Chemistry of Natural Compounds, Vol. 41, No. 4, 2005

HIGH-RESOLUTION ^{1}H AND ^{13}C NMR OF GLYCYRRHIZIC ACID AND ITS ESTERS

L. A. Baltina,[1] O. Kunert,[2] A. A. Fatykhov,[1] R. M. Kondratenko,[1] L. V. Spirikhin,[1] L. A. Baltina (Jr.),[1] F. Z. Galin,[1] G. A. Tolstikov,[3] and E. Haslinger[2]

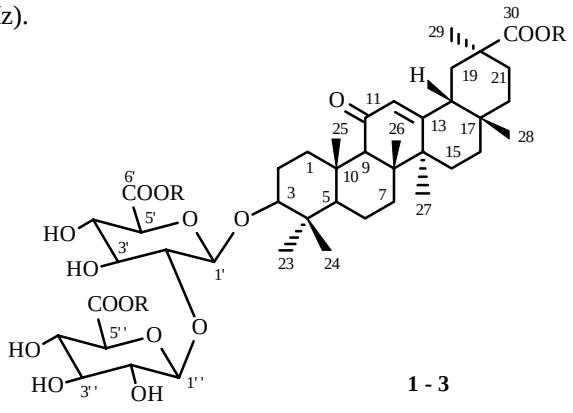
UDC 543.422.25:547.915

Resonances for protons and C atoms in the ^{1}H and ^{13}C NMR spectra of glycyrrhizic acid and its esters were assigned using high-resolution ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR methods.

Key words: glycyrrhizic acid, high-resolution NMR spectra.

High-resolution NMR spectroscopy is used to establish the structures of natural triterpenoids and their glycosides [1-3]. Glycyrrhizic acid (GA, **1**) is the principal saponin from roots of smooth (*Glycyrrhiza glabra* L.) and Ural licorices (*G. uralensis* Fisher) and is known to be highly parmacologically active [4]. It is interesting as a base for developing new antiviral [5, 6] and antitumor preparations [7].

The ^{13}C (22.5 MHz) and ^{1}H (300 MHz) NMR spectra of GA and several esters of **1** have been investigated [8, 9]. It was found that the glycoside bound to C-3 of the aglycon has the β-configuration. Signals for the C atoms were completely assigned. Later the stereochemistry and structure of GA was confirmed as 3β-hydroxy-11-oxo-18βH-olean-12-en-30-oic acid 3-O-[β-D-glucuronopyranosyl-(1→2)-β-D-glucuronopyranoside using ^{1}H (200 MHz) and ^{13}C (50 and 75.5 MHz) NMR spectra [10, 11]. Signals for the methyl protons and those located near the polar groups in the aglycon of GA (glycyrrhetic acid, GLA) were assigned [12, 13]. Signals for protons and C atoms of the 3-O-acetates of 18α- and 18β-GLA, in particular H-18, H-19, and H-28, were assigned using two-dimensional (2D) homonuclear ^{1}H—^{1}H COSY (45°) and heteronuclear CH spectroscopies [14]. However, signals for all protons in the PMR of GA have not yet been assigned owing to the use of instruments with low working frequencies (up to 300 MHz).



1 - 3

1: R = H
2: R = CH_3
3: R = CH_2-CH=CH_2

1) Institute of Organic Chemistry, Ufa Scientific Center, Russian Academy of Science, 450054, Ufa, prospekt Oktyabrya, 71, fax (3472)-35-60-66, e-mail: baltina@anrb.ru; 2) Institut fur Pharmazeutische Chemie und Pharmazeutische Technologie, Karl-Franzens-Universitat, Graz Universitatsplatz 1, A-8010 Graz, Austria, fax: +43-316-380-9846, e-mail: olaf.kunert@kfunigraz.ac.at; 3) N. N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Division, Russian Academy of Sciences, 630090, Novosibirsk, prospekt Akad. Lavrent′eva, 9. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 347-350, July-August, 2005. Original article submitted April 15, 2005.

 0009-3130/05/4104-0432

TABLE 1. ^{1}H and ^{13}C Chemical Shifts (δ, ppm) in NMR Spectra of the Aglycon of GA (**1**) and Its Esters **2** and **3** in Pyridine-d_5, TMS Internal Standard (313 K)

C atom	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	3.04; 1.06	39.6	3.06; 1.06	39.6	3.08; 1.09	39.6
2	2.29; 2.05	26.7	2.16; 2.03	26.7	2.19; 2.05	26.7a
3	3.35	89.3	3.30	89.5	3.31	89.4
4	-	40.0	-	40.0	-	40.0
5	0.76	55.6	0.77	55.6	0.79	55.5
6	1.51; 1.29	17.7	1.59; 1.41	17.7	1.62; 1.44	17.7
7	1.58; 1.25	33.1	1.60; 1.30	33.1	1.63; 1.32	33.1
8	-	45.6	-	45.6	-	45.6
9	2.45	62.2	2.45	62.2	2.48	62.2
10	-	37.4	-	37.4	-	37.4
11	-	199.4	-	199.4	-	199.4
12	5.94	128.8	5.83	128.8	5.84	128.8
13	-	169.4	-	168.9	-	168.9
14	-	43.6	-	43.5	-	43.5
15	1.73; 1.11	26.9	1.73; 1.09	26.9	1.74; 1.12	26.8
16	2.09; 0.96	26.7	1.99; 0.92	26.7	2.02; 0.94	26.6a
17	-	32.2	-	32.1	-	32.1
18	2.53	48.8	2.23	48.08	2.28	48.7
19	2.14; 1.75	41.8	1.92; 1.67	41.5	1.96; 1.70	41.4
20	-	44.1	-	44.3	-	44.3
21	2.27; 1.47	31.7	2.05; 1.37	31.4	2.10; 1.40	31.4
22	1.71; 1.45	38.5	1.41; 1.37	38.2	1.50; 1.39	38.2
23	1.41	28.2	1.32	28.0	1.33	28.0
24	1.21	16.7	1.15	16.5	1.16	16.7
25	1.20	16.9	1.29	16.8	1.31	16.8
26	1.06	18.9	1.11	18.9	1.12	18.9
27	1.42	23.6	1.38	23.5	1.41	23.5
28	0.81	28.7	0.78	28.6	0.80	28.6
29	1.34	28.8	1.16	28.2	1.20	28.2
30	-	179.1	-	176.8	-	176.0
R			3.66	51.6	4.72; 4.68	65.1
					5.97	133.2
					5.35; 5.19	118.1

Herein we report high-resolution ^{1}H and ^{13}C NMR spectra of **1** and its trimethyl (**2**) and triallyl (**3**) esters (Table 1) that were obtained on Varian Unity Inova high-resolution spectrometers at working frequency 600 MHz for proton spectra and 150 MHz for ^{13}C spectra. All signals for aglycon protons and the carbohydrate were assigned for GA and **2** and **3** using 1D ^{1}H and ^{13}C methods and 2D homonuclear ^{1}H—^{1}H and heteronuclear –CH– spectroscopies in the DQF—COSY, HSQC, HSQC—TOCSY, and HMBC modes, respectively [15]. Spin systems and quaternary C atoms were determined using HMBC experiments according to the literature method [16]. Use of a high-resolution spectrometer and 2D spectroscopy made it possible for the first time to assign completely proton siglans at strong field of 0.7-2.3 ppm and to refine assignments made previously for C atoms in the ^{13}C NMR spectra.

Table 1 lists the chemical shifts in the high-resolution ^{1}H and ^{13}C NMR spectra of GA and **2** and **3**. DQF—COSY experiments enabled most signals for each carbohydrate to be assigned starting with the obvious signals for anomeric protons at δ 5.03 and 5.37 ppm for GA with spin—spin coupling constant (SSCC) 7.9 Hz. This also confirms the β-configuration of the glycoside bonds for the GA carbohydrate. Anomeric protons in the PMR spectrum of the esters are observed at 4.94 and 5.32 ppm for **2** and at 4.94 and 5.33 ppm for **3** (SSCC 7.0-7.6 Hz). The signal for C1 in the 2D heteronuclear spectrum of **1** (39.6 ppm) couples with signals at 1.06 and 3.04 ppm that correspond to axial H_a and equatorial H_e on C1. Unusually strong screening of H_a is apparently bound not only with 1,3-diaxial interaction but also with anisotropic contribution of neighbour carbonyl group at C11. However, the resonances of all protons cannot be determind using only DQF—COSY experiments.

TABLE 2. ^{1}H and ^{13}C Chemical Shifts (δ, ppm) in NMR Spectra of the Carbohydrate of GA (**1**) and Its Esters **2** and **3** in Pyridine-d_5, TMS Internal Standard (313 K)

C atom	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1′	5.03 (d, J = 7.9)	105.1	4.94	105.0	4.94 (d, J = 7.6)	105.1
2′	4.24	84.5	4.12	84.5	4.13 (t, J = 8.0)	84.4
3′	4.36 (tr, J = 8.2)	77.8	4.24	77.6	4.27 (t, J = 9.0)	77.6
4′	4.52	73.0	4.36	72.7	4.40 (t, J = 9.0)	72.6
5′	4.54	77.3	4.41	76.8	4.45	76.9
6′	-	172.3	-	170.2	-	169.4
R			3.69	52.0	4.76; 4.76	65.5
					5.93	132.6
					5.40; 5.14	117.8
1″	5.37 (d, J = 7.9)	106.9	5.32	106.9	5.33 (d, J = 7.0)	106.9
2″	4.21	76.8	4.11	76.6	4.13 (tr, J = 8.0)	76.6
3″	4.28	77.7	4.19	77.5	4.22 (tr, J = 8.8)	77.5
4″	4.58	73.3	4.44	73.0	4.47	73.1
5″	4.58	78.4	4.45	77.7	4.50	77.9
6″	-	172.0	-	170.2	-	169.5
R			3.82	51.9	4.88; 4.88	65.8
					6.05	132.8
					5.47; 5.21	118.3

Therefore, signals of all protons were completely assigned using HSQC and HMBC experiments. The HSQC experiment correlates all proton resonances with the corresponding C atoms and enables signals of interglycoside bonds to be related [17]. Atom C19 (4.18 ppm) in the aglycon of GA corresponds to proton resonances at 2.14 and 1.75 ppm; C1 (39.6 ppm), 3.04 and 1.06 ppm; C22 (38.5 ppm), 1.71 and 1.45 ppm, etc. The carbohydrate part of the HSQC spectrum of GA confirms that there are two β-D-glucuronic acids in the carbohydrate chain and that they are (1→2)-bound (Table 2).

The strong-field doublet of methine proton H-5 (0.76 ppm) is coupled with a C signal at 55.6 ppm and has a single large vicinal constant $^{3}J_{HaHa}$ = 12.6 Hz with axial methylene proton H_a-8 (1.29 Hz). Such a magnitude for the vicinal constant indicates that the coupled protons are diaxial and, therefore, confirms the *trans*-fusion of rings A and B.

The stereochemically informative doublet—doublet signal for methine proton H-18 (2.53 ppm), which is coupled with a C signal at 48.8 ppm, has a single large constant $^{3}J_{HaHa}$ = 13.5 Hz with axial methylene proton H_a-19 (1.75 ppm) and a single small constant $^{3}J_{HaHa}$ = 3.8 Hz with equatorial proton H_e-19 (2.14 ppm).

The situation is analogous for the doublet—doublet signal for methine proton H-3 (3.35 ppm), which is coupled with a C signal at 98.3 ppm. The presence of one large and one small vicinal SSCC indicates that H-3 is axial and the glycside has the β-orientation.

Weak-field protons at 5.19, 5.35, and 5.97 ppm in the PMR spectrum of **3** are coupled with the olefinic C atoms of the carboxypropenyl moiety of the aglycon (118.1 and 133.2 ppm). Signals for protons at 5.14, 5.40, 5.93 and 5.21, 5.47, 6.05 ppm, respectively, belong to the carboxypropenyl moieties in the carbohydrate of **3**.

A comparison of the chemical shifts for C-8 (45.6) and C-14 (43.6) with the literature values for these atoms (43.87 and 45.83) [10] enabled the positions of these signals to be found in ^{13}C NMR spectra (150 MHz) of GA and its derivatives (Table 1). Analogous chemical shifts were found for these C atoms in 2D ^{13}C—^{1}H (COLOC) spectra of glycyrrhetic acid derivatives, the aglycon GA [18].

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded on a Varian Unity Inova 600 spectrometer at working frequency 600 and 150 MHz. Spectra of samples containing a solution of compound (20 mg) in pyridine-d_5 (0.72 mL) were recorded at 313 K using TMS as an internal standard. A detailed description of the experiments used to assign resonances in the high-resolution NMR spectra has been published [15].

We used crystalline GA (97%) prepared as before [11], mp 224-226°C (dec.), $[\alpha]_D^{20}$ +62.5° (*c* 0.02, EtOH), lit. [11] mp 222-223°C, $[\alpha]_D^{20}$ +62.2° (*c* 0.03, EtOH).

GA trimethyl ester (**2**) was prepared by methylation of GA using diazomethane by the usual method [19] and recrystallization twice from aqueous methanol, mp 282-283°C, $[\alpha]_D^{20}$ +52.5° (*c* 0.02, MeOH), lit. [19] mp 283-286°C, $[\alpha]_D^{20}$ +46.7° (*c* 0.02, EtOH).

GA triallyl ester (**3**) was synthesized as before [9] by treating a solution of GA in DMSO with allylbromide in the presence of KOH and recrystallizing from acetone:hexane, mp 156-157°C, $[\alpha]_D^{20}$ +68.5° (*c* 0.03, MeOH), lit. [9] mp 155-157°C, $[\alpha]_D^{20}$ +68° (*c* 0.025, EtOH).

ACKNOWLEDGMENT

The work was supported by grants of the Russian Foundation for Basic Research and Austria 03-03-20004 BNTS_a and the RF President NSh-1488.2003.3.

REFERENCES

1. H. Ageta, Y. Arai, H. Suzuki, T. Kiyotani, and M. Kitabayashi, *Chem. Pharm. Bull.*, **43**, 198 (1995).
2. N. Ullah, W. Seebacher, E. Haslinger, J. Jurenitsch, K. Rauchensteiner, and R. Weis, *Monatsh. Chem.*, **133**, 139 (2002).
3. Z. Bialy, M. Jurzysta, W. Oleszek, S. Piacente, and C. Pizza, *J. Agric. Food Chem.*, **47**, 3185 (1999).
4. G. A. Tolstikov, L. A. Baltina, E. E. Shul′ts, and A. G. Pokrovskii, *Bioorg. Khim.*, **23**, 691 (1997).
5. E. De Clerq, *Med. Res. Rev.*, **20**, 323 (2000).
6. H. Sato, W. Goto, J.-I. Yamamura, M. Kurokawa, S. Kageyama, T. Takahara, A. Watanabe, and K. Shiraki, *Antiviral Res.*, **30**, 171 (1996).
7. J. G. Chung, H. L. Chang, W. C. Lin, H. H. Wang, C. C. Yeh, C. F. Hung, and C. C. Li, *Food Chem. Toxicol.*, **38**, 163 (2000).
8. L. M. Khalilov, L. A. Baltina, L. V. Spirikhin, E. V. Vasil′eva, R. M. Kondratenko, A. A. Panasenko, and G. A. Tolstikov, *Khim. Prir. Soedin.*, 500 (1989).
9. L. A. Baltina, N. G. Serdyuk, E. V. Vasil′eva, L. V. Spirikhin, and G. A. Tolstikov, *Zh. Obshch. Khim.*, **30**, 1622 (1994).
10. G. G. Zapesochnaya, E. N. Zvonkova, V. A. Kurkin, E. V. Kazakova, L. N. Pervykh, V. I. Sheichenko, and V. A. Bykov, *Khim. Prir. Soedin.*, 772 (1994).
11. R. M. Kondratenko, L. A. Baltina, S. R. Mustafina, N. V. Makarova, Kh. M. Nasyrov, and G. A. Tolstikov, *Khim.-Farm. Zh.*, **35**, 39 (2001).
12. G. A. Tolstikov, L. M. Khalilov, L. A. Baltina, R. M. Kondratenko, A. A. Panasenko, and E. V. Vasil′eva, *Khim. Prir. Soedin.*, 645 (1985).
13. G. A. Tolstikov, Kh. O. Kim, L. F. Tolstikova, Kh. A. Alibaeva, S. M. Vasilyuk, V. P. Yur′ev, and M. N. Goryaev, *Izb. Akad. Nauk KazSSR, Ser. Khim.*, No. 2, 58 (1971).
14. L. M. Khalilov, E. V. Vasil′eva, A. A. Fatykhov, and G. A. Tolstikov, *Khim. Prir. Soedin.*, 363 (1991).
15. W. Seebacher, N. Simic, R. Weis, and O. Kunert, *Magn. Res. Chem.*, **41**, 626 (2003).
16. M. F. Summers, L. G. Marzilli, and A. Bax, *J. Am. Chem. Soc.*, **108**, 4285 (1986).
17. E. Breitmaier and W. Voelter, *Carbon-13 NMR Spectroscopy*, VCH, New York (1989).
18. N. I. Petrenko, V. Z. Petukhova, M. M. Shakirov, E. E. Shul′ts, and G. A. Tolstikov, *Zh. Org. Khim.*, **36**, 1013 (2000).
19. C. H. Brieskorn and H. Sax, *Arch. Pharm.*, **303**, 905 (1970).