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Pregnane glycosides from *Solanum nigrum*

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Two new pregnane saponins, solanigroside A (**1**) and solanigroside B (**2**), along with two known compounds (**3** and **4**), were isolated from 60% ethanolic extract of the dried herb of *Solanum nigrum* L. The structures of **1** and **2** were elucidated as 5α-pregn-16-en-3β-ol-20-one 3-*O*-β-D-xylopyranosyl-(1 → 3)-*O*-[α-L-arabinopyranosyl-(1 → 2)]-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-*O*-β-D-galactopyranoside (**1**) and 5α-pregn-16-en-3β-ol-20-one 3-*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-β-D-galactopyranoside (**2**), respectively, on the basis of extensive spectroscopic analysis as well as comparison with reported spectroscopic data of related compounds. This paper deals with the isolation and structural characterisation of pregnane glycosides from *S. nigrum* L.

Keywords: *Solanum nigrum*; Steroidal saponin; Pregnane saponin; Solanigrosides A and B

1. Introduction

Solanum nigrum L. is widely distributed in most regions of China. It has been used traditionally for the treatment of carcinoma of the lung, urinary bladder, larynx and vocal cords in combination with other herbs. The main components of *S. nigrum* L. reported were steroidal alkaloids [1] and flavonols [2]. Previously, our group analysed the constituents of this plant and found three steroidal glycosides, β_2-solamargine, solamargine and degalactotigonin [3]. Further phytochemical examination of *S. nigrum* was carried out with particular attention to total glycosides extract due to its potent cytotoxicity against U266 cells [4]. As a result, two new pregnane-type steroidal saponins solanigroside A (**1**) and solanigroside B (**2**), as well as two known compounds (**3** and **4**) were isolated. The first pregnane glycoside was isolated from *Paris polypylla* by Nohara *et al.* [5]. Recently, several pregnane glycosides along with other steroidal glycosides were obtained from *Tacca chantrieri* [6] and *Dioscorea spongiosa* [7]. This evidence indicated that the pregnane glycoside coexists in small amounts together with steroidal glycoside, and simultaneously

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means that pregnane glycoside could be biosynthesised from steroidal glycoside, because the intermediates from the spirostane to the pregnane in Marker's degradation procedure, 16-acylated pregnane derivatives, were also found [6,8,9]. This paper deals with the isolation and characterisation of pregnane glycosides from *Solanum nigrum* L.

2. Results and discussion

Compound **1** was obtained as an amorphous powder and positive to Liebermann-Burchardt and Molish tests, indicating its glycosidic nature. Its molecular formula was deduced by HRESI-MS (*m/z* 1073.4720 $[M + Na]^+$) as $C_{49}H_{78}O_{24}$. The ESI-MS spectrum showed positive ion fragment at *m/z* 1073 $[M + Na]^+$ and negative ion fragment at *m/z* 1049 $[M - H]^-$. The ^1H NMR spectrum of **1** showed the presence of four methyls at δ 0.85 (3H, s), 0.90 (3H, s), 2.24 (3H, s) and 1.73(3H, d, $J = 6.2$ Hz), five anomeric protons at δ 4.86 (1H, d, $J = 7.7$ Hz), 4.96 (1H, d, $J = 7.9$ Hz), 5.21 (1H, d, $J = 7.8$ Hz), 5.34 (1H, d, $J = 7.5$ Hz), and 6.30 (1H, br. s), and one olefinic proton at δ 6.61 (1H, m). In its ^{13}C NMR spectrum, 49 carbon signals were detected, which consisted of 4 methyls, 12 methylenes, 29 methines, and 4 quaternary carbons in combination with DEPT spectral analysis. One carbonyl carbon at δ 196.0, two olefinic carbons at δ 144.4 and 155.2 indicating the presence of an α, β-unsaturated carbonyl group in the molecule [10], as well as five anomeric carbons at δ 100.0, 101.5, 104.8, 105.2, 105.6 and four methyl groups at δ 12.1, 15.9, 26.9 and 18.3 were also confirmed in the ^{13}C NMR spectra.

The pregnane skeleton was determined by ^1H—^1H COSY, HSQC and HMBC spectra. A combination of ^1H—^1H COSY and HSQC experiments showed the following connectivities from C-1 to C-8, C-8 to C-12, and C-14 to C-16, starting from the well-resolved signals at δ 0.77 (H-1), 0.57 (H-9) and 6.61 (H-16). In the HMBC spectrum of **1**, ^{13}C—^1H long-range correlations were found for H_3-18 (δ 0.90)/C-12, 13, 14, 17; H_3-19 (δ 0.85)/C-1, 5, 9, 10; H_3-21 (δ 2.24)/C-17, 20 and H-16 (δ 6.61)/C-13, 14, 15, 17, 20. Full assignments of the proton and carbon signals of **1** are listed in table 1 based on the analysis of 1D and 2D NMR spectral data.

Acid hydrolysis of **1** with 2 M HCl afforded glucose, galactose, rhamnose, xylose, and arabinose, which were identified by thin-layer chromatography compared with authentic samples. Starting from the five anomeric protons, the exact identity of the monosaccharides and the sequence of the oligosaccharide chain were also determined by the analysis of a combination of DEPT, ^1H—^1H COSY, HSQC, HMBC, and TOCSY spectra. In the HMBC spectrum, long-range correlations were observed between H-1 (δ 4.86) of galactosyl and C-3 (δ 76.9) of the aglycone, H-1 (δ 4.96) of glucosyl and C-4 (δ 81.3) of galactosyl, H-1 (δ 6.30) of rhamnosyl and C-2 (δ 77.2) of galactosyl, H-1 (δ 5.34) of arabinosyl and C-2 (δ 81.3) of glucosyl, H-1 (δ 5.21) of xylosyl and C-3 (δ 87.7) of glucosyl. The ^{13}C NMR chemical shifts of the saccharide part were in good agreement with nigrumnin I reported in the literature [11]. Based on the chemical evidence and spectral analysis mentioned above, the structure of compound **1** (figure 1) was established as 5α-pregn-16-en-3β-ol-20-one 3-*O*-β-D-xylopyranosyl-(1 → 3)-*O*-[α-L-arabinopyranosyl-(1 → 2)]-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-*O*-β-D-galactopyranoside. It is a new compound named solanigroside A.

Compound **2** was obtained as an amorphous powder. The HRESI-MS showed the $[M + Na]^+$ ion at *m/z* 987.4390, corresponding to the molecular formula $C_{45}H_{72}O_{22}$. The ^1H NMR

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Table 1. ^{1}H NMR data of compounds **1**, **2** and ^{13}C NMR data of compounds **1**–**4** (C_5D_5N)[†].

	*Solanigroside A (**1**)*		*Solanigroside B (**2**)*			
Position	*^{1}H δ (J Hz)*	*^{13}C δ*	*^{1}H δ (J Hz)*	*^{13}C δ*	***3***	***4***
1	1.50	36.8	1.42	36.9	36.7	37.3
	0.77 (m)		0.73 (m)			
2	2.02 (m) 29.7		2.03 (m)	29.8	29.6	30.1
	1.76		1.59			
3	3.90	76.9	3.91 (m)	77.3	77.1	78.1
4	1.92 (m)	34.2	1.80 (m)	34.8	34.6	39.0
	1.65 (m)		1.36			
5	0.89	44.7	0.87 (m)	44.8	44.7	141.2
6	1.20 (2H, m)	28.7	1.14 (2H, m)	28.8	28.6	121.5
7	1.54	31.9	1.51 (m)	32.0	31.8	31.8
	0.85		0.84			
8	1.40 (m)	33.6	1.36	33.8	33.6	30.3
9	0.57 (m)	54.7	0.54 (m)	54.9	54.7	50.7
10		35.8		35.9	35.7	37.1
11	1.47	21.0	1.42	21.2	21.0	20.9
	1.32		1.30	35.2		
12	2.56 (m)	35.1	2.57 (m)		35.0	35.1
	1.32		1.32			
13		46.3		46.5	46.3	46.3
14	1.29	56.2	1.27	56.4	56.2	56.4
15	2.16	32.0	2.11	32.2	32.0	32.3
	1.87 (m)		1.87 (m)			
16	6.61	144.4	6.60 (d, 3.2)	144.6	144.6	144.8
17		155.2		155.2	155.2	155.2
18	0.90 (s)	15.9	0.90 (s)	16.1	15.9	15.9
19	0.85 (s)	12.1	0.64 (s)	12.1	12.0	19.2
20		196.0		196.1	196.0	196.3
21	2.24 (s)	26.9	2.25 (s)	27.1	26.9	27.1
Gal						Glc
1′	4.86 (d, 7.7)	100.0	4.90 (d, 7.9)	102.4	102.2	100.2
2′	4.39	77.2	4.44	73.2	72.9	77.9
3′	4.13	76.0	4.11	75.6	75.2	77.9
4′	4.50	81.3	4.61	80.2	79.7	78.6
5′	3.96	74.9	4.02	75.3	75.4	76.9
6′	4.73	60.2	4.71 (m)	60.6	60.4	61.2
	4.21		4.24			
Glc						Rha-I
1″	4.96 (d, 7.9)	105.2	5.17 (d, 7.9)	105.0	104.9	102.0
2″	4.23	81.3	4.41	81.4	81.1	72.4
3″	4.06	87.7	4.22	88.5	86.6	72.8
4″	3.80	70.2	3.86	70.8	70.2	74.0
5″	3.82	77.5	3.86	77.5	78.5	69.5
6″	4.52	62.7	4.55 (m)	62.3	62.3	18.6
	4.04		4.29 (m)			
Ara			Glc II		Glc II	Rha-II
1‴	5.34 (d, 7.5)	105.6	5.60 (d, 7.7)	104.9	104.6	102.8
2‴	4.41	73.1	4.07	75.3	76.0	72.5
3‴	4.02	74.5	4.20	78.6	77.5	72.7
4‴	4.17	69.5	4.18	71.6	70.8	73.9
5‴	4.67	67.1	3.87	78.6	77.4	70.4
	3.60					
6‴		4.55	62.3		62.7	18.5
		4.40				
Xyl			Glc III		Xyl	
1⁗	5.21 (d, 7.8)	104.8	5.31 (d, 7.8)	104.5	104.7	
2⁗	3.96	74.9	4.08	76.1	74.8	
3⁗	4.08	78.5	4.15	77.9	78.4	

Table 1 – *continued*

	*Solanigroside A (**1**)*		*Solanigroside B (**2**)*			
Position	*^{1}H δ (J Hz)*	*^{13}C δ*	*^{1}H δ (J Hz)*	*^{13}C δ*	**3**	**4**
4''''	4.12	70.5	4.25	70.9	70.5	
5''''	4.22	67.1	4.05	78.6	67.1	
	3.65					
6''''			4.52	63.0		
			4.04			
Rha						
1'''''	6.30 (br. s)	101.5				
2'''''	4.81	72.2				
3'''''	4.54	72.5				
4'''''	4.32	73.8				
5'''''	4.91	69.4				
6'''''	1.73 (d, 6.2)	18.3				

† Recorded on a Bruker-400 (100 MHz for ^{13}C) NMR spectrometer.

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spectrum of **2** displayed three tertiary methyl signals at δ 0.64 (3H, s), 0.90 (3H, s), 2.25 (3H, s) and four anomeric proton signals at δ 5.60 (1H, d, $J = 7.7$ Hz), 5.31 (1H, d, $J = 7.8$ Hz), 5.17 (1H, d, $J = 7.9$ Hz), 4.90 (1H, d, $J = 7.9$ Hz). Analysis of the ^{13}C NMR spectrum of **2** and comparison with that of **1** revealed that **2** possessed an aglycone moiety identical to that of **1**. The monosaccharides obtained after acid hydrolysis of **2** were detected as glucose and galactose by thin-layer chromatography in comparison with authentic samples. The β-configuration of glucose and galactose were determined by the large coupling value ($J_{H1-H2} > 7$) [12] of anomeric protons. The points of attachment of the saccharide part and interglycosidic linkage were established by HMBC experiment. The HMBC correlations were observed between H-1 (δ 4.90) of galactosyl and C-3 (δ 77.3) of the aglycone, H-1 (δ 5.17) of glucosyl I and C-4 (δ 80.2) of galactosyl, H-1 (δ 5.60) of glucosyl II and C-2 (δ 81.4) of glucosyl I, H-1 (δ 5.31) of glucosyl III and C-3 (δ 88.5) of glucosyl I. The ^{13}C NMR chemical shifts of the saccharide part were identical with those reported in the literature [13]. All the above data identified **2** as 5α-pregn-16-en-3β-ol-20-one 3-*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-β-D-galactopyranoside (figure 1). It is also a new compound named solanigroside B.

By similar means, compound **3** was identified as 5α-pregn-16-en-3β-ol-20-one lycotetraoside [9], namely, 5α-pregn-16-en-3β-ol-20-one 3-*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-β-D-galactopyranoside. By comparison with the published data [12], compound **4** was established as pregna-5,16-dien-3β-ol-20-one 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[α-L-rhamnopyranosyl-(1 → 4)]-*O*-β-D-glucopyranoside (figure 1).

3. Experimental

3.1 General experimental procedures

Melting points were determined with X-5 hot stage microscope melting point apparatus and are uncorrected. Optical rotations were obtained on a P-1020 digital polarimeter (JASCO Corp.). IR spectra were measured on a JASCO FT/IR-480 plus instrument. 1D and 2D NMR

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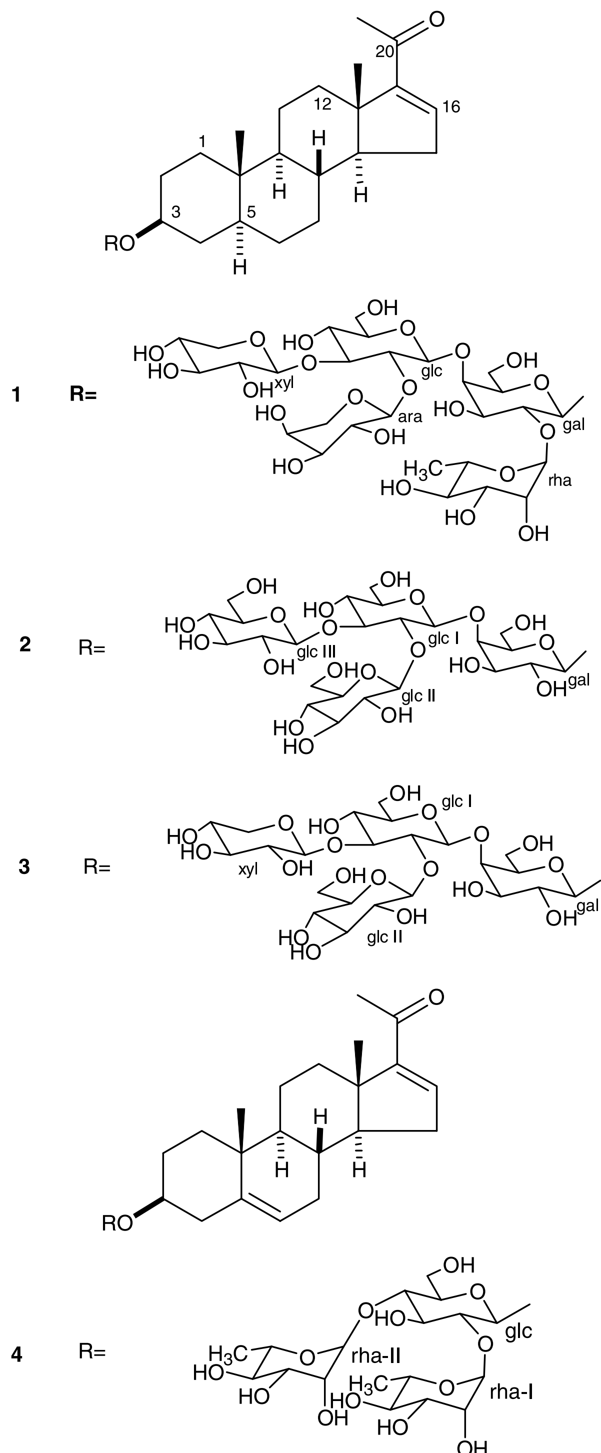

Figure 1. Structures of compounds **1**–**4**.

spectra were taken on a Bruker AV-400 (400 MHz for ^{1}H NMR) spectrometer in C_5D_5N solution. ESI-MS spectra were acquired using a Bruker Esquire 2000 mass spectrometer. HRESI-MS spectra were carried out on a Micromass Q-TOF mass spectrometer. Column

chromatography was performed with Diaion D-101 (Mitsubishi Kasei), silica gel (200–300 mesh, Qingdao Factory of Marine Chemical Industry, Qingdao, China) and ODS (40–63 μm, Merck). TLC was taken on Merck TLC plates precoated silica gel 60 F_{254} and the spots were detected by spraying with 10% H_2SO_4/EtOH and heating. Preparative HPLC was carried out on an ODS column (19 mm × 300 mm, 10 μm, XTerra Prep. Rp_{18}, Detector: RID).

3.2 Plant material

The herb of *Solanum nigrum* L. was collected from the outskirts of Shenyang, Liaoning Province in June 2003, and identified by Professor QiShi Sun (Division of Pharmacognosy, Shenyang Pharmaceutical University, China). A voucher specimen is available at the Department of Natural Products Chemistry, Shenyang Pharmaceutical University, China.

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3.3 Extraction and isolation

The dried whole plant of *S. nigrum* (19.8 kg) was extracted with 60% EtOH (200 L × 2). The solvent was removed under vacuum to yield the crude extract (3800 g). A suspension of the extract in water was centrifugalized and then applied to a D-101 macroresin column (120 mm × 1500 mm) and eluted with H_2O (40 L), 60% EtOH (40 L) and 95% EtOH (40 L) successively. The 60% EtOH eluate (130 g) was dried and then extracted with MeOH. The MeOH extract was separated by silica gel (3000 g) using $CHCl_3$/MeOH gradient mixtures (10:0–6:4) to give 10 fractions (fr. 1 to fr. 10). Fraction 9 (57 g) eluted with $CHCl_3$/MeOH (6:4) was further purified by octadecylsilanised (ODS) silica gel with MeOH/H_2O (7:3) and repeated preparative HPLC to afford compounds **1** (7.73 mg), **2** (3.36 mg), **3** (14.28 mg) and **4** (23.10 mg).

3.3.1 Compound 1. Amorphous powder. mp 205–207°C; $[\alpha]_D^{25}$ − 55.8 (*c* 0.043, MeOH); IR (KBr) ν_{max} 3424 (OH), 2930 (CH), 1655 (C=O), 1073 (glycosyl C—O) cm^{-1}. ^1H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) data: see table 1. ESI-MS (positive ion mode) *m/z* 1073 $[M + Na]^+$, 941 $[M + Na–Ara/Xyl]^+$, 795 $[M + Na–Ara/Xyl–Rha]^+$, 663 $[M + Na–Ara/Xyl–Rha–Ara/Xyl]^+$; ESI-MS (negative ion mode) *m/z* 1049 $[M − H]^−$, 917 $[M − H\ Ara/Xyl]^−$, 785 $[M − H\ Ara/Xyl–Ara/Xyl]^−$, 623 $[M − H\ Ara/Xyl–Ara/Xyl–Glc]^−$, 477 $[M − H\ Ara/Xyl–Ara/Xyl–Glc–Rha]^−$. HRESI-MS *m/z* 1073.4720 $[M + Na]^+$(calcd. for $C_{49}H_{78}O_{24}Na$,1073.4781).

3.3.2 Compound 2. Amorphous powder. mp 212–214°C; $[\alpha]_D^{25}$ − 17.8 (*c* 0.045, MeOH); IR (KBr) ν_{max} 3422 (OH), 2927 (CH), 1654 (C=O), 1073 (glycosyl C—O) cm^{-1}. ^1H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) data: see table 1. ESI-MS (positive ion mode) *m/z* 987 $[M + Na]^+$, 825 $[M + Na–Glc]^+$, 663 $[M + Na–Glc–Glc]^+$, 501 $[M + Na–Glc–Glc–Glc]^+$; ESI-MS (negative ion mode) *m/z* 963 $[M − H]^−$, 801 $[M − H–Glc]^−$, 639 $[M − H–Glc–Glc]^−$, 477 $[M − H–Glc–Glc–Glc]^−$. HRESI-MS *m/z* 987.4390 $[M + Na]^+$ (calcd. for $C_{45}H_{72}O_{22}Na$, 987.4413).

3.4 Acid hydrolysis of 1 and 2

Each saponin (1 mg) was refluxed with 5 ml 2 M HCl at 100°C for 2 h. The aglycon was extracted with EtOAc three times and the aqueous residue was concentrated and examined by HPTLC (silica gel) with two solvent systems (A: BuOH/Me_2CO/H_2O/HOAC = 4:5:1:1, B: EtOAc/BuOH/H_2O/HOAC = 4:4:1:1), compared with the authentic sugar samples [14].

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