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^{13}C NMR SPECTRA OF FUNCTIONALLY SUBSTITUTED 3β-CHLORODERIVATIVES OF CHOLESTEROL AND β-SITOSTEROL

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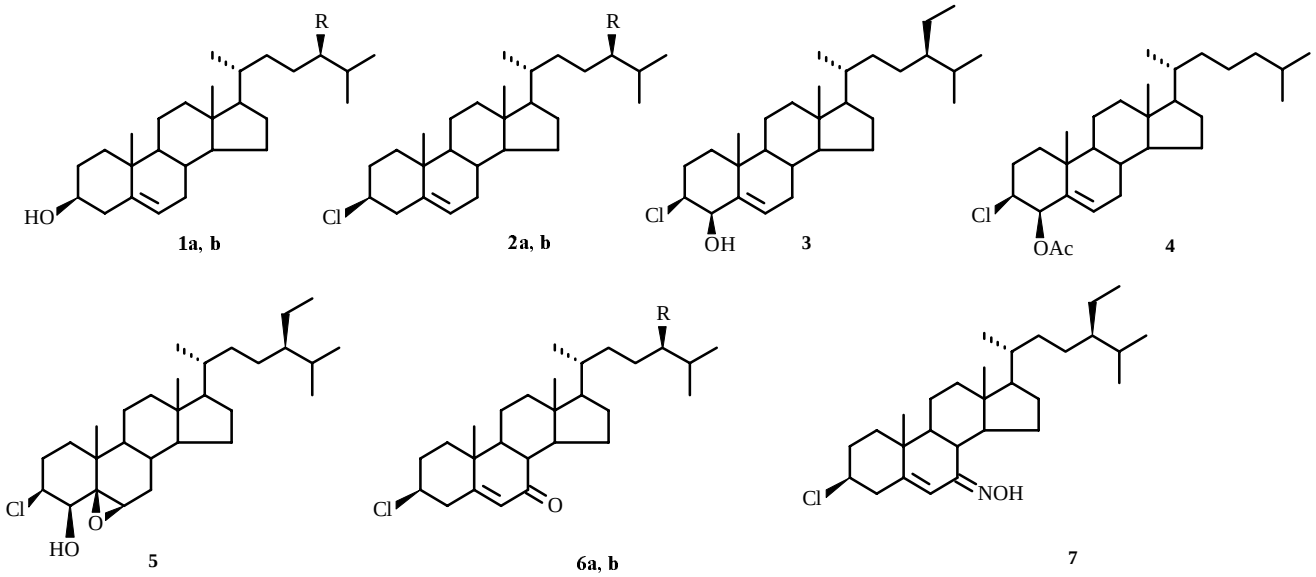
^{13}C NMR spectra were studied and signals were assigned for C atoms of steroids 1-7.

Key words: sterols, 3β-chlorosterols, ^{13}C NMR spectra.

We have previously studied the ^{13}C NMR spectra of several steroids oxidized in rings *A* and *B*, being derivatives of cholesterol (**1a**), β-sitosterol (**1b**), and stigmasterol. In the present article, which is a continuation of our work, we present data for ^{13}C NMR spectra of **2-7**. These compounds are 3β-chloroderivatives of sterols **1a** and **b**. We synthesized them by replacing the 3β-hydroxyls in **1a** and **b** by Cl and then oxidizing the resulting chloroderivatives **2a** and **b**. These compounds are interesting becuase some of them are typical insecticides for larvae of the Colorado beetle [3-6]. Further searches for active insecticides among 3β-chloroderivatives of sterols of more complicated structure depend on reliable methods for proving their structures. In our opinion, ^{13}C NMR spectroscopy is one such method.



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

^{13}C NMR chemical shifts of steroids **2b-7** are listed in Table 1. Analogous data for cholesterol (**1a**) [1, 2], β-sitosterol (**1b**) [1, 2], and 3β-chlorocholest-5-ene (**2a**) [7] are given for comparison. That fact that replacing the 3β-hydroxyl by Cl does not significantly change the chemical shifts of C atoms in rings *B*, *C*, and *D* and the side chains is interesting. In the spectra of **2a** and **b**, the chemical shifts for the C atoms of ring *A* only are different relative to their position in the spectra of the starting **1a** and **b**. Replacing the 3β-hydroxyl in cholesterol (**1a**) and β-sitosterol (**1b**) by Cl to give **2a** and **b** shifts the signal for C-3 in the ^{13}C NMR spectrum to strong field by 11.5-12.4 ppm mainly owing to the α-effect of the halogen. Signals for C-2 in the

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TABLE 1. Chemical Shifts of C Atoms in **1-7** (δ, ppm)

Atom	**1a**	**1b**	**2a[7]**	**2b**	**3**	**4**	**5**	**6a**	**6b**	**7**
C-1	37.2	37.3	39.3	39.2	39.3[a]	38.8	39.8	38.1	38.1	38.5
C-2	31.6	31.6	33.5	33.4	27.1	27.9	26.2	32.8	32.8	33.0
C-3	71.5	71.8	59.1	60.3	65.6	68.1	63.5	57.8	57.7	58.7
C-4	42.2	42.3	43.6	43.4	77.1	77.5	76.8	42.6	42.7	29.7
C-5	140.8	140.8	141.0	140.8	141.5	138.8	76.7	163.9	163.8	152.7
C-6	121.7	121.7	122.4	122.5	129.5	131.5	60.9	126.4	126.2	114.4
C-7	31.8	32.1	31.9	31.8	32.1	32.0	32.6	201.8	201.9	157.6
C-8	31.8	32.1	31.9	31.7	31.3	31.6	29.8	45.5	45.4	38.0
C-9	50.0	50.2	50.3	50.1	50.6	50.2	52.1	49.8	49.8	49.6
C-10	37.0	36.5	36.4	36.4	36.0	35.8	34.9	38.1	38.1	38.3
C-11	21.1	21.1	21.1	21.0	20.9	21.3	21.3	21.2	21.1	20.6
C-12	39.7	39.8	39.9	39.7	39.7[a]	39.4	39.7	38.6	38.6	43.1
C-13	42.2	42.3	42.4	42.3	42.5	42.3	42.2	42.6	43.1	42.8
C-14	56.0	56.8	56.8	56.7	57.0	56.7	56.3	49.8	49.9	49.9
C-15	24.2	24.3	24.3	24.3	24.3	24.2	24.3	26.2	26.1	27.1
C-16	27.9	28.3	28.3	28.3	28.2	27.9	28.2	28.4	28.5	28.3
C-17	56.1	56.1	56.3	56.1	56.2	56.1	56.2	54.8	54.7	54.6
C-18	11.8	12.0	12.0	12.0	12.1	11.8	12.0	12.1	12.0	12.2
C-19	19.4	19.1	19.3	19.3	20.5	20.3	17.9	17.2	17.2	17.8
C-20	35.7	36.2	35.8	36.2	36.2	35.8	36.1	35.6	36.1	36.1
C-21	18.7	18.8	18.9	18.8	18.8	18.7	18.8	18.8	18.9	19.1
C-22	36.0	34.0	36.3	34.0	34.0	36.2	34.0	36.2	33.9	33.9
C-23	23.8	26.2	24.0	26.1	26.2	23.8	26.2	23.8	26.3	26.1
C-24	39.5	45.2	39.6	45.8	46.0	39.5	45.9	39.3	45.8	45.8
C-25	28.2	29.2	28.0	29.1	29.2	28.2	29.3	27.9	29.1	29.1
C-26	22.4	18.9	22.7	19.1	19.1	22.5	19.1	22.5	19.0	19.1
C-27	22.7	19.1	23.0	19.9	19.8	22.8	19.8	22.7	19.8	19.8
C-28		23.1		23.1	23.2		23.2		23.0	23.0
C-29		11.9		11.9	11.9		11.7		12.0	12.0
CH_3CO						20.4				
$CH_3\underline{C}O$						169.9				

[a]Signals may be interchanged. Solvents: $CHCl_3$ (**1a**, **1b**, **4**, **5**, **6a**); CCl_4 (**2a**); $CDCl_3$ (**2b**, **3**, **6b**, **7**).

spectra of **2a** and **b** are shifted to weak field by 1.8-1.9 ppm compared with their positions in the spectra of **1a** and **b** owing to the β-effect of the Cl. Signals for C-4 are analogously shifted to weak field by 1.1-1.4 ppm in the spectra of **2a** and **b** compared with their positions in the spectra of **1a** and **b**. The shift to weak field by 1.9-2.3 ppm for C-1 that is observed in the spectra of **2a** and **b** compared with its position in the spectra of cholesterol and β-sitosterol can be explained by the γ-effect of the Cl.

The ^{13}C NMR spectrum of 3β-chloro-4β-hydroxysteroid **3** can be interpreted more conveniently by comparing it with that of 3β-chlorosteroid **2b**. According to Table 1, introducing an additional hydroxyl into **2b** shifts the signal for C-4 to weak field by 33.5 ppm owing to the α-effect. Simultaneously the 4β-hydroxyl shifts the signal for C-4 to weak field compared with its position in the spectrum of **2b** by 6.5 ppm owing to the β-effect. The signal for C-2 in the spectrum of **3** is observed at δ = 27.1 ppm. Such a shift to strong field by 6.4 ppm (compared with the spectrum of **2b**) is caused by the γ-effect of the 4β-hydroxyl. Of the remaining signals in the spectrum of **3**, the signal of the vinyl C-6 is interesting because it shifts to weak field by 7.0 ppm compared with its position in the spectrum of **2b**. This is caused by the allyl 4β-hydroxyl in **3**.

The chemical shifts of the C atoms in rings *A* and *B* in the ^{13}C NMR spectra of **3** and **4** differ insignificantly. This can be explained by both the different electronegativities of the hydroxy and acetoxy groups (acetylation effect) and the conformations of rings *A* in **3** and **4**. The latter is explained by the different volumes of the substituents on C-4, which experience a 1,3-diaxial interaction with the angular 19-methyl.

Epoxide **5** was prepared by epoxidation of allyl alcohol **3**. ^{13}C NMR spectra of **5** characteristically have C-5 and C-6 signals at 76.7 and 60.9 ppm, respectively. The same position is common for 5,6-epoxides of steroids [7]. Table 1 also shows

that replacing the 5-double bond by epoxy shifts the signals for C-7 and C-9 to weak field by 0.5 and 1.5 ppm, respectively, and the signal for C-8 to strong field by 2.5 ppm. However, the chemical shifts for C atoms of ring *A* change insignificantly.

Allyl oxidation by chromic acid at C-7 to give -Δ^5-7-ketones **6a** and **b** is yet another possible chemical transformation of **2a** and **b**. The presence in these molecules of the 7-ketone conjugated to the 5-double bond causes several characteristic changes in the ^{13}C NMR spectra. This occurs primarily for the position of the signal for C-7, which is observed at weak field at δ = 201.8-201.9 ppm. Signals for C-5 and C-6 appear at 163.8-163.9 ppm and 126.2-126.4 ppm, respectively. This indicates that a double bond conjugated to the 7-ketone is present at the 5- and 6-positions. Furthermore, the presence of the ketone in the position α to C-8 shifts its signal to weak field by ~13.6 ppm compared with this signal in the spectra of the starting **2a** and **b**. It is also interesting that signals for the quaternary C-10 in the spectra of **6a** and **b** shift to weak field by 1.7 ppm compared with their position in the spectra of **2a** and **b**. It should also be noted that the chemical shifts of ring *B* atoms in the spectra of **6a** and **b** are very similar to the corresponding characteristics in the spectrum of (24R)-3β-hydroxystigmast-5-en-7-one, which we studied earlier [2].

The signals for C-4 through C-8 undergo a characteristic shift to strong field compared with their positions in the spectrum of the starting compound in the ^{13}C NMR spectrum of oxime **7**, which was synthesized by the usual method from ketone **6b**.

Thus, ^{13}C NMR spectroscopy can be used effectively to establish the structures of various 3β-chlorosterols.

EXPERIMENTAL

Melting points were determined on a Kofler block. IR spectra were recorded in KBr pellets on a UR-20 instrument. Mass spectra were obtained on a Varian MAT-311 instrument with ionizing-electron energy 70 eV.

^{1}H NMR spectra of 5% solutions in $CDCl_3$ were obtained on a Bruker WM-360 NMR spectrometer at working frequency 360 MHz. Chemical shifts are given relative to TMS internal standard.

^{13}C NMR spectra of **1a** and **b**, **3-5**, and **6a** were recorded on a Bruker WM-360 NMR spectrometer at 90.56 MHz and 0.2 M. Chemical shifts are determined relative to TMS internal standard. The PGD (^{13}C{^{1}H}), GD (^{13}C), and in certain instances CW (off-resonance) regimes were used. The pulse duration was 30 µsec (30°), relaxation delay 1 sec, accumulation time 0.32 sec, number of scans for ^{13}C{^{1}H} 100, for ^{13}C, 1000. The accumulation was carried out at 16 Kb; transformation, 32 Kb of memory. The filter had an exponent with LB = 3 Hz.

^{13}C NMR spectra of **2b**, **6b**, and **7** were recorded on a Bruker AC-200 spectrometer at 50.32 MHz and 40-50 mg of compound in 0.5 mL of solution. Chemical shifts are given relative to TMS internal standard. The parameters are the same for ^{13}C{^{1}H} and ^{13}C except for the suppression regime. The spectral width is 12,195 Hz, relaxation delay 5 sec, recording time 0.67 sec, pulse duration 3.5 µsec (70°), digital resolution 1.5 Hz, filter exponent (LB = 1.5 Hz), number of scans 100 for ^{13}C{^{1}H} and 400 for ^{13}C. The CPD regime was used for suppression of proton coupling.

3β-Chlorocholest-5-ene (2a) and (24R)-chlorostigmast-5-ene (2b) were prepared from cholesterol (**1a**) and β-sitosterol (**1b**) by the literature method [8].

(24R)-3β-Chlorostigmast-5-en-4β-ol (3) was synthesized by allylic oxidation of **2b** by selenium dioxide in dioxane by the literature method [9].

3β-Chlorocholest-5-en-4β-ol acetate (4) was synthesized by the literature method [4].

(24R)-3β-Chloro-5,6β-epoxy-5β-stigmastan-4β-ol (5). A solution of **3** (1.4 g) in CH_2Cl_2 (50 mL) was stirred and treated with trifluoroperacetic acid (prepared by reacting 4.1 mL of 30% H_2O_2 with 5.0 mL of trifluoroacetic anhydride in 20 mL of CH_2Cl_2 with cooling in icewater). The reaction mixture was stirred at room temperature for 0.5 h and diluted with water (70 mL). The organic layer was separated and evaporated under vacuum together with aluminum oxide. The solid was placed on a silica-gel column and chromatographed with elution by a hexane—ether mixture (10:1). Yield 0.14 g of starting steroid **3** and 1.2 g of epoxide **5**.

Yield 92%, mp 127-129°C (hexane). IR spectrum (cm^{-1}): 3520 (OH). ^{1}H NMR spectrum (δ, ppm): 0.63 (18-Me, s), 0.81 (26/27-Me, d, J = 8 Hz), 0.84 (29-Me, J = 7 Hz), 0.89 (21-Me, d, J = 7 Hz), 1.18 (19-Me, s), 3.20 (H-4α, d, J = 1 Hz), 3.99 (H-3α, dt, J_1 = 12 Hz, J_2 = 3 Hz). Mass spectrum (*m*/*z*): 464, 466 (M^+).

3β-Chlorocholest-5-ene-7-one (6a). A solution of **2a** (10.1 g) in acetic acid (200 mL) and $CHCl_3$ (20 mL) was stirred at 50°C and treated with CrO_3 (10.1 g) in acetic acid (50%, 20 mL). The reaction mixture was stirred at 50°C for 2.5 h, cooled

to room temperature, diluted with water (200 mL), and extracted with ether (2×200 mL). The ether extract was treated with CH_3OH (20 mL), washed with water (200 mL), and evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by hexane. Yield of **6a,** 3.78 g, 36%, mp 141-143°C. Found, %: C 77.13, H 10.05, Cl 8.59. Calc. for $C_{27}H_{43}ClO$, %: C 77.38, H 10.34, Cl 8.46. IR spectrum (cm^{-1}): 1670 (C=O), 1635 (C=C). ^1H NMR spectrum (δ, ppm): 0.68 (18-Me, s), 0.86 (26-Me, d, J = 6.6 Hz), 0.87 (27-Me, d, J = 6.6 Hz), 0.92 (21-Me, d, J = 6.6 Hz), 1.22 (19-Me, s), 3.84 (H-3α, m, W/2 = 24 Hz), 5.68 (H-6, s).

(24R)-3β-Chlorostigmast-5-en-7-one (6b). A solution of **2b** (7.0 g) in acetic acid (150 mL) and $CHCl_3$ (30 mL) was stirred at 50°C and treated with CrO_3 (7.0 g) in acetic acid (50%, 14 mL). The reaction mixture was stirred at 50°C for 1.5 h, cooled to room temperature, treated with CH_3OH (10 mL), diluted with water, and extracted with ether. The ether extract was washed with water and evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by a ether—hexane mixture (1:10). Yield of **6b,** 1.6 g, 22%, mp 144.5-146°C (hexane). IR spectrum (cm^{-1}): 1680 (C=O), 1640 (C=C). ^1H NMR spectrum (δ, ppm): 0.69 (18-Me, s), 0.82 (26-Me, d, J = 7 Hz), 0.84 (27-Me, d, J = 8 Hz), 0.85 (29-Me, t, J = 8 Hz), 0.93 (21-Me, d, J = 7 Hz), 1.23 (19-Me, s), 3.88 (H-3α, m, W/2 = 22 Hz), 5.74 (H-6, br. s). Mass spectrum (*m*/*z*): 446, 448 (M^+).

(24R)-3β-Chlorostigmast-5-en-7-one oxime (7). A solution of **6b** (2.5 g) in pyridine (50 mL) was treated with hydroxylamine hydrochloride (2.4 g) and heated until it dissolved. After 66 h the solvent was evaporated under vacuum. The solid was chromatographed on a silica-gel column with elution by a ether—hexane mixture (1:10). Yield of **7,** 2.0 g, 78%, mp 141-143°C (hexane), lit. [10] mp 145°C. IR spectrum (cm^{-1}): 1645 (C=C–C=N–OH). ^1H NMR spectrum (δ, ppm): 0.70 (18-Me, s), 0.82 (26-Me, d, J = 7 Hz), 0.84 (27-Me, d, J = 8 Hz), 0.85 (29-Me, t, J = 8 Hz), 0.95 (21-Me, d, J = 7 Hz), 3.86 (H-3α, m, W/2 = 25 Hz), 6.62 (H-6, s). Mass spectrum (*m*/*z*): 461, 463 (M^+).

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