Chemistry of Natural Compounds, Vol. 48, No. 4, September, 2012 [Russian original No. 4, July–August, 2012]

GLYCOSYLATION OF SOLASODINE AND PHARMACOLOGICAL ACTIVITY OF THE PRODUCT

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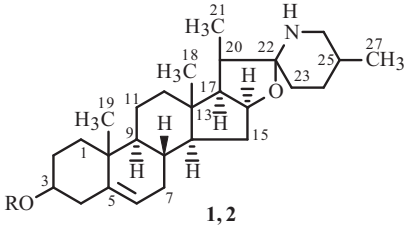
UDC 547.945+543.429.23

*Solasodine tetra-*O*-acetyl-3*β*-*D*-glucopyranoside was synthesized from tetra-*O*-acetylbromo-*D*-glucose by the Koenigs–Knorr reaction using solasodine isolated from cultivated* Solanum laciniatum. *The structure of the product was established using IR, mass, PMR, ^{13}C NMR, and 2D spectra. The pharmacological properties of the product were studied.*

Keywords: solasodine tetra-*O*-acetyl-3*β*-D-glucopyranoside, PMR and ^{13}C NMR spectroscopy, pharmacological properties.

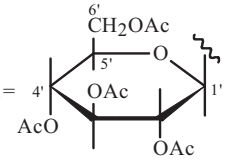
Solasodine glucosetetraacetate has now been synthesized. Solasodine tetra-*O*-acetyl-3*β*-D-glucopyranoside (**2**) of composition $C_{41}H_{61}NO_{11}$ was synthesized from tetra-*O*-acetylbromo-D-glucose [4] by a Koenigs–Knorr reaction [5] using solasodine (**1**) [1, 2] isolated from cultivated *Solanum laciniatum* [3] in order to prepare pharmacologically active compounds.



1, 2

1: R = H;

2: R =



The IR spectrum of **2** exhibited absorption bands for active amine H at 3410 cm^{-1}, –CH_2 and –CH_3 groups at 2840–2960 cm^{-1}, and an ester carbonyl at 1265 and 1760 cm^{-1}.

The mass spectrum of **2** showed principal peaks for ions with *m*/*z* 743 $[M]^+$, 729, 728, 725, 700, 701, 687, 630, 564, 397, 396, 368, 338, 331, 283, 282, 138, and 114 (100%), a part of which were identical to those for solasodine [6].

The PMR spectrum of **2** exhibited resonances corresponding to four methyls of the aglycon in the 18-, 19-, 21-, and 27-positions at 0.75 (s), 0.94 (s), 0.88 (d), and 0.78 (d) ppm, respectively. Protons of four acetate methyls resonated at weaker field of 1.94, 1.96, 1.99, and 2.01 ppm. The 0.8-2.3 ppm spectral range contained overlapping multiplets from methylene and methine protons. Resonances of the carbohydrate moiety appeared in the range 3.3–5.2 ppm. The glucose anomeric proton resonated at 4.53 ppm as a doublet with J = 8.0 Hz. The 2′-, 3′-, and 4′-protons of glucose C atoms appeared at 4.89 (dd), 5.14 (t), and 5.01 (t) ppm, respectively. The resonance of the glucose C-5′ proton formed a complicated multiplet at 3.61 ppm. The C-6′ methylene protons gave resonances at 4.05 and 4.19 ppm as two quartets. A 1H broad doublet characteristic of olefinic proton H-6 with J = 5.1 Hz was observed at 5.29 ppm.

The ^{13}C NMR spectrum of the studied compound in $CDCl_3$ contained 41 resonances. A comparison of the spectra of **2** and solasodine [7] (Table 1) revealed additional resonances. Four resonances assigned to acetate methyls were observed at strong field of 20.91–21.07. Five additional resonances characteristic of the carbohydrate ring were found in the range 62–74 ppm. The glucose anomeric carbohydrate C-1′ atom resonated at 99.92 ppm. Four resonances of carbonyls were recorded in the weak-field region at 169.59–170.97 ppm. A comparison of the ^{13}C NMR spectral characteristics of **1** and **2** indicated that C-3 experienced a substantial weak-field shift by 8.59 ppm that was caused by glycosylation of the hydroxyl.

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 0009-3130/12/4804-0610

TABLE 1. PMR and ^{13}C NMR Spectral Characteristics of **1** and **2** ($CDCl_3$, δ, ppm, J/Hz)

C atom	**2**			**1**
	DEPT	δ_C	δ_H	δ_C
1	CH_2	37.47	0.99*, 0.93*	37.16
2	CH_2	29.74	1.87*, 1.55*	31.99
3	CH	80.24	3.42 m	71.65
4	CH_2	39.19	2.12 (t, J = 11.4) 2.20 (ddd, J = 8.5, 4.8, 1.6)	42.22
5	C	140.69		140.91
6	CH	122.17	5.29 (br.d, J = 5.1)	121.40
7	CH_2	32.49	1.92*	32.08
8	CH	31.72	1.57*	31.54
9	CH	50.42	0.86*	50.03
10	C	37.18		36.57
11	CH_2	21.18	1.42*	20.79
12	CH_2	40.23	1.70*	39.85
13	C	40.83		40.45
14	CH	56.80	1.02*	56.46
15	CH_2	32.42	1.50*, 1.43*	32.08
16	CH	79.04	4.22 (br.q, J = 7.2)	78.91
17	CH	63.14	1.63*	62.73
18	CH_3	16.72	0.75 s	16.29
19	CH_3	19.68	0.94 s	19.30
20	CH	41.55	1.83*	41.20
21	CH_3	15.58	0.88 (d, J = 7.2)	15.13
22	C	98.57		98.25
23	CH_2	34.40	1.55*	33.92
24	CH_2	30.63	1.53*	30.12
25	CH	31.70	1.48*	31.34
26	CH_2	48.00	2.53 (t, J = 10.9) 2.60 (dd, J = 10.9; 3.8)	47.51
27	CH_3	19.62	0.78 (d, J = 6.4)	19.17
1′	CH	99.92	4.53 (d, J = 8.0)	
2′	CH	71.82	4.89 (dd, J = 9.6, 9.5)	
3′	CH	73.22	5.14 (t, J = 9.5)	
4′	CH	68.87	5.01 (t, J = 9.9)	
5′	CH	72.00	3.61 (ddd, J = 9.9, 4.8, 2.5)	
6′	CH_2	62.43	4.05 (dd, J = 12.2, 2.5) 4.19 (dd, J = 12.2, 4.8)	
		Acetyl resonances		
	CH_3	20.91, 20.94, 21.02, 21.07	1.94 s, 1.96 s, 1.99 s, 2.01 s	
	C=O	169.59, 169.69, 170.63, 170.97		

*Proton chemical shifts were established from a Hetcor experiment.

Resonances of protons and C atoms were assigned based on PMR and ^{13}C NMR spectra in addition to DEPT experiments and heterocorrelations (Hetcor) and by comparison of the spectral characteristics of **2** and solasodine [7].

Table 1 presents chemical shifts of C and H atoms and DEPT data of **2** and ^{13}C NMR of **1**.

Based on the results, glycosylation product **2** had the structure 2,3,4,6-tetra-*O*-acetyl-3*β*-D-glucopyranosylsolasodine.

Pharmacological Activity. A study of the overall activity and toxicity of **2** showed that its resorptive activity was manifested at a dose of 5 mg/kg upon s.c. administration as increased motor activity and reflexive stimulation. The reflexive stimulation at a dose of 50 mg/kg was manifested more prominently but was combined with muscular weakness and breathing suppression. The LD_{50} for s.c. administration was 186 ± 31 mg/kg; for i.v. administration, 33.6 (27.8–40.7) mg/kg.

Compound **2** at an i.v. dose of 2 mg/kg in acute tests on anesthetized cats caused a gradually increasing arterial pressure from 136 ± 11 to 170 mm Hg. The angiotensive activity peaked after 3–3.5 h, after which the arterial pressure

gradually decreased to the initial level after 6–7 h. The angiotensive activity was accompanied by pupil dilation, exophthalmy, and *in vivo* spleen suppression. This was consistent with activation of the sympathetic nervous system.

A single s.c. administration of **2** at a dose of 1–3 mg/kg enhanced the sexual activity of male rats that was manifested in an increased number of copulations and a decreased ejaculation latent period from the standard 15-minute time interval. The pharmacological index $K = LD_{50}/ED_{50}$ of **2** surpassed that of the known aphrodisiac Yohimbine.

EXPERIMENTAL

IR spectra of the studied compound were recorded in KBr on a Model System 2000 spectrometer (PerkinElmer). PMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Unity 400 plus spectrometer (Varian) at operating frequency 400 MHz for ^1H. The internal standard was HMDS. Chemical shifts in ^{13}C NMR spectra were given relative to the resonance of $CDCl_3$ (δ 77.36 ppm vs. TMS). DEPT and Hetcor experiments were carried out using standard spectrometer methods. Mass spectra were taken on a MX-1310 instrument at ionizing potential 40–50 V and 120–180°C. KSK silica gel was used for column (125–250 μm) and TLC (90 μm) chromatography. The eluents were $CHCl_3$ and $CHCl_3$:EtOH (5:1 and 5 mL per 5 drops, respectively).

Preparation of 2. Compound **2** (10 g) and silver acetate (9.6 g) were placed in a two-necked flask, treated with anhydrous benzene (400 mL, or $CHCl_3$ 200 mL and toluene 200 mL), refluxed, and condensed to a volume of ~300 mL. Then, crystalline tetra-*O*-acetylbromo-D-glucose (15 g) was added in portions and the distillation was continued, maintaining the solvent volume in the reaction mixture. The course of the glycosylation reaction was checked by TLC on silica gel using $CHCl_3$:EtOH (5 mL to 5 drops). When a satisfactory result was obtained, the reaction mixture was cooled, silver salts were separated, and the mixture was evaporated in vacuo at 40–50°C in a rotary evaporator to a dry solid. The reaction time was 14 h.

The dry solid was worked up with acetone (or MeOH). A mixture of crystals (7 g) was isolated and separated over a column of silica gel (2.5 × 120 cm, KSK, 125–250 μm, 1:30 ratio) with elution by $CHCl_3$ and after a negative reaction for alkaloids (Drafendorff's solution) by $CHCl_3$:EtOH (5:1) until complete elution of alkaloids. The eluate was collected in portions (50–100 mL). Each fraction was checked by chromatography on Silufol using $CHCl_3$:EtOH [5:1, or by TLC on KSK silica gel (90 μm) using $CHCl_3$:EtOH, 5 mL to 5 drops]. The initial $CHCl_3$ effluents (400–500 mL) afforded after work up by acetone (or MeOH) compound **2** (4.57 g), mp 213–216°C. The subsequent $CHCl_3$ effluent with a mixture of **2** and **1** was re-chromatographed over a SiO_2 column with elution by $CHCl_3$ and then $CHCl_3$:EtOH (5:1). Work up of the initial $CHCl_3$ effluents with acetone (or MeOH) afforded **2** (0.83 g); of the subsequent $CHCl_3$ effluents, a mixture of **2** and **1** (1.28 g).

The mother liquor (20 g) after separation of the mixture of crystals was chromatographed over a SiO_2 column by the method described above. Re-chromatography (4–5 times) of the initial $CHCl_3$ effluents afforded another 3.93 g of **2**; the subsequent $CHCl_3$ effluents, 1.64 g of a mixture of **2** and **1**.

In all instances, **1** and traces of other compounds (5.89 g) were obtained from the $CHCl_3$:EtOH effluents. The yield of **2** was 51.83%.

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