Two new compounds from Comastoma pedunlulatum

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Two new compounds from *Comastoma pedunlulatum*

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Two new compounds, a xanthonoid and a flavonoid *C*-glycoside, were isolated from the ethyl acetate extract of the dried herb of *Comastoma pedunlulatum*. The structures of the new compounds were elucidated, respectively, as 1,8-dihydroxy-3,5-dimethoxyxanthone 1-*O*-[2-(4′-hydroxy-3′,5′-dimethoxy-E-cinnamoyl)]-β-D-xylopyranosyl-(1-6)-β-D-glucopyranoside (**1**) and 6″-*O*-acetylisoorientin (**2**) on the basis of their spectroscopic and physicochemical properties.

Keywords: xanthonoid; flavonoid *C*-glycoside; *Comastoma pedunlulatum*

1. Introduction

The dried herb of *Comastoma pedunlulatum* (Rogle eX D. Dou) Holub (*Gentianaceae*), a traditional Tibetan medicine named Zangyinchen, is one of the most popular herbal medicines widely used in Tibetan prescriptions [1]. This plant naturally grows in highland areas between 3000 and 4800 m, mainly in Qinghai and Tibet regions. Zangyinchen possesses many biological functions, including clearing heat and anti-inflammatory, hepatoprotective, antioxidative, and normalizing gallbladder properties [2]. It is used mainly to cure hepatitis, liver fibrosis, and cholecystitis in Tibetan medicine for centuries, and some traditional Tibetan medicine patent prescriptions mainly containing Zangyinchen are widely used in the Tibetan area at present. Previous phytochemical studies of the plant have identified various compounds, including xanthones and xanthonoids [3,4]. In continuation of our studies on bioactive constituents of this plant, two new compounds were isolated from the active part of the ethyl acetate extract of the dried herb. In this paper, we report two new compounds, comastomaside A and 6″-*O*-acetylisoorientin, isolated from this plateau herbal medicine.

2. Results and discussion

Compound **1** was obtained as yellow powder. Compound **1** showed IR absorption bands at $3423\,cm^{-1}$ for hydroxyl groups, $1649\,cm^{-1}$ for a conjugated carbonyl, and 1605, 1565, and $1499\,cm^{-1}$ for an aromatic ring. Its UV absorption maxima at 257, 271, and 318 nm (MeOH) were due to the features of xanthone skeleton. The molecular formula was determined as $C_{37}H_{40}O_{19}$ by the pseudo-molecular ion in HR-ESI-MS at *m/z* 811.2066 $[M + Na]^+$.

The ^1H NMR spectrum (Table 1) suggested the presence of a tetrasubstituted xanthone moiety as aglycone, as

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Table 1.   ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data for compound **1** (in DMSO-d_6, δ in ppm).

Position	δ_C	δ_H (J, Hz)	Position	δ_C	δ_H (J, Hz)
Aglycone part			1-O-sugar Glc		
1	158.9		1″	100.4	5.02 (d, 7.5)
2	98.1	6.63 (br s)	2″	72.7	3.28
3	165.2		3″	76.0	3.32
4	94.5	6.65 (br s)	4″	69.6	3.11 (m)
4a	158.6		5″	75.2	3.59 (m)
4b	147.8		6″	68.6	3.96 (br d, 10.5)
5	142.1				3.59 (m)
6	120.2	7.39 (d, 9.0)	6″-O-Xyl		
7	104.3	6.81 (d, 9.0)	1‴	101.4	4.43 (d, 8.0)
8	149.9		2‴	73.0	4.63 (dd, 9.0 9.0)
8a	108.1		3‴	74.2	3.32
8b	104.0		4‴	69.4	3.38
9	180.8		5‴	65.5	3.76
3-O—CH$_3$	55.9	3.92 (s)			3.06 (dd, 11.0 11.0)
5-O—CH$_3$	56.3	3.81 (s)			
8-OH		13.24 (s)	2‴-O-substituent		
			1′	124.1	
			2′, 6′	105.6	6.86 (br s)
			3′, 5′	147.6	
			4′	137.9	
			7′	144.8	7.38 (d, 16.0)
			8′	114.7	6.34 (d, 16.0)
			9′	165.5	
			3′, 5′-O—CH$_3$	55.7	3.74 (s)

Note: Assignments were based on DEPT, ^{1}H–^{1}H COSY, HSQC, HMBC, and NOESY experiments. Glc = β-D-glucopyranose. Xyl = β-D-xylopyranose.

evident from the presence of two *o*-coupling aromatic protons at δ 7.39 (1H, d, $J = 9.0$ Hz, H-6) and 6.81 (1H, d, $J = 9.0$ Hz, H-7), two *m*-coupling aromatic protons at δ 6.65 (1H, br s, H-4) and 6.63 (1H, br s, H-2), respectively, as well as a singlet at δ 13.24 ascribable to proton signal of aromatic hydroxyl. The remaining part of the ^{1}H NMR spectrum showed proton signals of a *trans*-substituted cinnamoyl, a hexose, a pentose, and four methoxys, respectively. The signals of substituted cinnamoyl moiety are located at δ 6.86 (2H, s, H-2′, H-6′), 7.38 (1H, d, $J = 16.0$ Hz, H-7′), and 6.34 (1H, d, $J = 16.0$ Hz, H-8′), and the *trans*-olefinic bond was proved by the large coupling constant 16.0 Hz.

The resonance signals in the ^{13}C NMR spectrum (Table 1) included nine sp^2 O-substituted quaternary carbon signals,

three sp^2 quaternary carbon signals, eight sp^2 methine carbon signals, nine sp^3 O-substituted methine carbon signals, two sp^3 O-substituted methylene carbon signals and four methoxy carbon signals. These data confirmed the presence of a xanthone moiety, a disaccharide moiety with glucose and xylose, and a *trans*-substituted cinnamoyl moiety. The ^{13}C NMR chemical shift of C-1 at δ 158.9 revealed that the sugar moiety was attached to C-1 of the aglycon [3]. Acid hydrolysis of **1** yielded D-glucose and D-xylose. The 1D and 2D NMR spectra showed the presence of two anomeric signals at δ 5.02 (1H, d, $J = 7.5$ Hz, glu-H-1) and 4.43 (1H, d, $J = 8.0$ Hz, xyl-H-1) and two corresponding carbon signals at δ 100.4 and 101.4. The large $^3J_{H1-H2}$ coupling constant (7.5 and 8.0 Hz) suggested a β-anomeric configuration for the glucose unit and the

xylose unit [3,5]. The COSY experiment permitted the identification of disaccharide spin systems and assignments of their proton resonances (Table 1).

The HMBC experiment (Table 1) established the linkage position, the sequence of sugar chain, and the positions of substituents. The positions and the sequence of disaccharide moiety were defined unambiguously from the HMBC correlations between the proton signal at δ 5.02 (glu-H-1″) of β-glucose and C-1 at δ 158.9, and the proton signal at δ 4.43 (xyl-H-1‴) of β-xylose and C-6″ of the inner β-glucose at δ 68.6. The anomeric proton signal at δ 4.43 (xyl-H-1‴) of xylose also correlated with C-5‴ of xylose at δ 65.5. The cinnamoyl linked to C-2‴ of xylose was confirmed by HMBC correlation between the proton signal of xylose H-2‴ (δ 4.63) and the carbonyl at δ 165.5, together with the chemical shift of xylose H-2‴ (δ 4.63) and C-2‴ (δ 73.0). The methoxy substitution positions of xanthone skeleton and cinnamoyl moiety were also deduced from the HMBC correlations of the methoxyl at δ 3.92 with C-3 at δ 165.2, the methoxyl at δ 3.81 with C-5 at δ 142.1, and the methoxyl at δ 3.74 (6H, s) with C-3′/5′ at δ 147.6 (Figure 1). And this was confirmed by the NOESY correlations between 3-OCH₃ at δ 3.92 and two proton signals at δ 6.65 (H-4) and 6.63 (H-2), between 5-OCH₃ at δ 3.81 and H-6 at δ 7.39, and between 3′/5′-OCH₃ at δ 3.74 and the proton signal at δ 6.86 (2H, H-2′, 6′) (Figure 1).

Therefore, compound **1** was elucidated as 1,8-dihydroxy-3,5-dimethoxy xanthone 1-*O*-[2-(4′-hydroxy-3′,5′-dimethoxy-E-cinnamoyl)]-β-D-xylopyranosyl-(1-6)-β-D-glucopyranoside, named as comastomaside A (Figure 1).

Compound **2** was obtained as yellow powder. The IR spectrum indicated the presence of hydroxyl (3378 cm⁻¹) and carbonyl (1649 cm⁻¹) groups. Its UV spectrum exhibited absorption maxima at 257 nm (band II) and 354 nm (band I),
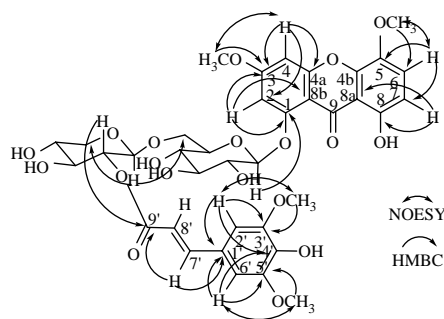

Figure 1. Structure and selected 2D NMR correlations of compound **1**.

which are characteristic absorption bands of a flavone skeleton. The molecular formula of **2** was established as $C_{23}H_{22}O_{12}$ by HR-ESI-MS at *m/z* 491.1191 [M + H]⁺.

The ¹H NMR spectrum (Table 2) exhibited a flavonoid pattern and showed signals of H-3 at δ 6.66 (1H, s) and H-8 at δ 6.46 (1H, s). The signals for three aromatic protons of B ring were observed at δ 7.39 (1H, br s, H-2′), 6.88 (1H, br s, H-5′), and 7.41 (1H, br s, H-6′), respectively. A hydroxyl proton signal at δ 13.58 (1H, s) appeared in the downfield. The singlet at δ 1.98 (3H, s) is evident for one methyl group. The signal at δ 4.58 was assigned to the anomeric proton (H-1″) with a coupling constant ($J = 9.5$ Hz), indicating a β-configuration. The proton signals at δ 4.34 (1H, d, $J = 11.5$ Hz) and 3.90 (1H, dd, $J = 11.5, 7.0$ Hz) were assigned to H-6″ₐ and H-6″ᵦ, respectively. The remaining sugar protons were overlapped in the region of δ 3.16–4.08.

The ¹³C NMR spectrum (Table 2) and DEPT NMR spectrum showed 23 carbon signals. Of the 23 carbons (Table 2), 15 were assigned to the aglycon part and 8 to the sugar moiety. The data of the 15 aglycon carbons, including six *O*-substituent sp^2 quaternary carbons, five sp^2 methine carbons, three sp^2 quaternary, and a quaternary carbon, were used to assign the aglycon moiety of **2** as having a

Table 2. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data for compound **2** (in DMSO-d_6, δ in ppm).

Position	δ_C	δ_H (J, Hz)	Position	δ_C	δ_H (J, Hz)
Aglycone part			6-*C*-sugar Glc		
2	163.7		1$''$	70.5	4.58 (d, 9.5)
3	102.9	6.66 (s)	2$''$	73.1	
4	181.9		3$''$	78.2	
5	160.9		4$''$	69.9	
6	108.6		5$''$	78.8	
7	163.4		6$''$	64.6	4.34 (d, 11.5)
8	93.6	6.46 (s)			3.90 (dd, 11.5, 7.0)
9	156.4				
10	103.4		6$''$-*O*-substituent		
1$'$	121.5		$-C = O$	170.5	
2$'$	113.3	7.39 (br s)	$-CH_3$	20.8	1.98 (s)
3$'$	145.8				
4$'$	149.8				
5$'$	116.1	6.88 (br s)			
6$'$	119.1	7.41 (br s)			
5-OH		13.58 (s)			

Note: Assignments were based on DEPT and HMBC experiments. Glc = β-D-glucopyranose.

flavonoid unit. The ^{13}C NMR chemical shift of C-6 (δ 108.6) suggested that **2** has a glycosyl linkage at C-6. The ^{13}C NMR signals at δ 70.5, 73.1, 78.2, 69.9, 78.8, and 64.6 indicated the presence of a hexose, and the hexose carbon data were in good agreement with the presence of glucose [6].

The HMBC spectrum showed the heterocorrelations between the anomeric proton of glucose moiety at δ 4.58 and carbons signals at δ 160.9 (C-5) and 163.4 (C-7) of aglycone moiety, indicating the glucose moiety on the C-6 position. This was confirmed by comparing the almost identical spectral data of **2** with those of isoorientin [6]. The correlation between the hydroxyl proton signal at δ 13.58 and carbon signals at δ 160.9 (C-5), 108.6 (C-6), and 103.4 (C-10) established the ascription of C-5 hydroxyl. The ^{13}C NMR spectrum exhibited the signals at δ 170.5 and 20.8 indicating the existence of an acetyl group. The acetyl linkage to C-6$''$ of glucose was revealed by the chemical shift of C-6$''$ (δ 64.6), moved toward downfield for 3.4 ppm, with contrast to the literature data, as well as the HMBC correlations between the carbonyl carbon at δ 170.5 and two proton signals H-6$''_a$ (δ 4.34) and H-6$''_b$ (δ 3.90). Therefore, compound **2** was elucidated as 5, 7, 3$'$, 4$'$-tetrahydroxy flavone 6-*C*-(6$''$-acetyl) glucopyranoside, also named as 6$''$-*O*-acetylisoorientin (Figure 2).

3. Experimental

3.1 General experimental procedures

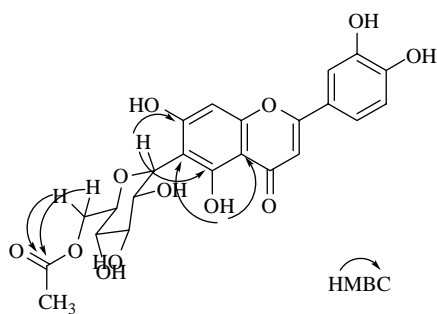Melting points were measured on an XT4-100 micromelting apparatus and are

Figure 2. Structure and selected HMBC correlations of compound **2**.

uncorrected. Optical rotations were obtained on a Perkin-Elmer 243B digital polarimeter. UV spectra were measured on a Labtech UV 2000 spectrophotometer. IR spectra were taken on a Bruker IFS-55 infrared spectrophotometer with KBr pellets. HR-ESI-MS and ESI-MS spectra were measured on an AutoSpec Ultima ETOF spectrometer in positive mode. NMR spectra were measured at 500 MHz for ^{1}H and 125 MHz for ^{13}C on an Inova 500 NMR spectrometer. Compounds were analyzed in DMSO-d_6 with TMS as an internal standard. Initial purification was carried out on silica gel (Qingdao Marine Chemical, Inc, Qingdao, China), AB-8 porous polymer resin (Tianjin University Chemical Corporation, Tianjin, China), Sephadex LH-20 resin (Amersham Biosciences, Piscataway, NJ, USA). Preparative HPLC was carried out on a Waters model 2487 instrument (Waters ODS, 7.8 i.d. 300 mm, Milford, MA, USA).

3.2 Plant material

The aerial parts of *C. pedunlulatum* were collected in Gonghe city, Qinghai Province of China, in August 2006. The identity of the plant material was verified by Prof. Peng-Cheng Lin and Chun-Lin Long, and a voucher specimen (HMH200608A) is deposited in the College of Life and Environmental Science, Minzu University of China.

3.3 Extraction and isolation

Dried and chipped aerial parts (5 kg) of *C. pedunlulatum* were extracted with boiling aqueous ethanol (70%). The solvent was filtered and evaporated *in vacuum*, and then the concentrated extract was successively partitioned with petroleum ether, ethyl acetate, and *n*-butanol. The ethyl acetate fraction (90.0 g) was successively purified on AB-8 porous polymer resin with gradient EtOH–H$_2$O as eluent to yield fractions of HEF (30.2 g), HES

(2.2 g), HEE (27.6 g), and HEN (16.9 g). Fr.HEF was chromatographed repeatedly on silica gel and eluted with CHCl$_3$– CH$_3$OH (1:0–0:1). The eluent was combined by monitoring with TLC to obtain fractions 1–13. Fraction 12 (231 mg) was subjected to Sephadex LH-20 eluted with CH$_3$OH–H$_2$O (1:1) and HPLC eluted with CH$_3$OH–H$_2$O (50:50) to yield **1** (6 mg). Fraction 13 (864 mg) was subjected to preparative TLC eluted with CH$_3$OH– H$_2$O (4:1) to yield **2** (18 mg).

3.3.1 Comastomaside A (**1**)

Yellow powder (acetone), mp 281–282°C, $[\alpha]_D^{20}$ −65.8 (*c* = 0.10, DMSO), UV (DMSO) λ_{max} (log ε): 257 (4.40), 271 (4.26), and 318 (4.34) nm; IR (KBr) ν_{max} (cm^{-1}): 3423, 1649, 1605, 1565, 1499, 1331; ^{1}H and ^{13}C NMR spectral data are shown in Table 1; ESI-MS (pos.) *m/z*:811 [M + Na]$^+$; HR-ESI-MS *m/z*: 811.2066 [M + Na]$^+$ (calcd for C$_{37}$H$_{40}$O$_{19}$Na, 811.2056).

3.3.2 6″-O-acetylisoorientin (**2**)

Yellow powder (acetone), mp 256–257°C, $[\alpha]_D^{20}$ −13.2 (*c* = 0.10, DMSO), UV (DMSO) λ_{max} (log ε): 257 (4.15), 272 (4.04), and 354 (3.95) nm; IR (KBr) ν_{max} (cm^{-1}): 3378, 1722, 1649, 1612, 1574, 1488; ^{1}H and ^{13}C NMR spectral data are shown in Table 2; ESI-MS (pos.) *m/z*: 491 [M + H]$^+$; HR-ESI-MS *m/z*: 491.1191 [M + H]$^+$ (calcd for C$_{23}$H$_{23}$O$_{12}$, 491.1184).

3.4 Acid hydrolysis of comastomaside A (**1**)

Compound **1** (1 mg) was heated in 3 ml of 10% HCl–dioxane (1:1) at 80°C for 4 h. After the dioxane was removed, the solution was extracted with EtOAc (3 ml) to yield the aglycon and the sugar, respectively. The sugar part was identified as glucose and xylose by co-TLC with monosaccharide standard.

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