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^{13}C NMR SPECTRA OF 6-HYDROXIMINOSTEROIDS OF THE STIGMASTANE SERIES

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*^{13}C NMR spectra were studied and signals of C atoms were assigned for 6-keto- and 6-hydroximinosteroids **1-10**.*

Key words: ^{13}C NMR spectra, 6-ketosteroids, 6-hydroximinosteroids.

We previously synthesized (24R,6E)-24-ethylcholest-6-hydroximino-4-en-3-one (**10**) [1, 2]. This compound, which was recently isolated from *Cinachyrella* marine sponges [3], is the first steroid oxime found in nature. We developed schemes [1, 2] for the preparation of **10** from β-sitosterol using 5-hydroxy-6-ketosteroids and their corresponding 6-ketoximes as intermediates.

The present article reports results from an investigation of ^{13}C NMR spectra of these compounds, primarily 6-hydroximinostigmastanes.

^{13}C NMR spectra of 5-hydroxy-6-ketosteroids of various structure have been published in several places [4-8]. However, ^{13}C NMR spectra of steroid oximes are less studied [3, 9]. Such compounds are also interesting because aromatase inhibitors [9] and active progestins [10] have been observed among them.

We interpreted ^{13}C NMR spectra of the steroids using mainly the chemical shifts of their signals and their multiplicity determined by the DEPT method. Steroids **1-10** were synthesized from β-sitosterol. Therefore, their structures are identical in rings *C* and *D* and the side chains. For this reason it is simple to assign unambiguously the signals for C atoms in these structure fragments by comparing their spectra with those of β-sitosterol and stigmastane steroids that we studied [7, 8, 11-13]. It should be noted that we have previously studied ^{13}C NMR spectra of **1**, **3**, and **9** [7]. However, the spectra of these compounds are described under different conditions (in particular, different concentrations, solvents, and recording parameters) in the present article. Therefore, their principal parameters differ, although not very substantially, from those previously reported [7]. Signals for the atoms in rings *A* and *B* in spectra of **1-10** were assigned assuming [14] that rather distant functional groups have an insignificant effect on their chemical shifts.

This made it possible to assign certain signals to the resonances of actual atoms by comparing the spectra of compounds of similar structure. Table 1 shows the principal spectral properties of **1-10**. Literature data for **10** are also given for sake of illustration [3]. It can be seen that the ^{13}C NMR spectra of **10** are similar to analogous spectra that have been previously reported [3].

One of the most important practical applications of ^{13}C NMR spectroscopy in the chemistry of steroids is establishing the type of fusion of rings *A* and *B*. The main criterion is the magnitude of the chemical shift of the angular 19-methyl C atom. It is known [14] that the signal for C-19 in spectra of *cis*-*A*/*B*-steroids appears at δ 22-23 ppm whereas it is situated at stronger field at δ 13-15 ppm in spectra of *trans*-*A*/*B*-steroids. Considering that the compounds we studied include several pairs of C-5 isomers, it seemed interesting to determine if ^{13}C NMR spectra could reliably prove the *A*/*B*-fusion in 5-hydroxy-6-ketosteroids and 5-hydroxy-6-hydroximinosteroids. Table 1 shows first of all that signals for C-19 in ^{13}C NMR of *cis*-*A*/*B*-steroids **5-8** are situated at δ 16.1-17.5 ppm. However, the signals for these same atoms in spectra of isomeric *trans*-*A*/*B*-steroids **1-4** have chemical shifts δ 13.7-14.4 ppm.

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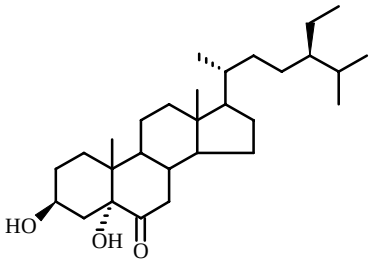

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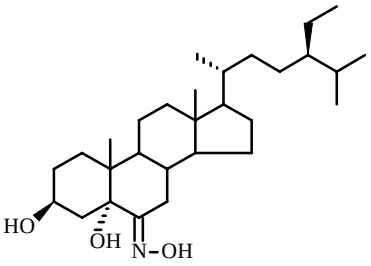

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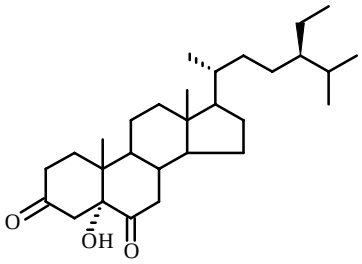

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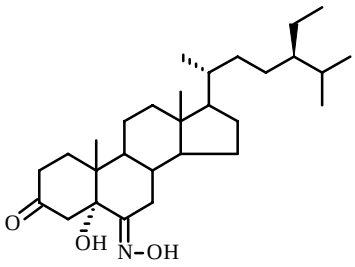

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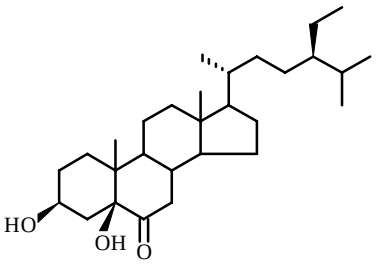

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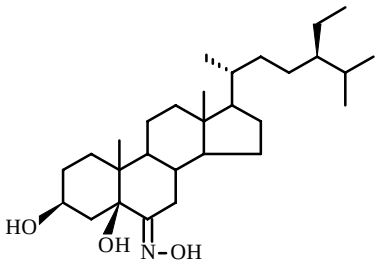

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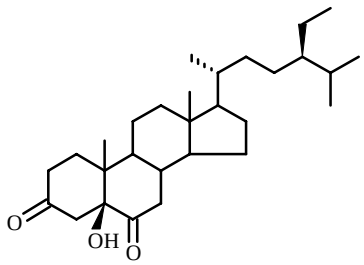

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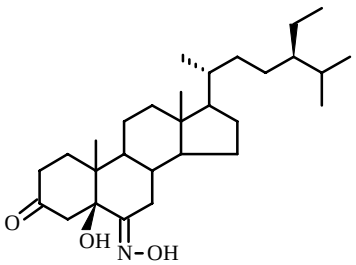

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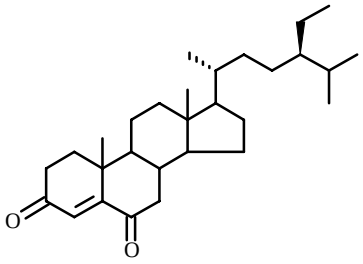

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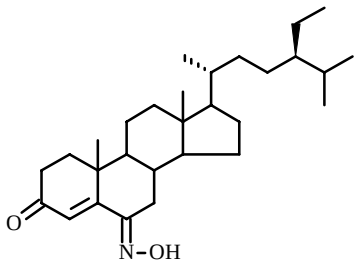

10

Therefore, we conclude that the difference in chemical shifts of C-19 in *trans*- and *cis*-*A*/*B*-isomers of steroids with 5-hydroxyls becomes insignificant. Apparently this occurs because of the differences in the signs of the effects produced by 5α- and 5β-hydroxyls on C-19. Thus, a 5α-hydroxyl exerts a γ-antiperiplanar effect on C-19 [4, 15]. As a result, the signal for C-19 shifts slightly to weak field in spectra of 5α-hydroxysteroids compared with its position in spectra of steroids without a 5α-hydroxyl.

A 5β-hydroxyl, in turn, has an ordinary γ-effect on C-19. Therefore, the signal for C-19 in ^{13}C NMR spectra of 5β-hydroxysteroids shifts to strong field compared with its position in spectra of compounds without a 5β-hydroxyl.

Conclusions about the relative positions of signals for equivalent atoms in 6-ketosteroids and their oximes can also be made on the basis of the data in Table 1. A preliminary analysis showed that the chemical shifts for C-5, C-6, C-7, and C-8, which are close to the O atom or hydroximino group, differed most. We composed Table 2 to estimate quantitatively these differences. The data were obtained by subtracting chemical shifts for equivalent atoms in the ^{13}C NMR spectra of the corresponding oximes and ketones without considering the solvent effect. However, it can still be concluded that replacing the 6-ketone by 6-hydryoxyimino in both *trans*-*A*/*B*- and *cis*-*A*/*B*-steroids shifts the signal for C-6 to strong field by 50-53 ppm.

TABLE 1. Chemical Shifts of C Atoms (δ, ppm) in ^{13}C NMR Spectra of **1**-**10**

Atom	1	2	3	4	5	6	7		8	9	10	10 [13]
C-1	31.7	30.1	32.2	32.3	24.9	25.5	31.5	32.1	31.7	35.6	34.8	34.8
C-2	30.7	30.1	37.7	38.0	27.9	26.6	37.0	37.4	37.4	34.0	33.9	33.6
C-3	66.8	67.2	210.5	211.4	65.5	66.9	207.1	207.9	212.7	202.3	201.8	201.0
C-4	37.6	37.9	45.2	47.5	37.2	39.4	48.0	48.8	49.7	125.5	122.5	122.6
C-5	80.3	77.0	82.4	78.9	81.9	78.0	83.8	85.1	80.6	161.1	162.9	162.3
C-6	213.6	162.6	212.1	160.0	212.7	159.4	210.2	210.2	160.2	199.5	155.5	156.0
C-7	42.8	25.1	42.2	25.8	41.5	28.5	41.4	42.4	28.4	46.8	29.6	29.6
C-8	37.6	35.0	37.7	35.4	37.4	35.0	37.4	37.1	34.4	34.2	32.6	32.7
C-9	44.7	44.9	45.0	45.6	42.9	42.8	43.9	43.2	43.4	51.0	51.2	51.2
C-10	42.2	41.1	43.2	42.0	44.1	42.6	44.4	44.4	42.3	39.8	38.7	38.7
C-11	21.9	21.6	21.9	22.0	21.6	21.9	22.0	22.2	21.9	20.9	20.8	20.8
C-12	40.1	40.0	39.9	40.2	39.5	39.9	39.4	39.7	39.7	39.2	39.3	39.3
C-13	43.3	43.2	43.4	43.1	43.1	43.1	43.2	43.2	43.0	42.6	42.5	42.5
C-14	56.8	56.5	56.4	56.5	57.0	56.7	56.9	56.5	56.9	56.6	56.6	56.6
C-15	24.1	24.2	24.2	24.4	24.0	24.4	24.0	24.1	24.0	24.0	24.1	24.0
C-16	28.4	28.4	28.4	28.5	28.0	28.5	28.0	28.5	28.2	28.0	28.2	28.1
C-17	56.2	56.3	56.2	56.3	56.0	56.3	56.0	56.2	56.0	55.9	55.9	55.9
C-18	12.2	12.0	12.2	12.2	12.0	12.2	12.0	12.2	12.0	12.0	12.0	12.0
C-19	14.2	14.4	23.7	14.2	17.0	17.5	16.1	16.7	16.1	17.5	16.5	16.6
C-20	36.4	36.3	36.4	36.4	36.0	36.4	36.1	36.4	36.1	36.1	36.1	36.2
C-21	18.9	18.9	19.0	19.0	18.7	19.0	18.7	19.0	18.7	18.7	18.7	18.7
C-22	34.1	34.1	34.2	34.2	33.8	34.2	33.8	34.2	33.9	33.8	33.8	33.8
C-23	26.4	26.3	26.4	26.4	26.1	26.5	26.1	26.5	26.1	26.1	26.1	26.4
C-24	46.1	46.0	46.1	46.1	45.8	46.1	45.6	46.1	45.8	45.8	45.9	46.1
C-25	29.4	29.3	29.5	29.5	29.1	29.5	29.1	29.5	29.1	29.2	29.2	29.0
C-26	19.2	19.1	19.3	19.3	19.0	19.3	19.0	19.3	19.0	19.0	19.0	19.0
C-27	20.0	19.9	20.0	20.0	19.8	20.0	19.8	20.0	19.8	19.8	19.6	19.6
C-28	23.4	23.2	23.4	23.4	23.1	23.4	23.1	23.4	23.1	23.1	23.1	23.1
C-29	12.2	12.2	12.2	12.2	12.0	12.2	12.0	12.1	12.0	11.9	12.0	12.3
Solvent	C_5D_5N	$CDCl_3$-CD_3OD (4:1)	C_5D_5N	C_5D_5N	$CDCl_3$	C_5D_5N	$CDCl_3$	C_5D_5N	$CDCl_3$	$CDCl_3$	$CDCl_3$	$CDCl_3$

An analogous shift by 44 ppm is observed in spectra of Δ^4-steroids. Such large differences in the position of the signals for C-6 in ^{13}C NMR spectra of 6-ketosteroids and their oximes are surely due to the different electronegativities of the 6-hydroximino and 6-ketone groups. It can also be noted that signals for C-7 in spectra of 6-hydroximinosteroids are shifted to strong field by 13-18 ppm compared with their positions in spectra of the corresponding 6-ketosteroids. Such a large shift is probably due to the *E*-geometry of the 6-hydroximino group. Signals for C-5 and C-8 in spectra of oximes of *trans*- and *cis*-*A*/*B*-6-ketosteroids are shifted to strong field by 3.2-3.9 and 2.3-3.0 ppm, respectively, compared with their positions in spectra of the corresponding 6-ketones. Also, analogous changes, although small, occur in spectra of Δ^4-steroids.

Thus, comparing ^{13}C NMR spectra of 6-ketosteroids and their oximes is a convenient method that significantly simplifies their interpretation.

TABLE 2. Differences in Chemical Shifts (δ, ppm) of C Atoms in ^{13}C NMR Spectra of 6-Ketosteroids and Their Oximes

Atom	*trans-A/B*-Steroids		*cis-A/B*-Steroids		Δ^4-Steroids
	Δ_{2-1}	Δ_{4-3}	Δ_{6-5}	Δ_{8-7}	Δ_{10-9}
C-1	-1.6	0.1	0.6	0.2	-0.8
C-2	-0.6	0.3	-1.3	0.4	-0.1
C-3	0.4	0.9	1.4	5.6	-0.5
C-4	0.3	2.3	2.2	1.7	-3.0
C-5	-3.3	-3.5	-3.9	-3.2	1.8
C-6	-51.0	-52.1	-53.3	-50.0	-44.0
C-7	-17.7	-16.4	-13.0	-13.0	-17.2
C-8	-2.6	-2.3	-2.4	-3.0	-1.6
C-9	0.2	0.6	-0.1	-0.5	0.2
C-10	-1.1	-1.2	-1.5	-2.1	-1.0
C-14	-0.3	0.1	-0.3	0	0
C-19	0.2	0.5	0.5	0	-1.0

EXPERIMENTAL

Melting points were determined on a Kofler block. IR spectra were recorded on a UR-20 instrument in the range 700-3600 cm^{-1} in KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 NMR spectrometer at working frequencies 200 and 50.32 MHz, respectively. Chemical shifts on the δ scale were determined relative to TMS internal standard. Details of the experiments have been published [12].

(24R)-24-Ethyl-5α-cholestan-3β,5-diol-6-one (**1**) and (24R)-24-ethylcholest-4-en-3,6-dione (**9**) were prepared by the literature method [16]; (24R,6E)-24-ethyl-5α-cholestan-6-hydroximino-3β,5-diol (**2**), (24R,6E)-5-hydroxy-24-ethyl-5α-cholestan-6-hydroximino-3-one (**4**), and (24R,6E)-24-ethylcholest-6-hydroximino-4-en-3-one (**10**), by the previous method [1]; (24R)-24-ethyl-5β-cholestan-3β,5-diol-6-one (**5**), (24R,6E)-24-ethyl-5β-cholestan-6-hydroximino-3β,5-diol (**6**), and (24R,6E)-5-hydroxy-24-ethyl-5β-cholestan-6-hydroximino-3-one (**8**), by the literature method [2].

(24R)-24-Ethyl-5α-cholestan-5-ol-3,6-dione (3). A solution of **1** (1.12 g) in THF (25 mL) was treated dropwise with stirring with chromic acid (2.0 mL, 8 N). After 15 min the reaction mixture was treated with more chromic acid (0.4 mL, 8 N). After 15 min the excess of oxidant was destroyed by adding 2-propanol (2.5 mL).

The mixture was filtered through a thin layer of aluminum oxide. The solvent was removed in vacua. The solid was crystallized from dioxane:ethanol.

Yield of **3**, 1.01 g (91%), mp 235-237°C, lit. mp 222-225°C [16]. IR spectra of this compound and an authentic sample that was synthesized previously [16] were identical. ^{1}H NMR spectra (C_5D_5N, δ, ppm, J/Hz): 0.67 (3H, s, 18-Me), 0.89 (3H, d, J = 6.0, 26-Me), 0.90 (3H, d, J = 6.0, 27-Me), 0.92 (3H, t, J = 6.0, 29-Me), 1.00 (3H, d, J = 6.0, 21-Me), 1.08 (3H, s, 19-Me), 2.31 (1H, dd, J_1 = 13.0, J_2 = 4.0, H-7β), 2.87 (1H, d, J = 15.0, H-4), 3.08 (1H, t, J = 13.0, H-7α), 3.23 (1H, d, J = 15.0, H-4), 8.12 (1H, s, 5α-OH).

(24R)-24-Ethyl-5β-cholestan-5-ol-3,6-dione (7). A solution of **5** (0.45 g) in THF (20 mL) was treated dropwise with stirring with chromic acid (1.0 mL, 8 N). After 10 min, the reaction mixture was treated with more chromic acid (0.1 mL, 8 N). After 10 min the excess of oxidant was destroyed by adding 2-propanol (1 mL). The mixture was filtered through a thin layer of aluminum oxide. The solvent was removed in vacua. The solid was crystallized from hexane. Yield 0.41 g (92%) of **7**, mp 171-174°C. IR spectrum (ν, cm^{-1}): 3450 (OH), 1730 (C=O).

^{1}H NMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.68 (3H, s, 18-Me), 0.79 (3H, s, 19-Me), 0.80 (3H, d, J = 6.0, 26-Me), 0.81 (3H, d, J = 6.0, 27-Me), 0.84 (3H, t, J = 6.0, 29-Me), 0.93 (3H, d, J = 6.0, 21-Me), 3.03 (1H, d, J = 15.0, H-4), 4.04 (1H, s, 5β-OH).

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