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^{13}C NMR SPECTRA OF BIOLOGICALLY ACTIVE COMPOUNDS. VIII. STEREOCHEMISTRY OF A TRITERPENEGLYCOSIDE – GLYCYRRHIZIC ACID – AND ITS DERIVATIVES

L. M. Khalilov, L. A. Baltina,
L. V. Spirikhin, E. V. Vasil'eva,
R. M. Kondratenko, A. A. Panasenko,
and G. A. Tolstikov

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Details of the ^{13}C NMR spectra of glycyrrhizic acid and four of its derivatives are given, and on their basis the configurations of the anomeric centers of the carbohydrate chain have been redetermined and the β-configuration of the C-1' carbon atom has been suggested.

There is no information in the literature on the use of ^{13}C NMR spectroscopy to establish the structure of a triterpene glycoside – glycyrrhizic acid – the active principle of an extract of liquorice Glycyrrhiza glabra.

We have previously [1] reported the ^{13}C NMR spectra of derivatives of the aglycon – glycyrrhetic acid. In the present paper we give details of the ^{13}C NMR spectra of glycyrrhizic acid (I) and four of its esters (II-V) as the result of a study in which a stereochemical configuration of the carbohydrate moiety of the molecule is proposed.

The stereochemistry of the disaccharide moiety of glycyrrhizic acid was established by Lythgoe and Trippett [2] by hydrolysis and the subsequent methylation of the hydrolysis products with the use of optical rotation results. It was shown [2] that the bond between the glucuronic acids of the disaccharide part of glycyrrhizic acid has the β-configuration and the bond with the aglycon the α-configuration.

Thus, according to results of previous work [2-4], glycyrrhizic acid has the structure of 3β-[O-α-D-glucopyranuronosyl-(1 → 2)-β-D-glucopyranuronosyloxy]-11-oxo-(18βH)-olean-12-en-30β-oic acid.

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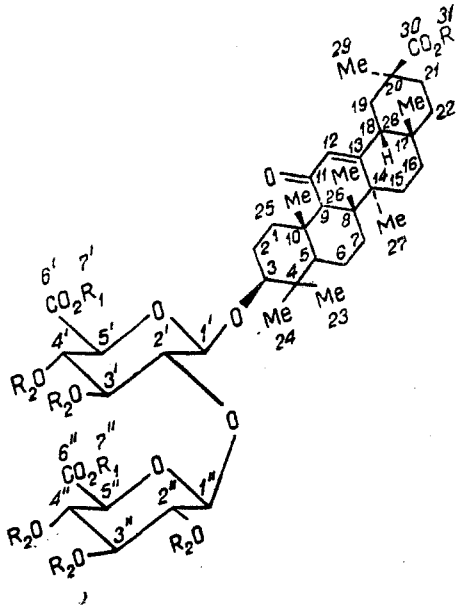

I. $R = R_1 = R_2 = H$
II. $R = R_1 = Me$, $R_2 = MeCO$
III. $R = R_1 = Me$, $R_2 = H$
IV. $R = R_1 = R_2 = Me$
V. $R = R_1 = Me$, $R_2 = PhCO$

We have investigated in detail the ^{13}C NMR spectra of the glycoside (I) and its derivatives (II-V). Table 1 gives the ^{13}C NMR chemical shifts for compounds (I-V). The multiplicity of the signals for compounds (II-V) fully agreed with the results for the spectrum of the acid (I).

The assignment of the signals of the C-atoms in the ^{13}C NMR spectra of glycoside (I) and its derivatives was made on the basis of information for the genin and its derivatives [1, 6] and literature information for corresponding glycosides [8-12]. The triterpene moiety of the glycyrrhizic acid molecule is easily identified in the spectrum of (I) from a comparison with the ^{13}C NMR spectrum of 18β-glycyrrhetic acid [1]. An exception is the signal of the C-3 carbinol carbon linked to the carbohydrate moiety of the molecule, which resonates at 90.74 ppm. In this case, the glycosylation effect amounts to 12 ppm and indicates the β-configuration of the O–C-3 bond [7], which is in harmony with the stereochemical structure of the aglycon of the glycyrrhizic acid molecule [3, 6].

The signals of the two carboxy groups of the carbohydrate moiety were observed in the weak-field region (172.29 pm). Weak-field signals at 106.01 and 105.10 ppm, respectively, corresponded to the C-1' and C-1" carbon atoms of the glucuronic acid residues. The signal of the C-2' carbinol carbon atom linked to the second glucuronic acid residue of the disaccharide moiety of the molecule appeared in a weaker field (83.78 ppm) than those of the other carbon atoms of the carbohydrate moiety of the glycoside (72.85-77.35 ppm).

On the basis of literature results [7, 10], increments of the influence of substituents on the chemical shifts of D-glucuronic acid derivatives were obtained and were used in the calculation of the chemical shifts of the carbon atoms of the carbohydrate moiety of the molecule of the α- and β-configurations of the C-1' anomeric center. A comparison of the calculated and experimental figures gave unambiguous evidence in favor of the β-configuration of the C-1' anomeric center. In the case of the α-configuration of this center, the C-3' and C-5' signals should be observed in a stronger field (δ_{calc} C-3' = 72.5 ppm and δ_{calc} C-5' = 71.9 ppm) because of the contribution of the steric 1,3-diaxial interaction, as follows from stereochemical models for the $^4C_1$ conformer that were considered. In the case of the $^1C_4$ conformer, diamagnetic shifts of practically all the carbinol signals would have been observed.

Similar information on the structure of the carbohydrate residue was obtained in a study of the ^{13}C NMR spectra of cyclosieversioside H – a triglycoside from Astragalus sieversianus [8].

More characteristic is the spectrum of the pentaacetate of the trimethyl ester of glycyrrhizic acid (II). The signals of the C-1' and C-1 anomeric protons (103.43 and 100.88 ppm, respectively) in the spectrum of (II) indicate the β-configurations of the glycosidic bonds.

TABLE 1. Parameters of the ^{13}C NMR Spectra* of Glycyrrhizic Acid (I) and Its Derivatives (II-V) ($CDCl_3$, δ, ppm, 25°C, 22.5 MHz, calculated figures given in parentheses)

C_l	I	II	III	IV	V
C1	40,15 t	39,18	39,52	39,17	39,23
C2	26,96 t	26,09	26,93	25,72	25,72
C3	90,74 d	91,18	90,40	90,66	90,87
C4	40,47 s	39,69	39,85	39,56	39,56
C5	56.40 d	55,48	55,78	55,49	55,36
C6	18,34 t	17,69	17,88	17,36	18,34
C7	32,87 t	32,94	33,29	32,77	32,77
C8	44.49 s	43,46	43,56	43,15	43,15
C9	62,99 d	62,01	62,20	61,82	61,88
C10	37,89 s	37,06	37,17	36,82	36,82
C11	202,52 s	200,00	200,56	200,14	200,14
C12	128,79 d	128,63	128,95	128,53	128,73
C13	171,90 s	169,42	169,55	169,55	169,07
C14	46,64 s	45,62	45,81	45,43	45,43
C15	27,48 t	26,74	26,93	26,44	26,44
C16	27,32 t	26,74	26,93	26,44	26,44
C17	32,87 s	32,10	32,24	31,86	31,86
C18	49,77 d	48,65	48,87	48,37	48,44
C19	42,27 t	41,42	41,64	41,06	41,12
C20	44,47 s	44,27	44,45	44,06	44,06
C21	31,92 t	31,40	31,64	31,14	31,14
C22	38,91 t	38,09	38,31	37,73	37,80
C23	28,20 q	27,60	27,85	27,48	27,87
C24	16,78 q	16,09	16,34	15,99	16,19
C25	16,97 q	16,50	16,93	16,32	16,45
C26	19,27 q	18,83	19,10	18,67	18,73
C27	23,83 q	23,49	23,87	23,37	23,43
C28	28,72 q	28,41	28,77	28,73	28,33
C29	29,15 q	28,63	28,93	28,33	28,53
C30	180,26 s	176,98	177,29	176,83	176,90
C31	—	51,88 q	52,20	51,76	51,76
C1′	106 01d	103,43	105,78	104,25	102,94
	(103,2)	(103,2)	(103,2)	(103,2)	(103,2)
C2′	83,78 d	77,15	83,95	86,95	76,51
	(82,9)	(79,0)	(82,9)	(89,7)	(79,0)
C3′	77,03 d	72,39	75,75	83,88	72,39
	(75,1)	(71,71)	(75,1)	(84,4)	(71,7)
C4′	72,85 d	71,38	71,81	77,48	70,11
	(72,3)	(70,5)	(72,3)	(76,5)	(70,5)
C5′	76,05 d	72,85	76,64	73,96	72,39
	(75,6)	(72,4)	(75,6)	(75,6)	(72,4)
C6′	172,29 s	166,88	169,68	169,00	166,59
C7′	—	52,91 q	52,99	52,29	52,68
C1″	105,10 d	100,88	104,41	102,88	100,33
	(104,3)	(99,9)	(104,3)	(104,3)	(99,9)
C2″	76,05 d	69,59	75,12	81,27	69,78
	(73,8)	(69,9)	(73,8)	(81,6)	(69,9)
C3″	77,35 d	72,63	76,64	85,84	73,18
	(76,5)	(73,1)	(76,5)	(85,8)	(73,1)
C4″	72,75 d	70,08	71,81	73,96	71,67
	(72,3)	(70,5)	(72,3)	(76,5)	(70,5)
C5″	77,09 d	74,12	76,64	80,68	74,15
	(75,6)	(73,8)	(77,0)	(77,0)	(73,8)
C6″	172,29 s	170,07	170,18	169,00	169,07
C7″		52,91	53,18	52,41	52,68

*Other signals – (II): 167.71; 169.42; 169.61; 169.66; 169.99 (C=O); 20.65; 21.02 (CH_3). (IV): 60.32; 60.84 (OCH_3). (V): 164.89; 165.08; 165.48; 166.59 (C=O); 128.27; 128.86; 130.49; 133.23; 134.53 (Ph).

A confirmation of the absence of an anomeric carbon atom of the α-configuration was also a comparison of the spectrum of compound (II) with that of cellobiose pentaacetate [10]. The figures calculated by additive schemes given in Table 1 also indicate the β-configuration of the C-1' anomeric center.

The signals of the carbinol carbon atoms of the trimethyl ester of glycyrrhizic acid (III) have chemical shifts close to those of the signals of the glycoside (I) itself. The signals of the carbonyl carbon atoms of the methoxycarbonyl groups (C-6', C-6") were observed in a weak field (169.00 ppm), and the signals of the methyl radicals (C-7' and C-7") of the

TABLE 2. Parameters of the ^1H NMR Spectra of the Carbohydrate Moiety of the Pentaacetate (II) and of the Pentabenzoate (V) (δ, ppm; $^3J_{HH}$, Hz; $CDCl_3$; 25°C; 300 MHz)

Proton	II		V	
	δ	$^3J_{HH}$	δ	$^3J_{HH}$
HC-1′	4,76	8,0	5,41	7,9
HC-2′	4,94	8,0; 9,5	5,52	9,6; 7,9
HC-3′	5,10	9,5; 9,8	5,87	9,6; 9,6
HC-4′	5,30	9,8; 9,8	5,70	9,6; 9,6
HC-5′	4,01	9,8	4,34	9,6
HC-1″	4,51	7,6	4,53	7,9
HC-2″	3,86	7,6; 9,5	3,89	7,9; 7,9
HC-3″	5,23	9,5; 9,5	3,80	7,9; 9,5
HC-4″	5,17	9,5; 9,5	5,17	9,5; 9,5
HC-5″	4,00	9,5	3,99	9,5

$COOCH_3$ groups also had close values (52.99 and 53.18 ppm, respectively).

Characteristic for the permethylate of glycyrrhizic acid (IV) are weak-field shifts of the signals of the carbinol atoms of up to 10 ppm in comparison with the spectrum of the acid (I), which is connected with the effect of O-methylation [10, 13].

The spectrum of the pentabenzoate of the trimethyl ester of glycyrrhizic acid (V) was characterized by additional signals of aromatic carbon atoms in the 128-134 ppm region.

The carbohydrate and aglycon parts of the spectrum were close to those of compound (II). The configurations of the anomeric centers of the glucuronic acids of the carbohydrate chain and of the O–C-3 bond of the aglycon were retained in the spectra of all the glycyrrhizic acid derivatives obtained.

On the basis of the ^{13}C NMR spectra of the glycoside (I) and its derivatives (II-V) considered above, glycyrrhizic acid should be ascribed structure (I): 3β-[O-β-D-glucopyranuronosyl-(1 → 2)-β-D-glucopyranuronosyloxy]-11-oxo-(18βH)-olean-12-en-30β-oic acid.

The results obtained by ^{13}C NMR spectroscopy were convincingly confirmed by a study of the proton magnetic resonance spectra of the pentabenzoate of glycyrrhizic acid (V). Table 2 gives the most informative signals of the nine protons directly linked to the diglucuronic acid residue. By using the double-resonance method we determined all the bonds between the proton systems and isolated two subsystems of five protons assigned to the different sugar residues. It must be mentioned that the values of all the vicinal spin-spin coupling constants, $^3J_{HH}$, were in the range of 7.9-9.6 Hz. This fact unambiguously determines the axial orientations of all the protons, including the stereochemically important ones – C-1′H, C-2′H, and C-1″H [17].

In the ^1H NMR spectrum of the pentaacetate (II) (see Table 2) in the region of protons linked to carbinol carbon atoms there are four doublet signals with values of the vicinal SSCC $^3J_{HH}$ = 7.6-9.8 Hz. Thus, the PMR spectra also provide evidence in favor of the β-configuration of the glycosidic bonds in the glycyrrhizic acid molecule.

EXPERIMENTAL

^1H NMR spectra were taken on a Bruker AM-30 spectrometer (300 MHz), and ^{13}C NMR spectra on a Jeol-FX 90 Q spectrometer (22.50 MHz) with broad-band and off-resonance suppression of proton interactions. The width of scanning was 6024 Hz and the resolution of the analog-digital converter 0.74 Hz.

The samples were prepared in $CDCl_3$ with TMS as internal standard.

The glycyrrhizic acid was isolated from liquorice extract by a published method [5] and was purified by conversion into the potassium salts [14] and repeated recrystallization from glacial acetic acid [15]. mp 217-219°C, $[\alpha]_D^{20}$ +55° (c 0.24; 50% EtOH). Lit. mp 216-220°C [16]. Compounds (II-V) were obtained by the methods described in [5] and were purified by repeated recrystallization or reprecipitation from aqueous ethanol and from acetone–hexane. The trimethyl ester (III) was isolated in the individual state with the aid

of chromatography on a column containing silica gel L (40/100 μm) with elution by chloroform–methanol–water (50:12:1).

All the compounds obtained were homogeneous according to thin-layer chromatography. The constants of derivatives (II-V) are given below:

Compound	mp, °C	$[\alpha]_D^{20}$, degrees	УФ, λ_{max}^{MeOH}, nm (lgε)
II	160–162	+47.5 (*c* 0.12; MeOH)	250 (4.11)
III	215–217	+43 (*c* 0.2; EtOH)	250 (4.21)
IV	273–275	+32 (*c* 0.13; $CHCl_3$)	249 (4.05)
V	177–179	+20 (*c* 0,02; MeOH)	230 (4.62) 257 (3.93)

SUMMARY

1. The ^{13}C NMR spectra of glycyrrhizic acid and its derivatives have been investigated and an assignment of the signals has been made.

2. On the basis of the results obtained, the configurations of the anomeric centers of the carbohydrate chain have been redetermined and the β-configuration of the C-1' carbon atom has been suggested.

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