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The Novel Structure of a Disaccharide Derivative from *Hemsleya amabilis*

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Abstract—Amabiose, a new disaccharide derivative with a novel skeleton, was isolated from the rhizomes of *Hemsleya amabilis*. Its structure was condensed from a glucose and a hemslose, which was elucidated unambiguously by spectroscopic means and chemical reaction, including 2D NMR spectroscopy and X-ray analysis.

Introduction

The southwest of China is the distribution center of the genus *Hemsleya* (Cucurbitaceae), which is relatively abundant in Yunnan and Sichuan. Recently, 31 species of this genus have been discovered, among which 20 species are found in Yunnan. *Hemsleya amabilis*, belonging to Cucurbitaceae, is distributed through central and western Yunnan and Guanxi.[1]

The rhizomes of this genus are known as folk medicines in the southwest of China, used in the treatment of bacillary dysentery, bronchitis and tuberculosis etc. Previously, it has been reported that dihydrocucurbitacin F and its 25-*O*-acetate were isolated from *H. amabilis* Diels.[2] In the present paper, we deal with the isolation and structure determination of amabiose from *H. amabilis* Diels.

Results and Discussion

The EtOAc fraction of the ethanol extract from the rhizomes of *H. amabilis* was subjected to repeated chromatography to afford compound **1** (amabiose). On acid hydrolysis, it

Table 1. ^{1}H, ^{13}C NMR and 2D NMR spectral of amabiose (in D_2O, ppm)

H	δ	J (Hz)	^{1}H–^{1}H COSY correlation with	C	δ (DEPT)	^{1}H–^{13}C COSY correlation with	COLOC correlation with
Glucose							
1	4.614 d	7.84	H-2.5a′	1	106.65(CH)	H-1	H-5′a, 5′b
2	3.304 dd	7.84, 9.68	H-1, 3	2	84.33(CH)	H-2	H-1, 3/H-1′
3	3.692 dd	8.82, 9.72	H-2, 4	3	75.78(CH)	H-3	H-2, 4
4	3.49 dd	8.82, 9.72	H-3, 5	4	72.26(CH)	H-4	H-3, 6a
5	3.580 m		H-4, 6a, 6b	5	80.25(CH)	H-5	H-4, 6a, 6b
6a	3.922 dd	2.2, 12.5	H-6b, 5	6	63.22(CH_2)	H-6a, 6b	H-4, 5
6b	3.780 dd	5.14, 12.5	H-6a, 5				
Hemslose							
1′	5.078 s			1′	115.41(CH)	H-1′	H-4b′, 5b′/H-2
2′				2′	88.59(C)		H-3′, 4b′, 5b′
3′	4.122 m		H-4′	3′	78.05(CH)	H-3′	H-4′
4′a	4.284 dd	4.24, 9.74	H-4′b, 3′	4′	78.06(CH_2)	H-4′a, 4′b	H-3′, 1′
4′b	4.107 dd	2.36, 9.76	H-4′a				
5′a	4.358 d	13.88	H-5′b, 5a′, 1	5′	73.99(CH_2)	H-5′a, 5′b	H-1
5′b	4.185 d	14.08	H-5′a, 1′				

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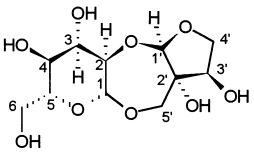
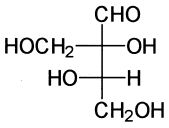


amabiose



hemslose

Figure 1. The chemical structure of amabiose and hemslose.

afforded D-glucose (Glc) and a new monosaccharide, named as hemslose.[3,4] The negative ion FAB mass spectrum exhibits a molecular ion peak at *m/z* 293$[M-1]^-$. The IR spectrum reveals the characteristic absorption of hydroxyl at 3400 cm^{-1}. The ^{13}C NMR shows 11 carbon atoms. DEPT spectrum divides these signals as 3×CH_2, 7×CH, 1×C (see Table 1). The ^1H NMR of **1** shows the presence of two anomeric proton signals at δ 5.07 (1H, s) and δ 4.61 (1H, d, J=7.8 Hz). This evidence suggests the presence of two glycoside bonds. The positions of C and H were assigned by ^1H-^1H COSY and ^1H-^{13}C COSY spectra. The position of the glycoside bond was established unambiguously by using COLOC experiments. The glucose C-1 signal at δ 106.65 shows long range correlation with the ^1H signals at 4.358 (H-5′a) and 4.185 (H-5′b). Thus, the first glycoside bond should be located on C-1 to C-5′. The long-range coupling of the carbonic signals at δ 115.4 (C-1′) with the proton signals at δ 3.304 (glucose H-2) was shown. Thus, the other glycoside bond should connect C-1′ with C-2. Therefore, the structure of compound **1** was established.

The EI-MS of acetylate of **1** show a molecular ion peak at *m/z* 505 $[M+1]^+$, which indicates that it is penta-acetylation of **1**. This evidence suggests the presence of two glycoside bonds. These results lead to the structure of **1** being assigned as β-D-glucopyranosyl(1→5′) β-D-hemslofuranosyl(1′→2). Finally, a single crystal X-ray analysis confirmed the structure deduced from the NMR spectroscopic data (Fig. 1).

Experimental

General procedures

All melting points are uncorrected. Optical rotations were measured in H_2O on a J-20C polarimrter. ^1H and ^{13}C NMR and 2D NMR spectra were recorded in D_2O with a Bruker AM-400 MHz instrument, using TMS as an internal standard. EI-MS: 70 eV, and FAB-MS spectra were measured with a VG AutoSpec mass spectrometer. IR spectra were measured on a Bio-Rad FTS 135 spectrophotometer, as KBr discs. CC was carried out with silica gel (200–300 mesh, Marine Chemical Industry Factory of Qingdao, China), D101 (60–80 mesh, Chemical Factory of Nankai University, Tianjin, China). The spots were visualized by spraying with 20% H_2SO_4, followed by heating.

Plant material

H. amabilis was collected at Songming, Yunnan, China. Specimen was taxonomically identified by Cheng-Yih Wu and deposited in the Herbarium of Kunming Institute of Botany, The Chinese Academy of Sciences.

Extraction and separation

The dried and powdered rhizomes (7.8 kg) were extracted with hot 95% ethanol three times. The ethanol extract (1.8 kg) was suspended in H_2O, and then was extracted with EtOAc. The EtOAc layer was concentrated to yield an EtOAc extract (404 g). The EtOAc extract was dissolved in water and filtered. The filtrate was concentrated to give the water extract (80 g).

The water extract (80 g) was subjected to macroporous absorption resin D-101, eluting with aq. Methanol (10, 20, 50, 80 and 100%), to give five fractions. Fr-2 (eluting with 20% MeOH, 5 g) was repeatedly chromatographed on silica gel, eluting with $CHCl_3$–CH_3OH (4:1) to give **1** (34 mg).

Compound (1). $C_{11}H_{18}O_9$ yield: 0.0084%, colorless prisms (from H_2O), mp 235°C (dec.), $[\alpha]_D^{28.1}$=+24.05° (H_2O, *c* 0.395), FAB-MS (negative) *m/z* (rel. Intensity): 385$[M-1+Gly]$(100), 293$[M-1]^-$. HRMS: *m/z* 293.0834 $[M-H]^-$, calcd. 293.0872 for $C_{11}H_{17}O_9$. IR ν (KBr) cm^{-1}: 3500–3000 (3445, 3416, 3359, 3255) (OH), 2973, 2929, 2905, 1300, 1109, 1048. ^1H and ^{13}C NMR (D_2O) data see Table 1.

Acid hydrolysis of 1

Compound **1** (3 mg) and sucrose (10 mg) were hydrolyzed with 20% H_2SO_4 in water for 10 h at 80–90°C, respectively. The reaction mixture was examined on TLC eluting with isopropyl alcohol–acetic ether–H_2O (7:1:2). This suggested the presence of glucose.

Test for Hemslose[4]

The reaction mixture (0.2 ml) was added, without mixing, to a 0.3% solution of β-naphthol in concentrated sulfuric acid (0.5 ml), after 3 min a green ring formed at the interface between the two solutions. This experiment shows that the hemslose and apiose have the very similar chemical properties.

Acetylation of 1. Compound **1** (6 mg) was dissolved in pyridine (1 ml) and Ac_2O (0.5 ml). The solution was allowed to stand for 2 days at room temperature. The reaction mixture was poured into ice water, and extracted with ether. The ether extract was dissolved in ethanol, and recrystallized give to colorless needles (**1**-Ac). EI-MS ($CDCl_3$) *m/z*(rel. Intensity): 505$[M+1]^+$, 463$[M+1-CH_3CO]^+$, 445, 403, 317, 289, 109(100), ^{1}HNMR ($CDCl_3$) ppm: δ 1.609 (3H, s, 6-$OCOCH_3$), 2.035, 2.066, 2.090, 2.117 (3H×4, s, 2′, 3′, 3, 4-$OCOCH_3$), 3.485 (1H, dd, J=7.8, 9.9 Hz, 2-H), 3.824 (1H, m, 5-H), 4.020 (1H, dd, J=5.8 Hz, 4-H), 4.029 (1H, d, J=13.2 Hz, 5′b-H), 4.122 (1H, d; J=13.3 Hz, 5′a-H), 4.168 (1H, dd, J=2.2, 12.4 Hz, 4′b-H), 4.239 (1H, dd, J=4.76, 12.4 Hz, 4′a-H), 4.293 (1H, dd, J=5.4, 10.14 Hz, 3-H), 4.534 (1H, d, J=7.8 Hz, 1-H), 4.999 (1H, m, 3′-H), 5.009 (1H, s, 1′-H), 5.028 (1H, dd, J=9.6, 12.1 Hz, 6b-H), 5.244 (1H, dd, J=9.6 Hz, 6a-H).

X-Ray crystal structure analysis of amabiose

$C_{11}H_{18}O_9$, M=294, monoclinic, space group $P2_1$,

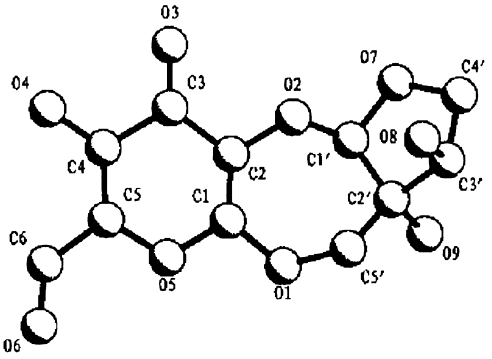


Figure 2. Crystal structure of amabiose.

a=8.777(1) Å, b=8.553 (1) Å, c=8.542(1) Å, β=83.174(4)°, V=636.7 (1) Å^3 or Z=2, D_c=1.530 g/cm^3. The structural relinement was carried out using a direct method (SHELXS-86) and all the C and O atoms were positioned from the difference Fourier method and full-matrix least squares. The final R_f=0.048, R_w 0.047. (w=1/$\sigma^2|F|$), GoF=7.345 for 1118 observed reflections. The X-ray structure of the molecule is shown in Fig. 2.

The results show that amabiose is condensed from glucose and hemslose, and there are inter-molecular and intra-molecular hydrogen-bonding interactions present in the crystal (Table 2).

Table 2. Hydrogen bonding parameters for compound **1**

Bonding atomic pair	Distance (Å)	Symmetry transformation
O3–O2	2.858	*X*, *Y*, *Z*
O3–O6	2.752	*X*, *Y*, 1+*Z*
O3–O9	2.820	1+*X*, *Y*, *Z*
O4–O8	2.721	1−*X*, 2−*Y*, 1−*Z*
O6–O7	2.867	*X*, *Y*, −1+*Z*
O8–O1	2.704	−*X*, 1/2+*Y*, 1−*Z*
O9–O7	2.987	*X*, *Y*, *Z*

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