1}) and aromatic ring (1614, 1514 and 831 cm^{-1}). The positive ESIMS spectrum of **1** exhibited a quasi-molecular ion peak at *m/z* 659[M+Na]$^+$, and the molecular formula was

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

determined as $C_{27}H_{40}O_{17}$ by HRESIMS at *m/z* 659.2178 $[M+Na]^+$ (calcd. for $C_{27}H_{40}O_{17}Na$ 659.2163). The ^1H NMR spectrum of **1** showed characteristic signals assignable to an isobutyl group at δ 0.71 and 0.87 (each d, 3H, J = 6.5 Hz, H_3-7 and H_3-8), 1.69 (m, 1H, H-6), 1.63 (dd, 1H, J = 14.0, 5.5 Hz, H_A-5) and 2.04 (dd, 1H, J = 14.0, 6.0 Hz, H_B-5), an isolated oxymethine at δ 4.36 (s, 1H, H-3) and a *para*-substituted benzyloxyl moiety at δ 7.03 and 7.27 (each br d, 2H, J = 8.0 Hz, H-3′, 5′ and H-2′, 6′), 5.00 and 5.15 (each d, 1H, J = 12.0 Hz, H_A-7′ and H_B-7′), as well as diagnostic signals attributed to two glycosyl units between δ 3.0 and 5.0 (**Table 1**). Beside the signals assignable to above moieties, the ^{13}C NMR and DEPT spectrum of **1** (**Table 1**) showed two carbonyl carbon signals at δ 173.8 and 174.4 and a sp^3 quaternary carbon at δ 85.5. The protons and protonated carbon signals in ^1H and ^{13}C NMR spectra were unambiguously assigned by the ^1H-^1H COSY, HMQC experiments. In the ^{13}C NMR spectrum of **1**, the signals due to both sugar units are in good agreement with those of glucopyranosyl groups[3,4]. After acidic hydrolysis of **1** with 2*N* HCl, the PC with authentic sugar samples confirmed that only glucose was released from **1**. In the ^1H NMR spectrum, the coupling constants of the anomeric protons at δ 4.85 (d, 1H, J = 7.5 Hz) and 4.88 (d, 1H, J = 7.5 Hz) indicated that the two glucopyranosyl units possessed β-configurations. The connections among above units were established by the HMBC experiment of **1** (**Figure 1**) which showed three-bond correlations from H-5 to C-1 and C-3, H-7′ to C-1, H-1″ to C-4′ and H-1‴ to C-2. Thus, the structure of compound **1** was determined as 2-β-D-glucopyranosyl-2-isobutyltartrate-1-(4-β-D-glucopyranosyloxybenzyl) ester, named as coelovirin C. The absolute configurations of C-2 and C-3 were assigned as (2*R*, 3*S*) by comparison of the optical specific rotation of the methanolysis product of **1** isobutyltartrate methyl diester[5] with those of (2*R*, 3*S*)-2-isobutyltartrate[6]. Dactylorin D was reported to possess the same structure, but the reported spectral data including FABMS and NMR data did not match the structure[6].

Figure 1 Structures and key HMBC correlations of **1** and **2**



1 **2**

Compound **2** was isolated as a white amorphous powder, $[\alpha]_D^{25}$ −57.1 (c 0.12, MeOH), UVλ_{max}(MeOH) nm (logε): 228(3.14), 270.4(2.90), 277(2.82). IR (KBr) cm^{-1}: 3425, 1732, 1612, 1514, 1388, 1371, 1232, 1074, 835. The positive ESIMS spectrum of **2** gave a quasi-molecular ion peak at *m/z* 637$[M+H]^+$, and the molecular formula $C_{27}H_{40}O_{17}$ was established by the HRESIMS at *m/z* 637.2310 (calcd. for $C_{27}H_{41}O_{17}$

637.2344). The ^{1}H, ^{13}C and DEPT spectral data of **2** (**Table 1**) were very similar to those of **1**. An extensive comparison of the ^{1}H and ^{13}C NMR data of **2** with those of **1** suggested that **2** is an isomer of **1**, and the difference between them is that the 4-β-D-glucopyranosyloxybenzyloxyl moiety is transferred from C-1 of the isobutyltartrate residue in **1** to C-4 of that in **2,** which was confirmed by the HMBC experiment of **2** showing obvious long range correlations from H_2-7′ to C-4, and H_2-5 to C-1 (**Figure 2**). Thus, the structure of **2** was determined as (2*R*, 3*S*)-2-β-D-glucopyranosyl-2-isobutyl-ltartrate-4-(4-β-D-glucopyranosyloxybenzyl) ester, named as coelovirin D.

Table 1 NMR data for compound **1** and **2**

No.	**1**		**2**	
	$^{1}H^{a}$	$^{13}C^{b}$	$^{1}H^{a}$	$^{13}C^{b}$
1		173.8 s		175.7 s
2		85.5 s		86.3 s
3	4.36 s	75.9 d	4.46 s	75.7 d
4		174.4s		172.3 s
5	1.63 dd (14.0, 5.5) 2.04 dd (14.0, 6.0)	47.2 t	1.68 dd (14.0, 5.5) 2.05 dd (14.0, 6.0)	46.9 t
6	1.69 m	24.9 d	1.79 m	25.1 d
7	0.71 d (6.5)	24.2 q	0.83 d (6.5)	24.1 q
8	0.87 d (6.5)	24.7 q	0.88 d (6.5)	24.8 q
Benzyl moiety				
1′		130.5 s		130.4 s
2′,6′	7.27 br. d (8.0)	131.4 d	7.30 br. d (8.5)	131.9 d
3′,5′	7.03 br. d (8.0)	117.7 d	7.04 br. d (8.5)	117.9 d
4′		159.3 s		159.5 s
7′	5.00 d (12.0) 5.15 d (12.0)	68.3 t	4.98 d (12.0) 5.19 d (12.0)	68.2 t
4′-O-glucosyl moiety				
1″	4.85 d (7.5)	102.2 d	4.89 d (6.5)	102.2 d
2″	3.40 m	74.9 d	3.40 m	74.9 d
3″	3.41 m	78.1 d	3.28 m	78.1 d
4″	3.42 m	70.0 d	3.41 m	71.4 d
5″	3.02 m	77.2 d	3.42 m	77.9 d
6″	3.64 dd (12.0, 5.5) 3.75 dd (12.0, 2.0)	61.3 t	3.64 dd (12.0, 6.0) 3.85 dd (12.0, 2.0)	62.5 t
2-O-glucosyl moiety				
1‴	4.88 d (7.5)	99.6 d	4.47 d (7.0)	99.4 d
2‴	3.19 dd (9.0,7.5)	75.5 d	3.12 dd (9.0, 7.0)	75.4 d
3‴	3.28 m	78.5 d	3.04 dd (9.0, 9.0)	78.1 d
4‴	3.42 m	71.4 d	3.25 m	70.2 d
5‴	3.37 m	78.0 d	2.74 ddd(10.0, 4.0, 2.5)	77.2 d
6‴	3.65 dd (12.0, 5.5) 3.83 dd (12.0, 2.0)	62.5 t	3.55 dd (12.0, 4.0) 3.62 dd (12.0, 2.5)	61.7 t

[a]Measured at 500 MHz in CD_3OD. Coupling constants (J) in Hz are given in parentheses. The assignments were based on ^{1}H-^{1}H COSY, HMQC and HMBC. [b]Measured at 125 MHz in CD_3OD. The assignments were based on DEPT, HMQC and HMBC.

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5. Methanolysis of **1** with 2% sodium methoxide in methanol yield isobutyltartrate methyl diester, $[\alpha]_D^{23}$ + 3.8 (*c* 0.13, MeOH), ^{1}H NMR (DMSO-d_6, 500 MHz) δ 4.20 (d, 1H, *J*=8.0 Hz, H-3), 1.57 (dd, 1H, *J*=13.5, 6.5 Hz, H-5a), 1.79 (dd, 1H, *J*=13.5, 6.0 Hz, H-5b), 1.63 (m, 1H, H-6), 0.76 (d, 3H, *J*=6.5 Hz, H-7), 0.88 (d, 3H, *J*=6.5 Hz, H-8), 3.68 (s, 3H, OMe), 3.58 (s, 3H, OMe). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 173.7 (s, C-1), 79.3 (s, C-2), 76.1 (d, C-3), 171.4 (s, C-4), 43.8 (t, C-5), 24.2 (t, C-7), 23.4 (d, C-6), 23.1 (q, C-8), 51.7 (q, OMe), 51.5 (q, OMe).
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