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CHEMICAL CONSTITUENTS OF THE RHIZOMES OF *Tetrastigma hemsleyanum*

Hong-Xin Liu,[1,2] Luan He,[1,2] Ri-Ming Huang,[1] and Sheng-Xiang Qiu[1*]

*A new phenolic glucoside, designated as hemsleyanumoide (**1**), along with nine known compounds, was isolated from the rhizomes of* Tetrastigma hemsleyanum. *The structure of compound **1** was determined on the basis of spectroscopic analysis.*

Keywords: *Tetrastigma hemsleyanum*, phenolic glucoside, hemsleyanumoide.

The genus *Tetrastigma* (Vitaceae) comprises about 100 species, and there are 44 species occurring in China [1]. The plant *Tetrastigma hemsleyanum* Diels et Gilg. is endemic in China. As a Chinese folk herb medicine, the whole herb or its rhizome possesses antipyretic, detoxifying, anti-inflammatory, circulation-improving, and pain-relieving properties. It is used for treating infantile febrile convulsion, pneumonia, asthma, hepatitis, rheumatism, irregular menstruation, and sore throat [2]. Previous phytochemical investigations of this species have revealed the occurrence of flavonoids and their glucosides, propenylphenols, and steroids [3–5]. In this study, an investigation towards clarifying the constituents of *T. hemsleyanum* roots was performed, which led to the isolation of a new phenolic glycoside, hemsleyanumoide (**1**), and nine known compounds (**2**–**10**). The structures of the known compounds were readily determined as 4-hydroxybenzoic acid 4-*O*-*β*-D-apiofuranosyl(1→2)-*O*-*β*-D-glucopyranoside (**2**) [6], 2-(4-hydroxy-3-methoxyphenyl)-ethyl-*O*-*β*-D-glucopyranoside (**3**) [7], 1-*β*-D-glucosyloxy-2-(3-methoxy-4-hydroxyphenyl)-propane-1,3-diol (**4**) [8], isorhamnetin-3-*O*-*β*-D-rutinoside (**5**) [9], tachioside (**6**) [10], isotachioside (**7**) [10], gentisic acid 5-*O*-*β*-glucoside (**8**) [11], 6-hydroxy-1-methyl-1,2,3,4-terahydro-*β*-carboline (**9**) [12], and 2-hydroxynaringin 5-*O*-*β*-D-glucopyranoside (**10**) [13].

Hemsleyanumoide (**1**) was obtained as yellowish needle crystals, and its molecular formula was established as $C_{19}H_{26}O_{13}$ based on the HR-ESI-MS at *m/z* 461.1303 $[M - H]^-$. The ^{1}H NMR spectrum of **1** (Table 1) showed signals for a 1,3,4-trisubstituted [δ 7.62 (1H, s, H-2), 7.18 (1H, d, J = 8.4 Hz, H-5), 7.64 (1H, d, J = 8.4 Hz, H-6)] phenyl ring and two anomeric protons [δ 5.12 (d, J = 7.7 Hz, H-1′)], 5.57 (d, J = 1.3 Hz, H-1″)] for the diglycosidic moiety. The ^{13}C NMR spectrum of **1** (Table 1) exhibited 19 carbon signals, including 11 carbon signals, which was ascribed to a diglycosidic moiety (δ 100.3, 77.3, 78.8, 71.3, 78.1, 62.4, 110.2, 77.9, 80.8, 75.5, 66.2), one methoxy group (δ 56.4), and a carbonyl group (δ 170.6). Moreover, the remaining six carbons (δ 125.9, 114.2, 150.3, 151.8, 115.7, 124.5) indicated that there was a phenyl unit. HMBC correlations (Fig. 1) from H-2 to C-7 and OMe, H-5 to C-1, H-6 to C-4, and OMe to C-3 and C-4 indicated that the aglycone moiety was 4-hydroxy-3-methoxybenzoic acid.

The ^{1}H–^{1}H COSY spectrum of **1** revealed the sequential *trans*-1,2-diaxial relationship of H-1′/H-2′/H-3′/H-4′/H-5′ and two correlated methylene signals (H-6′a, b), indicating the presence of a glucopyranosyl moiety in **1**. The remaining pentose moiety was established to be apiose by comparison of the ^{1}H and ^{13}C NMR signals with those of the known compound 4-hydroxylbenzoic acid 4-*O*-*β*-D-apiofuranosyl (1→2)-*O*-*β*-D-glucopyranoside, which was also isolated from this plant [6].

1) Program for Natural Product Chemical Biology, Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, 510650, Guangzhou, P. R. China, fax: 86 20 37081190, e-mail: sxqiu@scbg.ac.cn; 2) University of Chinese Academy of Sciences, 100049, Beijing, P. R. China. Published in *Khimiya Prirodnykh Soedinenii*, No. 6, November–December, 2015, pp. 929–930. Original article submitted March 27, 2014.

TABLE 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Data of Compound **1** (CD_3OD, δ, ppm, J/Hz)

C atom	δ_C	δ_H	C atom	δ_C	δ_H
1	125.9 C	–	5′	78.1 CH	3.47 (1H, m)
2	114.2 CH	7.62 (1H, s)	6′	62.4 CH_2	3.91 (1H, m)
3	150.3 C	–			3.70 (1H, m)
4	151.8 C	–	1″	110.2 CH	5.57 (1H, d, J = 1.3)
5	115.7 CH	7.18 (1H, d, J = 8.4)	2″	77.9 CH	3.98 (1H, d, J = 1.2)
6	124.5 CH	7.64 (1H, d, J = 8.4)	3″	80.8 C	–
7	170.6 C	–	4″	75.5 CH_2	4.20 (1H, d, J = 9.7)
1′	100.3 CH	5.12 (1H, d, J = 7.7)			3.79 (1H, m)
2′	77.3 CH	3.77 (1H, m)	5″	66.2 CH_2	3.54 (2H, s)
3′	78.8 CH	3.64 (1H, m)	OMe	56.4 CH_3	3.90 (3H, s)
4′	71.3 CH	3.43 (1H, m)			

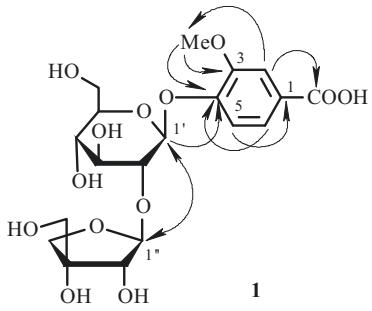

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Fig. 1. Key 2D NMR correlations of compound **1**.

The HMBC spectrum also exhibited correlations between H-1″ of apiose and C-2′ of the glucopyranosyl moiety and between glucopyranosyl H-1′ and C-4 of the 4-hydroxy-3-methoxybenzoic acid, suggesting that the apiosyl moiety was connected to the glucosyl C-2′ position. Since apiose is known to be biosynthesized from D-glucose via decarboxylation of UDP-D-glucuronic acid [14], both sugar components in **1** were assumed to be in the D-form. Therefore, compound **1** was elucidated as 3-methoxy-4-hydroxybenzoic acid 4-*O*-*β*-D-apiofuranosyl (1→2)-*O*-*β*-D-glucopyranoside and given the trivial name hemsleyanumoide.

EXPERIMENTAL

Optical rotations were measured on a PerkinElmer 341 polarimeter. UV spectra were recorded in MeOH on a PerkinElmer Lambda 35 UV-vis spectrophotometer. 1D and 2D NMR spectra were recorded on a Bruker DRX-400 spectrometer with TMS as internal standard. HR-ESI-MS data were obtained on a Bruker Bio TOF IIIQ mass spectrometer. All solvents were of analytical grade (Shanghai Chemical Plant, Shanghai, People′s Republic of China). Silica gel (200–300 mesh) was used for column chromatography, and precoated silica gel GF254 plates (Qingdao Haiyang Chemical Plant, Qingdao, People′s Republic of China) were used for TLC. C18 reversed-phase silica gel (150–200 mesh, Merck), MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd.), and Sephadex LH-20 gel (Amersham Biosciences) were also used for column chromatography. TLC spots were visualized under UV light and by dipping into 5% H_2SO_4 in alcohol followed by heating.

The rhizomes of *T. hemsleyanum* were collected from Leye County of Guangxi Province, China, in June 2010 and identified by Dr. Fa-Guo Wang of SCBG. A voucher specimen was deposited at the Laboratory of Natural Product Chemical Biology, SCBG.

The air-dried powder of the plant material (15 kg) was exhaustively extracted with MeOH three times, and the extract (1.1 kg) was suspended in H_2O and partitioned with *n*-hexane and EtOAc. The H_2O part (1 kg) was subjected to resin D101 chromatography eluting with a gradient of increasing EtOH in H_2O (0–95%) to afford three fractions. Fraction A (8 g,

EtOH–H_2O, 10:90) was subjected to reversed-phase column chromatography over C_{18} silica gel eluting with a gradient of increasing MeOH in H_2O (5–95%) to afford two fractions. Fraction A1 was subsequently chromatographed on a Sephadex LH-20 column (MeOH) and repeatedly on a silica gel column to afford **1** (5 mg), **2** (7 mg), **3** (8 mg), **6** (4 mg), **7** (4 mg), and **8** (6 mg). Fraction A2 was subsequently chromatographed on a Sephadex LH-20 column (MeOH) and repeatedly on a silica gel column to afford **4** (10 mg) and **9** (5 mg). Fraction B (3 g, EtOH–H_2O, 50:50) was chromatographed on a Sephadex LH-20 column (MeOH) to afford two fractions. Fraction B2 was purified by repeated column chromatography over silica gel to provide **5** (50 mg), **10** (10 mg).

Compound 1. Yellow needle crystals, $[\alpha]_D^{20}$ –69.6° (*c* 0.05, MeOH). UV (MeOH, λ_{max}, nm) (log ε): 254.9 (2.68). For ^1H and ^{13}C NMR, see Table 1. ESI-MS (neg.) *m/z* 461 ($[M - H]^-$); HR-ESI-MS (neg.) *m/z* 461.1303 $[M - H]^-$ (calcd for $C_{19}H_{25}O_{13}$, 461.1373).

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