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PMR AND ^{13}C NMR SPECTRA OF BIOLOGICALLY ACTIVE COMPOUNDS. XII.* TARAXASTEROL AND ITS ACETATE FROM THE AERIAL PART OF *Onopordum acanthium*

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Crystalline taraxasterol and its acetate were isolated for the first time from Onopordum acanthium. *Two-dimensional COSY, HSQC, and HMBC NMR experiments were carried out for complete assignment of signals in the PMR and ^{13}C NMR. Chemical shifts of stereochemically important methyl C atoms C-28 and C-29 were measured.*

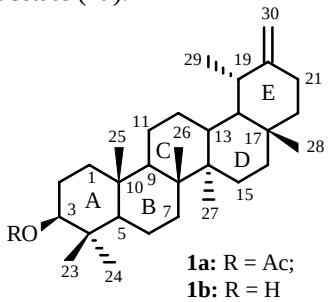
Key words: *Onopordum acanthium* L., two-dimensional spectrum, PMR, ^{13}C NMR, HSQC, HMBC, COSY, TOCSY, ROESY spectra.

Scotch thistle (*Onopordum acanthium* L., Asteraceae) is a biennial herbaceous plant. It has application in medical practice as a bactericide, cardiotonic, and hemostatic agent and is used against hypotonicity [2-4]. The lactones arctiopicrin and onopordopicrin [2] were isolated from leaves of Scotch thistle; alcohols lupeol and amyrin as their acetates [5] from seeds.

Our goal was to isolate and establish the structure of new biologically active terpenoids from *Onopordum acanthium* using PMR and ^{13}C NMR spectra.

The aerial part of *Onopordum acanthium* was investigated for the presence of biologically active compounds. Extraction by $CHCl_3$ and chromatography over a silica-gel column using ethylacetate:hexane afforded pure crystalline compounds. IR spectra and ^{13}C NMR spectra of one of the compounds were similar to those of taraxasteryl acetate **1a** [6, 7]. However, the melting point of the isolated compound (232-234°C) differed substantially from the literature value (256-257°C [8]).

Therefore, we investigated the NMR spectra of the isolated compounds. Table 1 lists the principal PMR and ^{13}C NMR spectral properties for taraxasterol (**1b**) and its acetate (**1a**).



1a: R = Ac;
1b: R = H

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TABLE 1. Chemical Shifts of ^{1}H (500 MHz) and ^{13}C (125 and 75 MHz) for compounds **1a** and **1b** (δ, ppm)

Atom	**1a**				**1b**	
	CS ^{13}C, ppm. $CDCl_3$ [6]	CS ^{13}C, ppm $CDCl_3$ (75 MHz)	CS ^{13}C, ppm C_5D_5N	CS ^{1}H, ppm (J/Hz) C_5D_5N	CS ^{13}C, ppm $CDCl_3$ [6]	CS ^{13}C, ppm $CDCl_3$ (75 MHz)
1	38.4	38.11	38.55	1.64, 0.92	38.7	38.71
2	23.6	23.67	24.07	1.75, 1.67	27.3	27.53
3	80.8	80.94	80.78	4.70 dd (11.8; 4.9)	78.9	79.12
4	37.7	37.77	38.01	-	38.7	38.98
5	55.4	55.42	55.62	0.78	55.3	55.49
6	18.1	18.16	18.42	1.46, 1.35	18.2	18.41
7	33.9	33.97	34.26	1.35, 1.35	34.0	34.20
8	40.8	40.89	41.11	-	40.8	41.02
9	50.3	50.37	50.54	1.33	50.4	50.61
10	37.0	37.02	37.21	-	37.0	37.26
11	21.4	21.44	21.62	1.48, 1.21	21.3	21.56
12	25.5	26.12	26.42	1.65, 1.08	25.5	25.60
13	38.8	39.13	39.40	1.56td (12.2; 3.3)	38.7	38.42
14	41.9	42.15	42.24	-	41.9	42.15
15	26.6	26.62	26.93	1.65, 0.94	26.6	26.78
16	39.1	38.84	38.55	1.26, 1.17	39.1	39.26
17	34.4	34.50	34.69	-	34.4	34.50
18	48.6	48.61	48.81	0.99	48.5	48.82
19	38.3*	39.36d	39.62	2.15 q (6.8)	38.2*	39.49
20	154.4	154.62	154.82	-	154.4	154.71
21	25.4	25.59	25.93	2.48, 2.23	25.4	25.60
22	39.3*	38.27t	39.09	1.41, 1.41	39.2*	38.43
23	27.8	27.92	28.04	0.91	27.9	28.08
24	16.4	16.48	16.77	0.91	15.2	16.01
25	15.4	15.86	16.42	0.86	15.8	15.46
26	16.2	16.32	16.02	0.99	16.1	16.38
27	14.6	14.70	14.89	0.96	14.6	14.86
28	26.1**	19.41	19.78	0.94	26.1**	19.59
29	19.4**	25.47	25.60	1.08	19.3**	25.74
30	107.0	107.11	107.56	4.79, 4.74	107.0	107.23
OAc	21.1; 170.8	21.30; 170.98	21.12; 170.66	2.04		

*and**, literature [6] values of signals re-assigned using data from ^{13}C JMOD and 2D NMR experiments.

Table 1 shows that the chemical shifts (CS) for most C atoms are the same in the literature [6] and the experimental results, which unambiguously characterize the ursane skeleton of taraxasterol with five cyclohexane rings, seven methyls, one *exo*-methylene group on C-20, and an alcohol (**1b**) or acetate (**1a**) on C-3.

Signals in the PMR and ^{13}C NMR spectra were assigned completely using one- and two-dimensional (2D) NMR methods (JMOD, COSY, TOCSY, ROESY, HSQC, and HMBC).

The weak-field region of the PMR of compound **1a** contains signals for three protons: two broad singlets at 4.79 and 4.74 ppm and one doublet of doublets at 4.70 ppm. Based on cross-peaks in the HSQC, the first two protons belong to C-30 with CS 107.56 ppm. The doublet of doublets at 4.70 ppm corresponds with the proton on C-3 (80.78 ppm) of ring A. The large vicinal spin—spin coupling constant (SSCC) $^{3}J_{HH}$ = 11.8 Hz is consistent with an axial position for H-3 and an equatorial orientation for the acetate. Cross-peaks in the COSY spectrum with H-2 and H-2′ at 1.75 and 1.67 ppm, respectively, correspond with the proton. Cross-peaks with H-1 and H-1′ at 1.64 and 0.92 ppm are observed in the TOCSY spectrum in addition to those mentioned above. Atoms C-2 and C-1 have CS 24.07 and 38.55 ppm, respectively. Cross-peaks with C-2 and a quaternary C atom at 37.21 ppm (C-4) are found in the HMBC spectrum for H-3. A correlation with the C atom of the carboxylic group at 170.66 ppm is also observed. This gives an indication of the position of the acetate on C-3. Cross-peaks with protons at 0.78 ppm (1H, triplet; corresponds with the C atom with CS 55.62 ppm), 0.91 ppm (6H singlet), and H-2, H-2′, and H-1 characterize C-3.

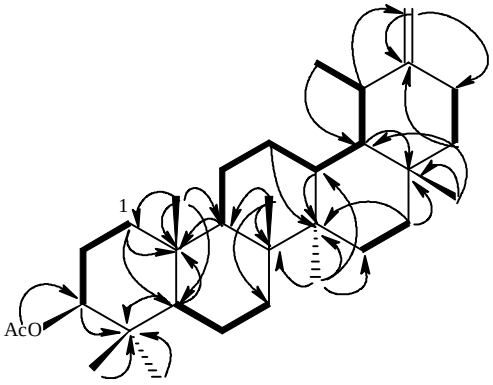

Fig. 1. COSY (H—H) and HMBC (H→C) correlations.

The protons with CS 0.91 ppm in the HSQC correspond with signals for C atoms with CS 28.04 and 16.77 ppm of the *gem*-dimethyls C-23 and C-24. Their protons also correlate with tertiary (55.62 ppm) and quaternary (38.01 ppm) C atoms C-5 and C-4, respectively. Signals for H-6 and H-7 of ring B are assigned using their correlations with H-5 in the COSY and TOCSY spectra. Their characteristic atoms C-6 (18.42 ppm) and C-7 (34.26 ppm) are found using the HSQC spectra. Protons H-1 and H-5 and protons of the methyl with CS 0.86 ppm give correlations in the HMBC spectrum with a quaternary C atom with CS 38.01 ppm. These are C-10 and methyl C-25.

This assumption is also confirmed by HMBC spectra, in which correlations of the C-25 protons with C-5, C-1, and methine C-9 with CS 50.54 ppm are observed. In the HSQC spectrum, the last signal correlates with H-9 with CS 1.33 ppm which, in turn, correlates with C-10 and yet another quaternary C atom with CS 41.11 ppm (C-8). C-8 couples with methyl protons at 0.99 ppm (on C-26 with CS 16.02 ppm).

Signals for protons and C atoms in rings C, D, and E (Fig. 1) were assigned analogously. Signals for methyls were assigned based on coupling of their protons with the corresponding C atoms, as shown in Fig. 1.

Because of an erroneous stereochemical interpretation for important signals in **1a** [6], the assignment of signals in ring E was examined in more detail. According to ^{13}C APT (JMOD, test for number of bound protons), the signal at 39.09 ppm corresponds with methylene C-22 of ring D whereas that at 39.62 ppm belongs to methine C-19. Therefore, the previous assignment [6] of signals at 38.3 (C-19) and 39.3 (C-22) ppm should be reversed (Table 1).

The doublet for methyl protons H-29 at 1.08 ppm in the HSQC spectrum corresponds with the C atom at 25.60 ppm (C-29). Corresponding cross-peaks in the HMBC spectrum between methyl protons in ring D at 1.08 ppm and C atoms at 39.62 (C-19) and 154.82 ppm (C-20) in addition to C-29 at 25.60 ppm and a proton at 2.15 ppm (H-19) confirm that the assignment is correct. Methyl protons with CS 0.94 ppm, which correspond in the HSQC spectrum with the C atom at 19.78 ppm (C-28), have correlations in the HMBC spectrum with C atoms at 34.69 and 48.81 ppm. These were previously assigned to C-17 and C-18, respectively. Correlations between C-28 and protons at 1.41, 1.26, and 0.99 ppm are also observed. These were assigned to H-22, H-16, and H-18, respectively. It should be noted that the strong-field position of the signal for C-28 indicates that it has an axial orientation. The *trans*-fusion of rings A-E was established using 2D ROESY and HMBC spectra and were confirmed by an x-ray structure analysis.

Thus, signals for protons and C atoms were assigned completely using one- and two-dimensional PMR and ^{13}C NMR experiments. Assignments for C-19, C-22, C-28, and C-29 were revised.

EXPERIMENTAL

Onopordum acanthium L. was collected in the Republic of Bashkortostan about 100 km to the west of Ufa. The plants were identified taxonomically using the herbarium collection of the Institute of Biology of the Ufa Scientific Center of the Russian Academy of Sciences.

Ground and air-dried raw material (flower receptacles) collected during the second half of flowering was exhaustively extracted with $CHCl_3$. The total extracts were chromatographed over a silica-gel (KSK) column (1:50 ratio of extract to silica gel).

TLC monitoring used SiO_2 plates (Silufol) and development by iodine vapor.

IR spectra were recorded on a Specord 75-IR spectrometer as mineral-oil mulls.

PMR and ^{13}C NMR spectra were recorded on a Bruker AMX-300 (75 MHz working frequency for ^{13}C in $CDCl_3$) instrument. ^{13}C spectra were edited using a standard JMOD regime.

2D NMR spectra were recorded on a DRX-500 (Bruker) instrument in deuteropyridine at 30°C. All experiments used standard Bruker methods. 2D COSY, HSQC, and HMBC spectra were recorded using a gradient method. The CS were measured to an accuracy of 0.01 and 0.05 ppm for protons and C atoms, respectively.

Rotation angles were measured on a Perkin—Elmer 141 polarimeter. Melting points were determined on a Boetius heated microstage.

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