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^{13}C NMR SPECTRA OF β-SITOSTEROL DERIVATIVES WITH OXIDIZED RINGS A AND B

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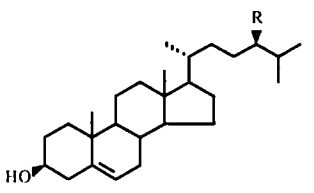
^{13}C NMR spectra of stigmastane steroids are studied. Signals are assigned to C atoms.

^{13}C NMR spectroscopy is one of the most important methods for establishing and proving the structures of such complicated organic compounds as steroids [1]. Important information about structural features of steroids that cannot be obtained using other physical methods can be derived from ^{13}C NMR spectra.

We previously synthesized steroids **3b**, **4**, **5a**, **5b**, **6b**, **7**, and **8**, which contain hydroxyls and ketones in rings *A* and *B*, starting from β-sitosterol (**1b**) [2]. It should be noted that certain of these compounds, namely the triol **3b**, the dihydroxyketone **5a**, and the diketones **6b** and **8**, occur in nature. Further research [3, 4] demonstrated that dihydroxyketone **5a** is a convenient starting material for preparing various polyhydroxysteroids. The hydroxyketone **9**, which we synthesized from β-sitosterol through the acetate **2**, is also interesting.



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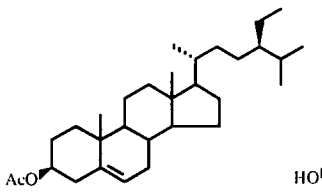

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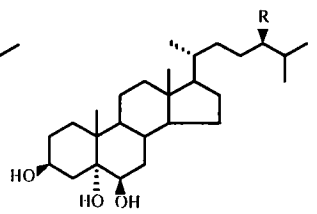

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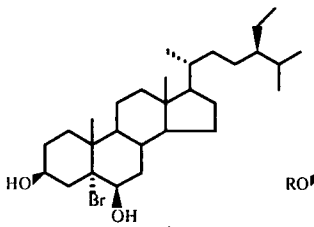

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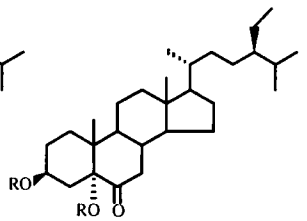
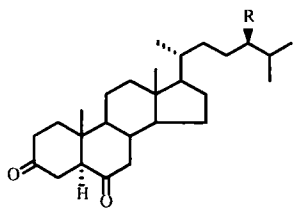
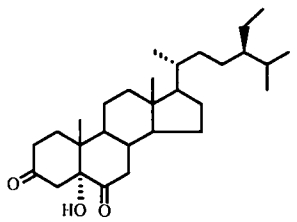


5a: R=H; **b**: R=Ac



6a: R=H; **b**: R=Et



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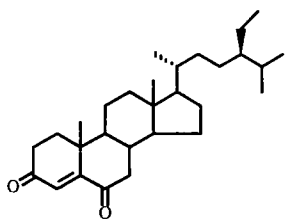

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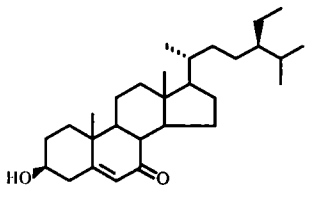

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 0009-3130/99/3506-0646$22.00

TABLE 1. ^{13}C {^{1}H} NMR Spectra (δ, ppm) Compounds of **1-9**

Atom	1a	1b	2	3a*[10]	3b	4	5a	5b	6a[11]	6b	7	8	9
C-1	37.2	37.3	38.2	32.5	32.4	30.8	32.5	30.7	37.0	37.4	37.5	35.5	36.4
C-2	31.6	31.6	27.9	33.4	33.6	33.0	33.4	28.0	37.4	37.0	37.3	34.2	31.2
C-3	71.5	71.8	73.9	67.6	67.9	67.4	67.7	69.9	208.9	209.0	210.7	199.2	70.4
C-4	42.2	42.3	39.8	42.1	43.0	47.3	35.8	35.9	38.2	38.1	53.4	125.5	41.9
C-5	140.8	140.8	139.7	76.0	76.3	64.0	76.6	88.7	57.6	57.5	82.8	161.0	165.3
C-6	121.7	121.7	122.6	76.3	76.7	69.1	?*	196.1	211.0	211.1	210.7	202.2	126.1
C-7	31.81	32.1	32.0	35.8	35.9	37.0	42.8	42.8	46.6	46.6	41.9	46.8	202.2
C-8	31.8	32.1	32.0	31.3	31.6	30.1	31.3	38.5	38.1	38.1	31.6	33.9	45.4
C-9	50.0	50.2	50.2	46.0	46.1	55.0	46.3	45.4	53.6	53.5	45.9	51.0	50.0
C-10	37.0	36.5	37.1	39.2	39.3	39.6	39.2	43.9	41.2	41.2	39.5	39.8	38.4
C-11	21.1	21.1	21.1	22.0	21.9	21.5	21.9	21.5	21.7	21.7	21.6	20.9	21.2
C-12	39.7	39.8	39.8	40.8	40.9	40.0	40.8	39.6	39.5	39.4	39.5	39.1	38.8
C-13	42.2	42.3	42.4	43.2	43.3	43.1	43.3	43.2	43.0	43.0	43.2	42.6	43.1
C-14	56.0	56.8	56.8	56.8	56.9	56.6	57.0	56.7	56.7	56.6	56.4	56.6	50.0
C-15	24.2	24.3	24.4	24.7	24.7	24.0	24.8	23.9	23.8	24.0	24.0	24.0	23.1
C-16	27.9	28.3	28.3	28.7	27.4	28.1	28.8	28.5	28.0	28.1	28.1	28.0	28.5
C-17	56.1	56.1	56.2	56.8	56.9	56.0	57.0	56.1	56.2	56.0	56.1	55.9	54.8
C-18	11.8	12.0	11.9	12.6	12.5	12.0	12.6	11.9	12.0	12.0	12.0	11.9	12.0
C-19	19.4	19.1	19.1	17.3	17.3	16.7	17.3	13.8	12.6	12.6	13.9	17.5	17.3
C-20	35.7	36.2	36.7	36.3	36.9	36.1	36.6	35.9	35.7	36.1	36.1	36.0	36.1
C-21	18.7	18.8	18.8	19.2	19.3	18.7	19.2	18.5	18.7	18.7	18.7	18.7	19.0
C-22	36.0	34.0	34.1	36.6	34.4	33.9	34.5	34.1	36.1	33.9	33.9	33.9	34.0
C-23	23.8	26.2	26.3	24.4	26.6	25.9	26.8	26.1	24.0	26.1	26.1	26.0	26.3
C-24	39.5	45.2	46.0	39.9	46.4	45.7	46.1	45.9	39.5	45.8	45.9	45.8	45.9
C-25	28.2	29.2	29.1	28.4	29.7	29.1	29.8	29.2	28.0	29.2	29.2	29.1	29.2
C-26	22.4	18.9	19.3	22.9	20.1	19.1	19.5	19.1	22.5	19.0	19.1	19.0	19.8
C-27	22.7	19.1	19.8	22.9	19.4	19.8	20.1	19.8	22.8	19.8	19.8	19.8	19.0
C-28		23.1	23.2		23.6	23.1	23.7	23.2		23.1	23.1	23.1	23.1
C-29		11.9	11.9		12.3	12.0	12.3	11.9		12.0	12.0	11.9	12.0
CH_3CO			21.4					21.1; 21.5					
CH_3OH			170.4					170.0; 170.6					

Solvent: $CDCl_3$ unless noted*(C_5D_5N). **Signal not observed.

These features prompted us to study the ^{13}C NMR spectra of these compounds. In our opinion, the results have independent significance and are discussed in the present article.

Assigning signals in the ^{13}C NMR spectra to resonances of individual C atoms in steroids is a rather complicated problem [1]. We solved this problem by using data for the chemical shifts and multiplicities determined from ^{13}C spectra. Comparison of the analyzed spectra with those of compounds of similar structure was very helpful.

We began the study by comparing the spectra of cholesterol (**1a**) and β-sitosterol (**1b**). The chemical shifts of C atoms in these compounds are listed in Table 1. It should be noted that they agree well with the analogous values reported in the literature for cholesterol [1, 5, 6] and β-sitosterol [6-8].

Table 1 shows that the chemical shifts of C atoms in the cyclic part of cholesterol and β-sitosterol are practically identical. However, the positions of the signals for atoms in the side chains differ markedly. β-Sitosterol has an additional 24-ethyl group, in contrast with cholesterol. The differences can be considered to arise owing to the presence of this group. The corresponding data are listed in Table 2. It can be seen that the signal for C-24 in the spectrum of β-sitosterol is shifted to downfield by +5.7 ppm. This shift is primarily due to the fact that C-24 in cholesterol is secondary whereas it is tertiary in β-sitosterol. Furthermore, the 24-ethyl group produces an α-effect on the chemical shift of C-24 in the spectrum of β-sitosterol.

TABLE 2. Chemical Shifts (δ, ppm) of C Atoms in Side Chains of Cholesterol (**1a**) and β-Sitosterol (**1b**)

Atom	1a	1b	Δ
C-20	35.7	36.2	+0.5
C-21	18.7	18.8	+0.1
C-22	36.0	34.0	-2.0
C-23	23.8	26.2	+2.4
C-24	39.5	45.2	+5.7
C-25	28.2	29.2	+1.0
C-26	22.4	18.9	-3.5
C-27	22.7	19.1	-3.6
C-28		23.1	
C-29		11.9	

The downfield shifts of the signals for C-23 and C-25 in the spectrum of β-sitosterol compared with their position in the spectrum of cholesterol are due to the β-effect of the 24-ethyl group. Analogously, the upfield shifts of the signals for C-22, C-26, and C-27 in the spectrum of compound **1b** compared with their positions in the spectrum of cholesterol are due to the γ-effect of the 24-ethyl group. However, Table 2 shows that the chemical shifts of C-20 and C-21, which are distant from C-24, are practically identical. Considering that the literature contains much data for the $^{13}C\{^1H\}$ NMR spectra of cholesterol derivatives, the features noted above can be used to assign signals in spectra of the corresponding β-sitosterol derivatives.

The acetate **2** is the simplest derivative of β-sitosterol that we studied. Signals of C atoms in its $^{13}C\{^1H\}$ NMR spectrum can be easily assigned. Table 1 shows that the chemical shifts of C atoms in rings *B*, *C*, and *D* and the side chains of compounds **1b** and **2** are practically identical. The differences in the chemical shifts of atoms in ring *A* can be understood by examining literature data [9] for the shifts of signals upon converting alcohols to acetates. Thus, the signal for C-3 in the spectrum of acetate **2** should shift to downfield by ~3 ppm owing to the α-effect of the acetoxy group compared with its position in the spectrum of alcohol **1b**. The signals for C-2 and C-4 in the spectrum of acetate **2** shift to upfield owing to the β-effect of the acetoxy group.

The analysis of the $^{13}C\{^1H\}$ NMR spectrum of the 3β,5α,6β-triol **3b** is simplified by the fact that the spectrum of the corresponding cholestane derivative (**3a**) has been reported [10]. The data are given in Table 1. Using the literature data for the side chain, we were able rather simply to assign signals for all atoms in the spectrum of triol **3b**. Certain differences in the chemical shifts of C atoms in rings *C* and *D* and the side chain in the spectrum of triol **3b** compared with their positions in the spectra of β-sitosterol (**1b**) and its acetate (**2**) are surely due to solvent effects.

The bromohydrin **4** has a structure similar to triol **3a**. Therefore, the $^{13}C\{^1H\}$ NMR spectra of these compounds are rather similar. Only the chemical shifts of atoms in rings *A* and *B* differ owing primarily to the different electronegativities of the hydroxy and bromo groups on C-5.

The ^{13}C NMR spectra of the dihydroxyketone **5a** and its diacetate **5b** [3] were determined analogously by comparing them with the analogous spectra of compounds **1-4**.

The assignment of signals in the spectrum of the 3,6-diketone **6b** was significantly simplified because the $^{13}C\{^1H\}$ NMR spectrum of the corresponding cholestane derivative **6a** has been published [11]. The data are given in Table 1. Using these data and taking into account changes in the chemical shifts of C atoms in the side chains (Table 2), we assigned all signals in the spectrum of diketone **6b**. However, we believe that the signal for C-1 should appear at lower field than that for C-2, in contrast with the literature data [11] for diketones **6a** and **6b**. The deciding argument in favor of this hypothesis is the reported ^{13}C NMR spectrum of 5α-cholestan-3-one [1], in which these atoms appear in just this order.

The hydroxyketone **7** has a structure very similar to that of diketone **6b**. For this reason, signals for atoms in the $^{13}C\{^1H\}$ NMR spectrum of hydroxydiketone **7** are most easily assigned by comparing them with the analogous signals in the spectra of diketones **6a** and **6b**. Naturally, the effect on the chemical shifts of C atoms in close proximity to the 5α-hydroxy group should be considered.

Table 1 shows that the additional 5α-hydroxy group in compound **7** cause a significant downfield shift of the signal for

C-5 owing to the α-effect compared with its position in the spectra of diketosteroids **6a** and **6b**. The signal for C-4 also shifts to downfield as a result of the β-effect. The fact that the 5α-hydroxy group in steroid **7** has no significant effect on the chemical shifts of the C atoms of the 3- and 6-ketones is interesting.

We compared the ^{13}C {^1H} NMR spectrum of the Δ^4-3,6-diketone **8** with that of cholest-4-en-3-one [1] in order to assign the signals for C atoms in ring *A*. Naturally, we took into account the effect of the 6-ketone, which shifts the signal for C-4 to downfield and that for C-5 to upfield. Table 1 contains the data for the spectrum of steroid **8** that were obtained taking into account the changes in the positions of the C atoms in the side chain. The analysis suggests that the additional double bond in steroid **8** compared with diketone **6b** produces unpredictable changes in the chemical shifts of the C atoms in rings *A* and *B*. These changes are so significant that the ^{13}C {^1H} NMR spectra of compound **6b** can hardly be used to analyze the spectrum of Δ^4-3,6-diketone **8**.

We compared the spectrum of 3β-hydroxy-Δ^5-7-ketone **9** with that of β-sitosterol (**1b**) in order to assign unambiguously the signals of atoms in rings *A*, *C*, and *D* and the side chain. However, chemical shifts of atoms in ring *B* of compound **9** were determined by comparing them with the analogous shifts in the spectrum of 3β-acetoxycholest-5-en-7-one [1, 9]. This enabled the spectrum to be fully interpreted (Table 1).

Thus, we completely assigned signals of C atoms in the studied compounds. This will make it much easier in the future to use ^{13}C {^1H} NMR spectroscopy to prove the structure of other stigmastane polyhydroxysteroids.

EXPERIMENTAL

All ^{13}C NMR spectra were obtained in $CDCl_3$ and C_5D_5N (99.8 D) solutions on a Bruker WM-360 NMR spectrometer at 90.56 MHz for ^{13}C and ~0.2 M. Chemical shifts are given relative to an internal standard of TMS. We used PGD (^{13}C {^1H}), GD (^{13}C), and in certain instances CW off-resonance regimes. The pulse length was 30 μs (30°), relaxation delay 1 s, accumulation time 0.32 s, number of scans ~100 for ^{13}C {^1H}, ~1000 for ^{13}C. A total of 16 K memory was used for accumulation; 32 K, for transformation. The filter exponent was LB = 3 Hz.

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