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Two novel oligosaccharides from *Solanum nigrum*

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ABSTRACT

Phytochemical analysis of *Solanum nigrum* has resulted in the isolation of two novel disaccharides. Their structures were determined as ethyl β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranoside **(1)** and ethyl β-D-thevetopyranosyl-(1→4)-α-D-oleandropyranoside (**2**), respectively, by chemical and spectroscopic methods.

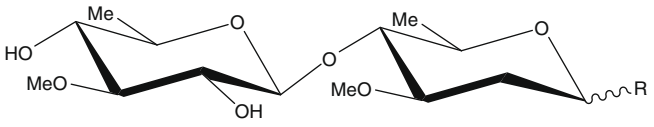
Oligosaccharides of 2-deoxy sugars have been reported to possess immunomodulating, anticomplementary, antitumor, and anticancer activities.[1–4] Many oligosaccharides of 2-deoxy hexoses are known to occur in nature as the glycone part of the biologically active pregnanes and cardiac oligoglycosides.[5–7] The plant *Solanum nigrum* L. (Solanaceae), a popular medicinal herb in China, is believed to have various therapeutic properties, especially against certain types of cancer. Its therapeutic mechanism remains unknown. This paper describes the structure elucidation of two novel oligosaccharides (**1**, **2**) (Fig. 1), isolated from the extract of the aerial parts of the plant. Their structures were determined as ethyl β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranoside and ethyl β-D-thevetopyranosy-(1→4)-α-D-oleandropyranoside, respectively, by chemical and spectroscopic methods including ESIMS, ^{1}H, ^{13}C, and 2D NMR spectroscopy.

Compound **1**, $C_{16}H_{30}O_8$ (ESIMS *m/z* 349 $[M-H]^-$), gave a positive color test in the Keller–Kiliani reaction[8] indicating the presence of rare, 2,6-dideoxy hexose(s). The presence of two anomeric carbon signals at δ 101.68 and 99.16 in the ^{13}C NMR spectrum and two anomeric proton signals at δ 4.49 (d, *J* 8.1 Hz, 1H) and 4.45 (dd, *J* 9.7 and 1.7 Hz, 1H) in the ^{1}H NMR spectrum of **1** showed it to be a disaccharide. The large coupling constants (8.1 and 9.7 Hz) of the two anomeric signals indicated that both sugars were present in the $^4C_1$(D) conformation joined through β-glycosidic linkages. The ^{1}H NMR spectrum of **1** also showed the signals for other ring protons of the sugar moieties. The presence of carbon signals at δ 15.12 and 64.65 in conjunction with a triplet (3H, *J* 7.1 Hz) at δ 1.23 and two double-quartets (*J* 7.1, 9.4 Hz) at δ 3.92 (1H) and 3.52 (1H) suggested that these signals were due to a $-OCH_2CH_3$ group present at the anomeric position. The ^{1}H NMR spectrum of **1** contained two singlets of three protons each for two methoxy groups (δ 3.66 and 3.38) confirming the presence of two methylated sugars. The ESIMS/MS spectrum of **1** also showed that the $[M-H]^-$ fragment sequentially lost one OCH_2CH_3 and one molecule of CH_3OH giving a fragment-ion peak at *m/z* 271 which showed the ethyl glycosidic structure and the presence of a methoxy group in **1**.

The ^{1}H and ^{13}C NMR spectroscopic data ascribed to the first sugar moiety of **1** were the same as those reported for the D-oleandrose moiety,[9] and the ^{1}H and ^{13}C NMR spectroscopic data ascribed to the second sugar moiety of **1** were the same as those reported for the D-thevetose moiety,[10] which were further confirmed by examination of the corresponding HMBC, HSQC, and ROESY spectra (Figs. 2 and 3).

In the light of the foregoing evidence, the structure of **1** was established as ethyl 6-deoxy-3-*O*-methyl-β-D-glucopyranosyl-(1→4)-2,6-dideoxy-3-*O*-methyl-β-D-*arabino*-hexopyranoside (**1**).

Compound 2, $C_{16}H_{30}O_8$ (ESIMS *m/z* 373 $[M+Na]^+$, 723 $[2M+Na]^+$), gave positive color tests in Keller–Kiliani reaction[8] indicating the



1 R = OEt
2 R = OEt

Figure 1. Structure of **1** and **2**.

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Figure 2. Key HMBC interactions in **1**.

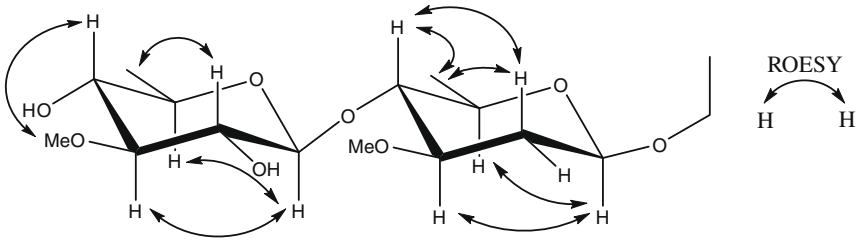

Figure 3. Key ROESY interactions in **1**.

presence of rare, 2,6-dideoxy hexose(s). The ^{1}H and ^{13}C NMR spectroscopic data of **2** were very similar to that of **1** except for some signals of the D-oleandrose moiety.

The broad doublet (2.7 Hz) of the H-1 and two double doublets of doublets of the two protons of the H-2 each at δ 2.30 (13.0, 5.0, 1.3 Hz) and δ 1.56 (13.0, 11.3, 3.8 Hz) confirmed that the glycosidic linkage between D-oleandrose moiety and $-OCH_2CH_3$ group was α-oriented. The ^{1}H and ^{13}C NMR spectroscopic data of **2** are given in Table 1. In light of the foregoing evidence, the structure of **2** was thus established as ethyl 6-deoxy-3-*O*-methyl-β-D-glucopyranosyl-(1→4)-2,6-dideoxy-3-*O*-methyl-α-D-*arabino*-hexopyranoside (**2**).

1. Experimental

1.1. General

Column chromatography (CC) was carried out using silica gel (SiO_2, 200–300 mesh) from Qingdao Marine Chemical Plant, Qingdao, PR China. Sephadex LH-20 was purchased from GE Healthcare Bio-Sciences AB. YMC*GEL® ODS-A was obtained from YMC Co., Ltd. TLC-precoated silica gel G plates were from Qingdao Marine Chemical Plant, Qingdao, PR China. ^{1}H, ^{13}C, and 2D NMR spectra were determined using a Bruker AV-500 spectrometer; chemical shifts are in δ units relative to Me_4Si. Spin–spin coupling constants (*J*) are in hertz. Mass spectra were determined using an Agilent-1100-JC/MSD-Trap (ESIMS) spectrometer.

1.2. Plant material

The powered water extraction of aerial parts of *S. nigrum* was purchased from Nanjing Zelang Pharmaceutical Co., Ltd, PR China. A voucher specimen was identified by Professor Xue-Hua Song (China Pharmaceutical University) and has been deposited with the Herbarium of China Pharmaceutical University, Nanjing, PR China (reference number: 20080911).

Table 1
NMR data (500 MHz $CDCl_3$) of **1** and **2**

Carbon	**1**		**2**		
	δ_C	δ_H	HMBC (H to C)	δ_C	δ_H
Oleandrose-1					
1	99.16	4.45 (dd, *J* 9.7, 1.7 Hz)	C-2, $-OCH_2CH_3$	96.50	4.88 (d, *J* 2.7 Hz)
2	35.69	2.38 (ddd, *J* 10.5, 4.5, 1.7 Hz) 1.49 (dt, *J* 11.2, 10.7 Hz)	C-1, C-3, C-4	34.46	2.30 (ddd, *J* 13.0, 5.0, 1.3 Hz) 1.56 (ddd, *J* 13.0, 11.3, 3.8 Hz)
3	78.83	3.38–3.40 (m)	C-4, C-5	76.56	3.42–3.45 (m)
4	79.72	3.34–3.36 (m)	C-3, C-5, C-1′	80.28	3.36–3.39 (m)
5	71.31	3.34–3.37 (m)	C-4, C-6	66.85	3.36–3.39 (m)
6	18.47	1.37 (d, *J* 5.4 Hz)	C-3, C-4, C-5	18.42	1.34 (d, *J* 6.1 Hz)
OMe	55.79	3.38 (s)	C-3	56.03	3.41 (s)
Thevetose-2					
1′	101.68	4.49 (d, *J* 8.1 Hz)	C-4, C-3′, C-5′	101.56	4.51 (d, *J* 8.0 Hz)
2′	73.27	3.47 (t, *J* 8.6 Hz)	C-1′, C-3′	73.28	3.50 (t, *J* 8.6 Hz)
3′	85.45	3.09 (t, *J* 9.0 Hz)	C-2′, C-4′, OMe	85.48	3.12 (t, *J* 9.0 Hz)
4′	74.82	3.16 (t, *J* 9.0 Hz)	C-3′, C-5′, C-6′	74.85	3.19 (t, *J* 9.0 Hz)
5′	71.89	3.34–3.36 (m)	C-1′, C-4′, C-6′	71.88	3.36–3.39 (m)
6′	17.76	1.31 (d, *J* 6.1 Hz)	C-4′, C-5′	17.76	1.33 (d, *J* 6.3 Hz)
OMe	60.57	3.66 (s)	C-3′	60.54	3.69 (s)
$-OCH_2CH_3$	64.65	3.92 (dq, *J* 7.1, 9.4 Hz) 3.52 (dq, *J* 7.1, 9.4 Hz)	C-1, $-OCH_2CH_3$	62.56	3.73–3.77 (m) 3.66–3.69 (m)
$-OCH_2CH_3$	15.12	1.23 (t, *J* 7.1 Hz)	$-OCH_2CH_3$	15.03	1.21 (t, *J* 7.1 Hz)

1.3. Extraction and isolation

The dried powered water extraction material (1 kg) was suspended in water, which was further extracted with *n*-butanol (1 L). After concentrating the *n*-butanol extract in vacuo to afford a residue (18 g), the residue was separated by column chromatography (ODS-A) using a gradient of 10:90–60:40 MeOH–H_2O, to afford 30 fractions (Fr. 1). Fr. 1 (21–30) was subjected to additional chromatography (Sephadex LH-20, 1:1 MeOH–H_2O) to yield 178 fractions (Fr. 2). Fr. 2 (54–85) was further chromatogramed by column chromatography (SiO_2, 1:1 petroleum ether–EtOAc) to afford compounds **1** (10 mg) and **2** (14 mg).

1.4. Characterization of compounds 1 and 2

1.4.1. Ethyl β-D-thevetopyranosyl-(1-4)-β-D-oleandropyranoside (1)

Colorless gel. It gave a positive color test in the Keller–Kiliani reaction.[8] ^1H, ^{13}C NMR, and HMBC spectroscopy: Table 1. The key correlations of HMBC and ROESY are presented in Figure 1. ESIMS (negative ion): 349 ($[M-H]^-$), 271 ($[M-H-OCH_2CH_3-CH_3OH]^-$).

1.4.2. Ethyl β-D-thevetopyranosyl-(1-4)-α-D-oleandropyranoside (2)

Colorless gel. It gave a positive color test in the Keller–Kiliani reaction.[8] ^1H and ^{13}C NMR: Table 1. ESIMS (positive ion): 373 ($[M+Na]^+$), 723 ($[2M+Na]^+$).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2009.06.013.

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