^1H AND ^{13}C SPECTRA OF BIOLOGICALLY ACTIVE COMPOUNDS X. TWO-DIMENSIONAL HH COSY 45° AND CH HET CORR SPECTRA OF THE 18α- AND 18β-ISOMERS OF GLYCYRRHETIC ACID 3-ACETATE

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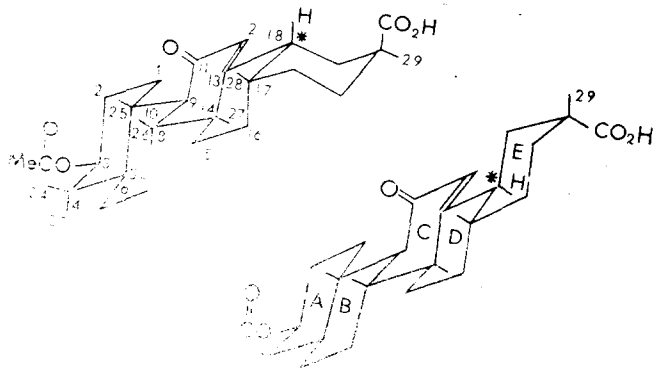
An assignment of ^1H and ^{13}C NMR signals has been made by the methods of homonuclear two-dimensional spectroscopy, HH COSY (45°), and heteronuclear correlation spectroscopy, CH HET CORR. It has been shown that the range of diastereomeric effects in the ^{13}C NMR spectra substantially exceeds the effects due to solvents, in contrast to the proton spectra in which these ranges overlap.

Assignments of the ^{13}C signals for a number of derivatives of pentacyclic triterpenoids (the 18α- and 18β- isomers of glycyrrhetic acid) have been made in [1-4] and also in our own publication [5]. The proton magnetic resonance spectra of compounds of this series were first obtained by Mousseron-Canet and Crauzet [6]. Later [7], the class of triterpenoids studied was substantially expanded and an assignment was made of the signals of the protons located near the polar groups. However, the assignment of the signals of the ring protons in the 0.7-2.1 ppm region is a fairly complex task for instruments with a low working frequency (≤ 100 MHz).



In the present work, by the methods of two-dimensional homonuclear spectroscopy, HH COSY (45°), and heteronuclear CH spectroscopy we have made an assignment of the signals of the protons and of the carbon nuclei for the stereoisomeric pairs of 18α- and 18β- glycyrrhetic acid acetate.

Figure 1 gives the line ^1H spectrum of the 18β isomer of glycyrrhetic acid 3-acetate (I) taken at a working frequency of 300 MHz in deuteropyridine solution. In the weak field are found signals assigned previously [6, 7] of the hydroxylic proton of the acid group in the form of a broadened singlet at 6.16 ppm, the singlet of a proton on a double bond at C-12 (5.95 ppm), and the signal of the equatorial proton at C-1 (3.12 ppm) linked by geminal ($^2J_{H,H}$ = 13.5 Hz) and vicinal ($^3J_{H,H}$ = 4.3 Hz) constants with the protons at C-2. The assignments of the proton signals in the strong-field region are more problematical and require detailed consideration. With this aim, by the standard procedure [8] on a Bruker AM 300 instrument we obtained the two-dimensional HH NMR spectrum in the COSY (45°) variant that is given in Fig. 2. The spectrum is limited to the 0.6-6.0 ppm region and consists of an image of a linear spectrum in the form of the diagonal section and the cross-peaks symmetrical with respect to it showing the nature of the interaction between the corresponding protons

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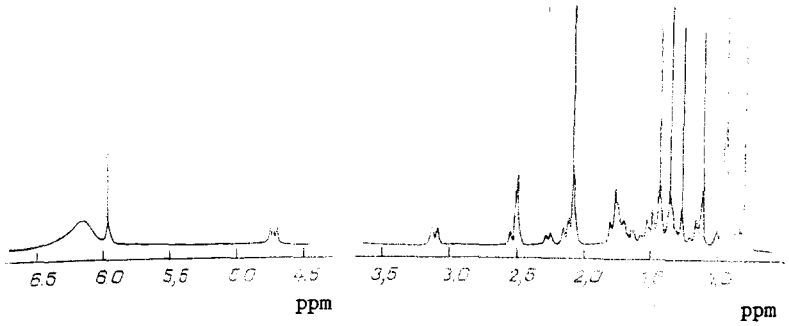

Fig. 1. ^{1}H NMR spectra of the 18β- isomer of glycyrrhetic acid 3-acetate (deuteropyridine, TMS).

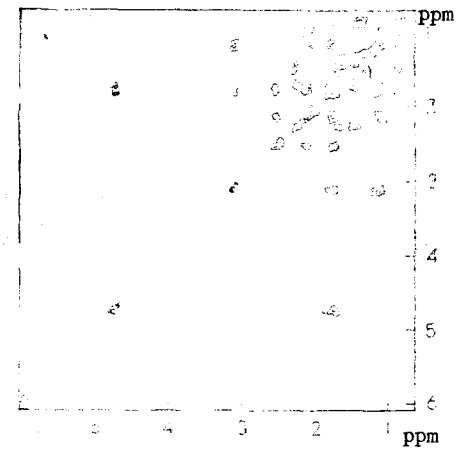

Fig. 2

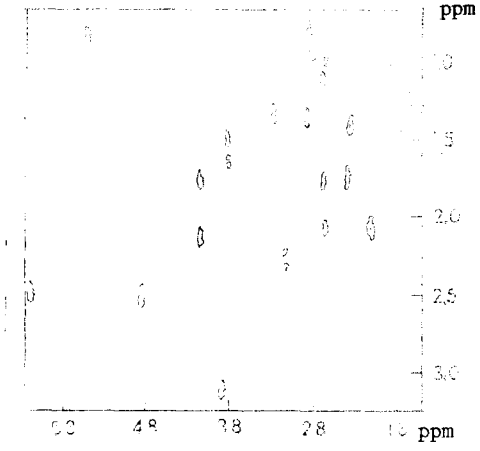

Fig. 3

Fig. 2. Contour map of the HH COSY (45°) two-dimensional homonuclear NMR spectrum of the 18β- isomer of glycyrrhetic acid 3-acetate.

Fig. 3. Contour map of the CH CORR two-dimensional heteronuclear NMR spectrum of the 18β- isomer of glycyrrhetic acid 3-acetate.

Thus, it can be seen from Fig. 2 that the proton giving a signal at 3.12 ppm interacts with the proton giving a signal at 1.16 ppm (the geminal constant from the linear spectrum, $^{2}J_{H,H}$ is −13.5 Hz) and has an additional interaction with the protons giving a signal at 1.75 ppm. The results of a detailed analysis of the two-dimensional spectrum show that the assignment of the other proton signals remains extremely problematical. In view of this circumstance, we turned to a two-dimensional $^{13}C-^{1}H$ heteronuclear correlation experiment in the standard variant CH COOR of the firm of Bruker [9]. The general form of the spectrum is illustrated in Fig. 3. Along the x axis is shown the file for the ^{13}C NMR spectrum (δ ^{13}C, ppm), and along the y axis the file for the proton spectrum (δ H, ppm). In this case, cross-peaks show the existence of a direct link between the protons and the corresponding carbon nuclei (^{13}CH).

Knowing the assignment of the signals in the ^{3}C NMR spectrum that we made earlier [5], which corresponded to literature information [2, 4], it was possible to refine the assignment of the signals of the protons. Thus, to the signal of the C-1 carbon atom (39.07 ppm) correspond the following interacting protons: the axial C-1 Ha at 1.16 ppm ($^{2}J_{HH}$ = 13.5, $^{3}J_{HaHa}$ = 11.3, $^{3}J_{HaHe}$ = 4.5 Hz) and the equatorial C-1 He at 3.12 ppm ($^{2}J_{HH}$ = 13.5, $^{3}J_{HaHe}$ = 4.5 Hz). It must be mentioned that the screening of the axial proton takes place not only through 1,3-diaxial interactions of steric nature but also through the anisotropic contribution of the carbonyl group at C-11. And conversely, the equatorial proton C-1 He falls into the paramagnetic region of the cone of the anisotropy of the carbonyl group, and its signal

is observed in a weaker field. The diastereotopic effects of the protons amount to 1.96 ppm. The signals of the protons at C-23 and C-24 have the same shifts in the ^1H NMR spectrum (0.93 ppm). It is interesting to note that the carbon atoms of the diastereotopic methyl groups are then resolved at 14 ppm. The axial C-23 methyl group, screened through 1,3-diaxial interaction of steric nature, corresponds to the stronger-field signal.

The HC-18, H_2C-19 spin system is also stereochemically informative. The signal of the proton at C-18 linked with a carbon signal having $\delta^{13}C$ = 48.73 ppm forms a doublet at 2.52 ppm with an axial constant of $^3J_{HaHa}$ = 13.6 Hz slightly overlapped by the singlet signal of the proton at C-9 (δ = 2.48 ppm). The two protons attached to the C-19 carbon atom are located in the strongly overlapping regions of 1.15 ppm (C-19 Ha) and 2.13 ppm (C-19 He), which, in its turn, confirms the assignment of the C-19 signal in the carbon spectrum [5].

Analogous experiments were performed for the 18α- epimer of 3-O-acetylglycyrrhetic acid, in which one- and two-dimensional spectra in deuteropyridine solution were obtained.

In order to study the detailed differences in the PMR spectra, the following experiment was carried out. An equimolar mixture of the 18α- and 18β- epimers of glycyrrhetic acid 3-acetate (0.1 mole/dm^3) was prepared, and for this linear ^1H and ^{13}C NMR spectra and also HH COSY and CH COOR two-dimensional spectra were recorded. The parameters of the ^{13}C and ^1H NMR spectra are given in Tables 1 and 2, respectively. Figure 4 shows a map of the two-dimensional CH correlation experiment in which is illustrated in the form of a rectangle the diastereomeric effects of the chemical shifts in the ^1H and ^{13}C NMR scale for the C-19 H, C-18 H, and C-28 H spin systems. The pattern for C-19 H, in which there is no overlapping of the signals, appears most clearly and unambiguously.

The diasteromeric effects in the ^{13}C NMR scale that are given in Table 1

$$\Delta\delta_{^{13}Ci} = \delta_{^{13}Ci18\beta} - \delta_{^{13}Ci18\alpha}, \quad (1)$$

exceed by more than an order of magnitude the range of diastereomeric effects of the protons (Table 2) connected with the corresponding carbon nuclei

$$\Delta\delta_{^1HI} = \delta_{^1Hi18\beta} - \delta_{^1Hi18\alpha}. \quad (2)$$

We then studied the concentration dependence of the diastereomeric effects of the CSs of the carbon nuclei and protons. For the HC-19 and HC-18 nuclei, which are of stereochemical interest, graphs of these relationships are given in Figs. 5 and 6, respectively. A fourfold dilution of an equimolar mixture of the 18α- and 18β- epimers led to a change in $\Delta\delta_{C-9}$ of about 0.06 ppm. On the protein scale, these changes are also small.

TABLE 1. Diastereomeric Effects of the ^{13}C NMR Chemical Shifts of an Equimolar Mixture of the 18α- and 18β- Epimers of Glycyrrhetic Acid 3-Acetate (d, ppm, deuteropyridine, TMS, 75.5 MHz).

Ci	18α	18β	Δδ Ci	Ci	18α	18β	Δδ Ci
1	39,00	39,00	0,00	17	35,66	32,10	−3,56
2	23,90	23,90	0,00	18	40,59	48,65	8,06
3	80,47	80,47	0,00	19	32,43	41,61	9,18
4	38,16	38,35	0,19	20	42,68	44,04	1,38
5	55,02	55,07	0,05	21	36,31	31,50	−4,81
6	17,69	17,61	−0,08	22	29,29	38,21	8,92
7	33,75	32,72	−1,03	23	28,12	28,12	0,00
8	43,94	43,46	−0,48	24	17,04	16,97	−0,07
9	60,70	61,91	1,21	25	16,83	16,64	−0,19
10	37,11	37,32	0,21	26	18,59	18,75	0,16
11	198,97	199,41	0,44	27	20,69	23,52	2,83
12	124,22	128,56	4,34	28	16,06	28,68	12,62
13	166,01	169,91	3,90	29	21,10	28,68	7,58
14	45,13	45,49	0,36	30	1,0,84	179,08	−1,76
15	26,91	23,58	−0,33	31	170,58	170,58	0,00
16	37,63	26,76	−10,87	32	21,10	21,10	0,00

TABLE 2. Diastereomeric Effects of the ^1H NMR Chemical Shifts of an Equimolar Mixture of the 18α- and 18β- Epimers of Glycyrrhetic Acid 3-Acetate in Deuteropyridine (δ, ppm, TMS, 300 MHz).

HCi	300° K		
	18α	18β	$\Delta\delta_{Ci}$
1	2,99	3,10	0,11
3	4,72	4,72	0,00
9	2,36	2,48	0,12
12	5,76	5,95	0,19
18	2,34	2,52	0,18
22	2,18	2,27	0,09
23	0,93	0,93	0,00
24	0,93	0,93	0,00
25	1,32	1,26	—0,06
26	1,11	1,10	—0,01
27	1,33	1,45	0,12
28	0,72	0,80	0,08
29	1,43	1,35	—0,08
30	0,45	0,45	0,00
32	2,07	2,07	0,00

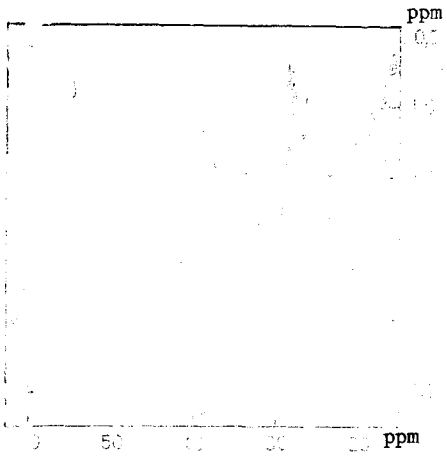

Fig. 4. Contour map of the CH CORR two-dimensional heteronuclear NMR spectrum of a mixture of the 18α- and 18β- isomers of glycyrrhetic acid 3-acetate.

The temperature dependences of the diastereomeric effects for the C-18, H-18, C-9, H-9, and C-18, H-28 nuclei are shown in Figs. 7 and 8. With a rise in the temperature by 37°C there is a more paramagnetic change in Δδ, which amounts to one fifth of the absolute magnitudes of the effects.

The concentration and temperature dependences of the diastereomeric effects reflect changes in the screening of the magnetic nuclei due to variations in the solvent molecule environment of the magnetic nuclei. In the ^{13}C NMR spectra, when deuteropyridine was replaced by the less polar deuterochloroform the changes in the values of the CSs were fairly small, while in the proton spectra these changes were comparable with the values of the diastereomeric effects. The facts observed permit the use of the ^{13}C NMR diastereomeric effects for carrying out stereochemical assignments of conformationally rigid systems over fairly wide ranges of temperatures and concentrations.

The ^1H and ^{13}C NMR spectra were recorded on an AM 300 spectrometer at working frequencies of 375 MHz, respectively. The solvents were $CDCl_3$ and C_5D_5N, with TMS as internal

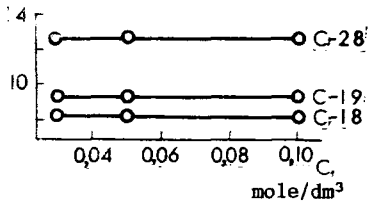

Fig. 5

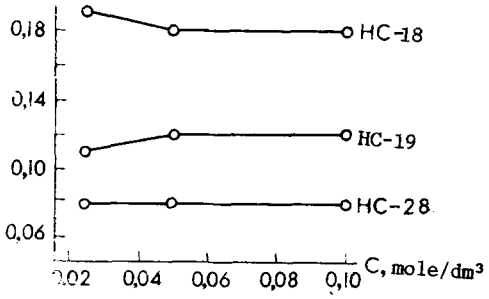

Fig. 6

Fig. 5. Dependence of the diastereomeric effects of the carbon atoms of the 18β- isomer of glycyrrhetic acid 3-acetate on the concentration of the solution.

Fig. 6. Dependence of the diastereomeric effects of the protons of the 18β- isomer of glycyrrhetic acid 3-acetate on the concentration of the solution.

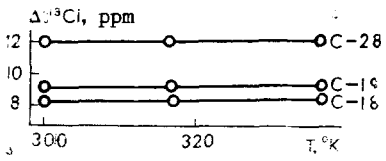

Fig. 7

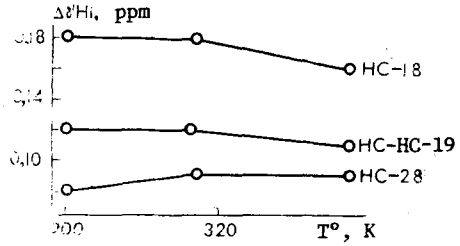

Fig. 8

Fig. 7. Temperature dependence of the diastereomeric effects of the carbon atoms of the 18β- isomer of glycyrrhetic acid 3-acetate.

Fig. 8. Temperature dependence of the diastereomeric effects of the protons of the 18β- isomer of glycyrrhetic acid 3-acetate.

standard. The homonuclear HH COSY (45°C) experiments [8] and the heteronuclear HC HET CORR experiments [9] were carried out in accordance with the standard programs of the Bruker firm.

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