Chemistry of Natural Compounds, Vol. 45, No. 1, 2009

PMR AND ^{13}C NMR SPECTRA OF BIOLOGICALLY ACTIVE COMPOUNDS. XIII.* STRUCTURE AND STEREOCHEMISTRY OF A NEW PHENYLPROPANOID GLYCOSIDE ISOLATED FROM *Onopordum acanthium* SEEDS

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UDC 547.97.581.184.19: 61.5.322(07)

*The structure of a new compound was determined using PMR and ^{13}C NMR spectroscopy (HHCOSY, HSBC, HMBC, ROESY) as 2-[3′-methoxy,4-*O*-β-D-galactopyranos-1-yl)benzyl]-3-(3″,4″-dimethoxybenzyl)-4-hydroxybutyric acid, which was isolated for the first time from seeds of Scotch thistle* Onopordum acanthium *L.*

Key words: *Onopordum acanthium* L., Asteraceae, 1D and 2D PMR and ^{13}C NMR spectroscopy, extraction, stereochemistry.

Various parts of Scotch thistle [*Onopordum acanthium* L. (Asteraceae)] have previously yielded taraxasterylacetate from the flower thalamus and taraxasterol from leaves and stems [1, 2]. In continuation of this research, we investigated ripe seeds of Scotch thistle growing in the European part of Russia, Western Siberia, Central Asia, and in the Caucasus and Urals, including Bashkirya, which are used as a medicinal agent for heart diseases and as bactericidal, hemostatic, and antitumor agents [3, 4]. The plant is the basis of the preparation Cardiodoron drops, which regulate the activity of the cardiovascular system, are effective for poor blood circulation, and exhibit a regenerative effect [5].

The 1D ^{13}C NMR spectrum of a compound isolated from *O. acanthium* seeds contained 27 resonances including 4 at strong field of 33-46 ppm; 10, at 50-100 ppm and δ 178.38 ppm. The others were located in the spectral region corresponding to aromatic systems. The structure was established by studying the isolated compound using high-resolution PMR and ^{13}C NMR spectroscopy.

We assigned 6 resonances in the middle region (five doublets and a triplet at 69.71 ppm) to the glycoside fragment C(1′)-C(6′). The chemical shifts were similar to those of *β*-D-galactose [6]. Besides these resonances, this spectral region contained a single triplet with δ_C 70.67 ppm (Table 1). This resonance became the starting point for finding vicinal couplings from 2D HHCOSY and HSQC spectra (Fig. 1, Table 2) and was designated C-1. The spectral analysis was able to find the spin system belonging to the aliphatic acid fragment.

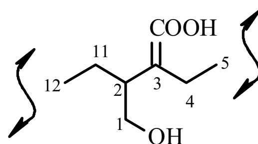

Fig. 1. Aliphatic fragment of the molecule established from HHCOSY, HSQC, and HMBC spectra.

*For No. XII, see [1].

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TABLE 1. ^{13}C NMR Chemical Shifts of **1** (DMSO-d_6)

Glycoside fragment		Aliphatic fragment		Aromatic fragment I		Aromatic fragment II	
C atom	δ, ppm	C atom	δ, ppm	C atom	δ, ppm	C atom	δ, ppm
1′	100.31 d	1	70.68 t	5	131.83 s	12	131.20 s
2′	73.24 d	2	40.76 d	6	113.92 d	13	112.48 d
3′	76.88 d	3	45.56 d	7	147.37 s	14	148.73 s
4′	69.70 d	4	33.54 t	8	145.36 s	15	148.72 s
5′	76.99 d	11	36.86 t	9	115.24 d	16	111.93 d
6′	61.71 t	18	178.38 s	10	121.34 d	17	120.45 d
				19	55.60 q	20	55.53 q
						21	55.56 q

TABLE 2. PMR Chemical Shifts of Phenylpropanoid Glycoside (DMSO-d_6)

Glycoside fragment		Aliphatic fragment		Aromatic fragment I		Aromatic fragment II	
H atom	δ, ppm, J/Hz	H atom	δ, ppm, J/Hz	H atom	δ, ppm, J/Hz	H atom	δ, ppm, J/Hz
1′	4.85 (d, ^3J = 6)	H_a-1	3.87 (t, ^3J = 6.0);	5	-	12	-
2′	3.20-3.30 m	H_b-1	4.01 (t, ^3J = 6.0)	6	6.79 s	13	6.66 s
3′	3.20-3.30 m	2	2.40-2.50 m	7	-	14	-
4′	3.18 m	3	2.71 m	8	-	15	-
5′	3.20-3.30 m	H_a-4	2.78 m	9	7.01 (d, ^3J = 5.0)	16	6.82 (d, ^3J = 5.0)
H_a-6′	3.48 m,	H_b-4		10	6.69 (d, ^3J = 5.0)	17	6.61 (d, ^3J = 5.0)
H_b-6′	3.68 m	H_a-11	2.50 m	19-OCH_3	3.73 s	20-OCH_3	3.70 s
		H_b-11	2.52 (dd, ^2J = 13.0, ^3J = 8)			21-OCH_3	3.70 s

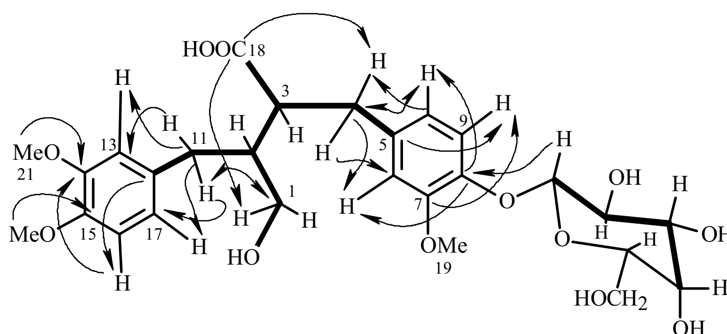
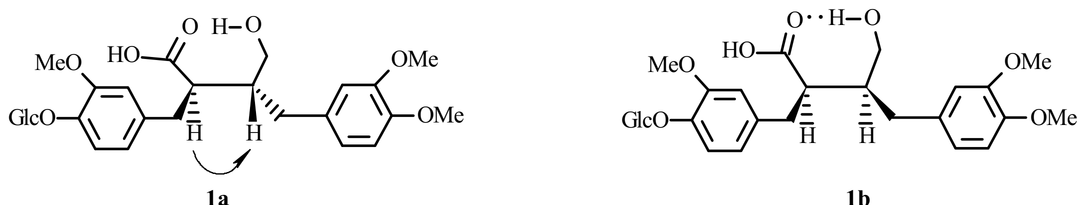


Fig. 2. C–H correlations according to 2D HMBC.

Thus, the fourth and eleventh C atoms were bonded to different aromatic fragments, which were 3,4-disubstituted aromatic rings with similar chemical shifts (Table 1, aromatic fragments I and II). Two pairs of doublets with coupling constants 5.0 Hz and singlets with δ 6.79 and 6.67 ppm were unambiguously assigned to these structures in the 1D PMR spectrum and 2D homo- and heteronuclear correlation spectra. Obviously, the substituents in the aromatic ring included methoxyls because singlets were observed in the corresponding region of the PMR spectrum with δ_H 3.70 and 3.73 and in the ^{13}C NMR spectrum with δ_C 55.60, 55.53, and 55.56 ppm. The integrated intensity of resonances in the proton spectrum and the number of resonances in the ^{13}C NMR spectrum were consistent with the presence of three such substituents, one of which in the *p*-position of the aromatic ring of fragment I was a glycoside fragment because a cross-peak was observed in the HMBC spectrum corresponding to C-8—H-1′ coupling. Figure 2 also shows cross-peaks illustrating the desired bonds between C atoms with δ_C 33.54 and 36.86 ppm (C-4,11) and fragments I and II. The 2D ROESY spectrum confirmed the proposed structure (Table 2).

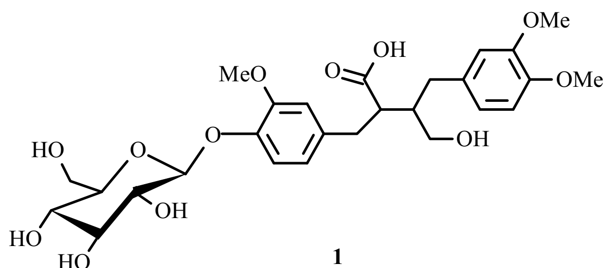
We also determined the relative configuration of the two asymmetric centers C-2 and C-3. Thus, diasterotopic protons on C-1 appeared as two triplets (Δδ 0.26 ppm) in the PMR spectrum recorded in DMSO-d_6 solution. This multiplicity is due to free rotation around the single C_1–C_2 bond. However, the shape of the observed resonances in $CDCl_3$ solution was transformed into a doublet of doublets (see Experimental) whereas the difference of the chemical shifts between the observed protons H_a-1 and H_b-1 became 0.7 ppm, which indicated limited rotation possibly due to a H-bond between hydroxyl and carboxyl groups on C-1 and C-3, respectively.

Therefore, a conformer with an intramolecular H-bond dominated in $CDCl_3$. A shift of conformational equilibrium in the nonpolar solvent was also consistent with the strong-field shift by Δδ 0.3 ppm of the resonance for the C-3 proton and the significant change of chemical shifts experienced by protons of aromatic fragment II. The ROESY spectrum in $CDCl_3$ was recorded in order to determine the relative configuration of chiral centers C-2 and C-3 for the two possible diastereoisomers (*threo*- and *erythro*-) **1a** and **1b**. The spectrum of the compound with configuration **1b** should exhibit a cross peak between the second and third protons, in contrast with **1a**.



As it turned out, there was no H_2—H_3 correlation in the ROESY spectrum ($CDCl_3$). Therefore, the molecule had the *threo*-configuration of the two asymmetric centers relative to each other.

Elemental analysis and mass spectrometric data confirmed the proposed structure of the isolated compound as 2-[3′-methoxy,4-*O*-*β*-D-galactopyranos-1-yl)benzyl]-3-(3″,4″-dimethoxybenzyl)-4-hydroxybutyric acid (**1**).



Isolated compound **1** contained two Ph–C_3– structural units, which classified it as a phenylpropanoid [7]. It is possible that the resulting glycoside was the combination product of simple phenylpropanoids, which are very often found as unsaturated compounds. For example, glycosides of cinnamic alcohols such as vimalin and triandrin from *Rhodiola rosea* L. [8] and of cinnamic acids (chlorogenic [9] and 1,5-dicaffeoylquinic [7]) and *p*-coumaric acid 4-*O*-*β*-D-glucopyranoside [7] and caffeic acid 3-*O*-*β*-D-glucopyranoside [10] are known. Certain phenylpropanoid glycosides based on phenylethanoids include *trans*-ethylene fragments and saturated phenylpropanes. These include echinacoside, which was isolated from *Echinacea angustifolia* Moench roots [11], verbascoside and plantamajoside [12], which exhibit antimicrobial properties [10]. It is known that plants store lignin structural blocks in cambium cells as such glycosides [13].

Considering that this compound was isolated from *O. acanthium* L., we called it aconiside.

EXPERIMENTAL

PMR and ^{13}C NMR spectra in DMSO-d_6 and $CDCl_3$ were recorded on a Bruker DRX-500 instrument (operating frequency for ^1H, 500.13 MHz; for ^{13}C, 125.76 MHz). 2D HHCOSY, ROESY, HSQC, and HMBC spectra were obtained using

standard methods. Chemical shifts are given on the δ scale relative to the used solvents (internal standard). Melting points were determined on a Boetius miniature heating stage; specific rotation, on a Perkin—Elmer 141 spectropolarimeter.

GC—MS was performed in a Finnigan 4021 instrument using a glass capillary column (50,000 × 0.25 mm), HP-5 stationary phase, He carrier gas, programmed temperature from 50 to 300°C at 5°C/min, 280°C vaporizer, and 250°C ion source.

Plants were collected in Tujmazy Region of the Republic of Bashkortostan in the second year of growth (September). The plant was identified by Candidate of Biological Sciences A. A. Muldashev (Institute of Biology, USC RAS).

2-[3′-Methoxy,4-*O*-*β*-D-galactopyranos-1-yl)benzyl]-3-(3″,4″-dimethoxybenzyl)-4-hydroxybutyric Acid (1). Ground air-dried raw material (457 g) was exhaustively extracted in a Soxhlet apparatus with $CHCl_3$ (300 mL) for 2 d. The material (*O. acanthium* seeds) to be extracted was placed in a paper cup in the extractor. The resulting $CHCl_3$ extract was evaporated in a rotary evaporator (to 200 mL) and treated at room temperature with *n*-hexane (30 mL). The solution was left for 7 d at 10°C. The resulting crystals were filtered and recrystallized. The precipitation was repeated three times to afford 2-[3′-methoxy,4-*O*-*β*-D-galactopyranos-1-yl)benzyl]-3-(3″,4″-dimethoxybenzyl)-4-hydroxybutyric acid (**1**) (0.46 g) in 0.1% yield.

2-[3′-Methoxy,4-*O*-*β*-D-galactopyranos-1-yl)benzyl]-3-(3″,4″-dimethoxybenzyl)-4-hydroxybutyric Acid (1). Mass spectrum (*m*/*z*, *I*, %): 390 [M^+H - Gly], 326 [M - GlyOH + CO_2 + H_2], 281, 248, 235 (17), 221 (11), 208 (13), 177 (71) [GlyO - H_2], 163 (17), 151 (93) $[(OCH_3)_2C_6H_3CH_2]^+$, 137 (100) $[(OCH_3)_2C_6H_3]^+$, 122 (50), 107 (58), 91 (33), 65 (18), mp 80-81°C, $[\alpha]_D^{25}$ –23.2° (*c* 4.082, $CHCl_3$). $C_{27}H_{36}O_{12}$. UV spectrum (λ_{max}, $CHCl_3$, nm): 281. IR spectrum (ν, cm^{-1}): 750, 1410, 1450, 1500, 1580, 1750, 2900, 3000, 3400. Tables 1 and 2 list the PMR and ^{13}C NMR spectra.

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 2.42 (1H ,m, ^3J = 7.0, H-2), 2.47 (1H, m, H-3), 2.50 (1H, m, H_a-11), 2.58 (1H, dd, ^3J = 13.0, ^3J = 8.0, H_b-11), 2.81 (2H, br.s, CH_2-4), 3.36 (1H, m, H-3′), 3.57 (3H, m, H-2′,4′,5′), 3.69 (3H, s, OCH_3-19), 3.73 (3H, s, OCH_3-20), 3.74 (2H, m, $\underline{CH_2}$-6′), 3.76 (1H, m, H_a-1) and 3.76 (3H, s, $OC\underline{H_3}$-21), 4.06 (1H, dd, ^2J = 15.0, ^3J = 7.0, H_b-1), 4.78 (1H, d, ^3J = 6.0, H-1′), 6.50 (1H, d, ^3J = 5.0, H-17), 6.56 (1H, d, ^3J = 5.0, H-10), 6.70 (1H, d, ^3J = 5.0, H-16), 6.90 (1H, d, ^3J = 5.0, H-9).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 34.27 (t, C-4), 37.79 (t, C-11), 41.06 (d, C-4), 46.24 (d, C-3), 55.79 (q, O–CH_3-19,20,21), 61.25 (t, C-6′), 69.27 (d, C-4′), 71.04 (t, C-1), 73.09 (d, C-2′), 75.85 (d, C-3′), 75.97 (d, C-5′), 101.58 (d, C-1′), 111.45 (d, C-16), 113.19 (d, C-6), 116.73 (d, C-9), 120.57 (d, C-17), 121.73 (d, C-10), 130.31 (s, C-12), 132.79 (s, C-5), 145.01 (s, C-8), 147.76 (s, C-7), 148.94 (s, C-15), 149.10 (s, C-14), 178.59 (s, C-18).

ACKNOWLEDGMENT

We thank A. Z. Khalilova and A. A. Muldashev for help in collecting and identifying the plant material and Doctor of Chemical Sciences Prof. A. S. Shashkov for supplying the 2D NMR results.

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