Chemistry of Natural Compounds, Vol. 35, No. 6, 1999

^{13}C NMR SPECTRA OF STEROL DERIVATIVES, INTERMEDIATES IN THE SYNTHESIS OF ECDY- AND BRASSINOSTEROIDS

N. V. Kovganko, Zh. N. Kashkan, and E. V. Borisov

UDC 547.92

The ^{13}C NMR spectra of a series of steroids used to synthesize ecdy- and brassinosteroids are studied.

^{13}C NMR spectroscopy is widely used to elucidate the structures of various steroids [1]. This method until now has been used in the chemistry of ecdysteroids mainly to determine the structures of natural compounds [2, 3]. Recently the capabilities of ^{13}C NMR spectroscopy in the chemistry of brassinosteroids have been demonstrated [4, 5]. The use of ^{13}C NMR spectra to establish the structures of intermediates in the chemical synthesis of ecdy- and brassinosteroids is very promising. Only isolated examples of this use have been reported in the literature [6-8].

Results from a study of the ^{13}C {^{1}H} NMR spectra of a series of steroids prepared by us earlier during the chemical synthesis of ecdy- and brassinosteroids are reported in the present article. We used mainly data for the chemical shifts and multiplicities determined from ^{13}C NMR spectra to assign signals to resonances of specific C atoms. Comparison of the analyzed spectra with those of compounds with similar structures was very helpful.

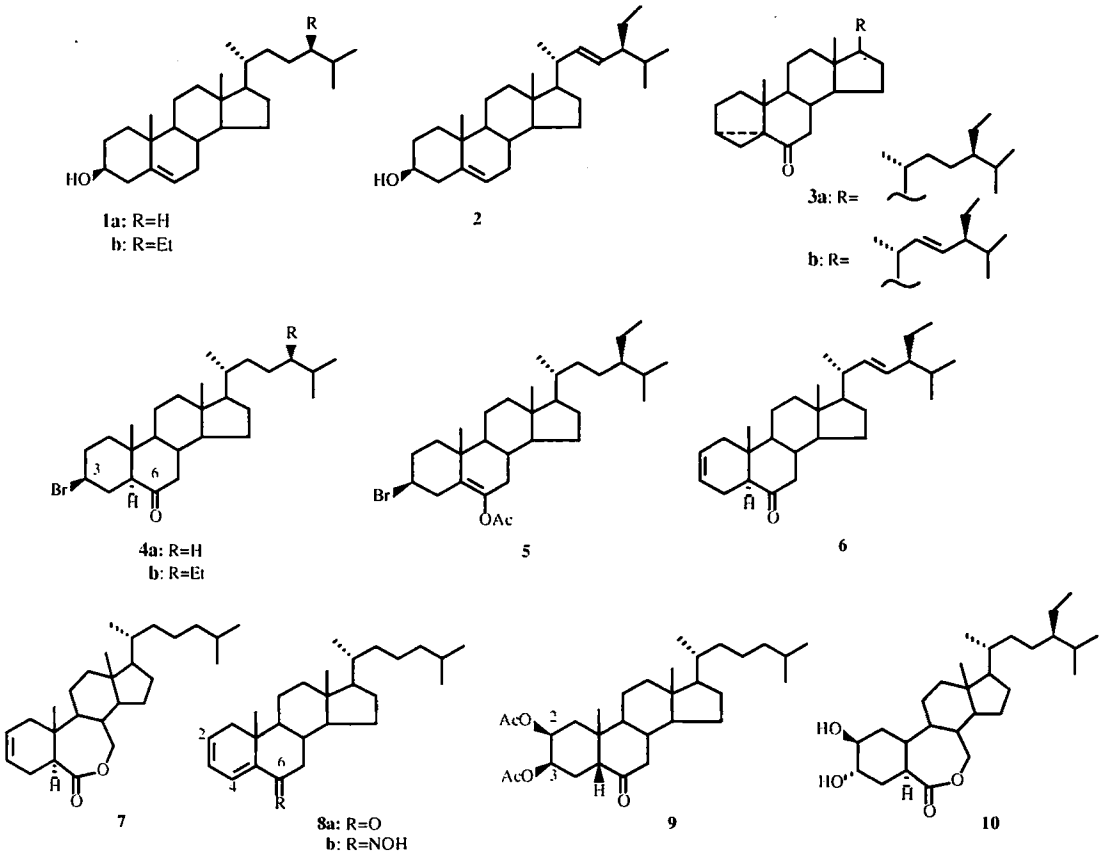
The starting compounds for the syntheses were cholesterol (**1a**), β-sitosterol (**1b**), and stigmasterol (**2**). Therefore, we began the study by assigning the signals in the spectra of these compounds. The results are listed in Table 1. It should be noted that these spectra agree well with those reported in the literature for cholesterol [1, 9, 10], β-sitosterol [10-12], and stigmasterol [10, 13] respectively. Data from the ^{13}C {^{1}H} NMR spectra of compounds **1a**, **1b**, and **2** significantly simplified the assignment of signals in the spectra of **3-10** (Table 1). For this we compared chemical shifts of C atoms in rings *C* and *D* and the side chains of the studied steroid with the analogous values of the corresponding sterol from which the compound was prepared. Thus, the ^{13}C {^{1}H} NMR spectrum of 3α,5-cyclo-6-ketosteroid **3a** was compared with that of β-sitosterol **1b**, from which it was synthesized [14]. Table 1 shows that the chemical shifts of atoms in rings *C* and *D* and the side chains of these compounds are practically identical. However, signals for C atoms in rings *A* and *B* of steroid **3a** were assigned by comparing them with the corresponding reported values for 3α,5-cyclo-5α-cholestan-6-one [6].

Signals of all C atoms in the ^{13}C {^{1}H} NMR spectrum of 3α,5-cyclo-6-ketone **3b** [15, 16] could be assigned analogously by comparing them with those in the spectra of stigmasterol (**2**) and 3α,5-cyclo-5α-cholestan-6-one [6]. A number of conclusions about the effect of the three-membered ring in compounds **3a** and **3b** on the chemical shifts of C atoms situated close to it can be made on the basis of the data in Table 1. The significant upfield shift of the signals for C-3, C-4, and C-5 is particularly interesting. The signal for C-4 is especially strongly shifted (to 11.7 ppm).

We also studied the ^{13}C {^{1}H} NMR spectra of 3β-bromo-6-ketosteroids **4a** and **4b**, which have been previously synthesized [15-18]. Signals in the spectrum of compound **4a** were assigned by comparing them with analogous values for 3β-hydroxy-5α-cholestan-6-one [1]. Chemical shifts of the C atoms in rings *B*, *C*, and *D* and the side chains agree well in the spectra of both compounds. The differences in the chemical shifts of C atoms in ring *A* of these compounds can be explained by considering the different electronegativity of the Br atom and the O atom of the hydroxy group.

Institute of Bioorganic Chemistry of the National Academy of Sciences of Belarus, 220141, Belarus, Minsk, ul. Acad. Kuprevicha, 5/2. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 751-756, November-December, 1999. Original article submitted June 21, 1999.

 0009-3130/99/3506-0642$22.00



The spectrum of 3β-bromo-6-ketone **4b**, a derivative of β-sitosterol, was analyzed by comparing it with that of compound **4a** taking into account changes in the chemical shifts of C atoms in the side chain that are caused by the presence of the 24-ethyl group. The enolacetate **5** was prepared from the 6-ketosteroid **4b** [14]. Ring *A* in these compounds has the same structure. Therefore, as can be seen from Table 1, the chemical shifts of C-1, C-2, C-3, and C-4 in the spectra of compounds **4b** and **5** are similar in principle. Signals of C atoms in ring *B* can be assigned by comparing the spectrum of enolacetate **4b** with that of compound **1a**. Naturally, the presence of the 6-acetoxy group in the former should be taken into account. This produces a downfield shift for the signals of C-5, C-6, and C-7 in enolacetate **4b** compared with their positions in the spectrum of compound **1a**.

Unsaturated 6-ketosteroids are widely used in the synthesis of ecdy- and brassinosteroids. This type of compounds includes the $\Delta^{2,22}$-6-ketone **6** [15, 16]. The $^{13}C\{^1H\}$ NMR spectrum (Table 1) enables the presence of the principal structural elements [6-ketone, 2(3)- and 22(23)-double bonds] in this molecule to be reliably proved. Signals for atoms in rings *B*, *C*, and *D* of steroid **6** were assigned by comparing them with analogous signals in the spectra of 6-ketones **4a** and **4b**. Chemical shifts of C atoms in the side chain of steroid **6** were determined by comparing them with the analogous values in the spectrum of stigmasterol (**2**). Comparison of the spectra of steroid **6** and 5α-cholest-2-ene [1] enabled the chemical shifts of the C atoms in ring *A* to be found.

Unsaturated lactone **7** [18] is formally a derivative of Δ^2-6-ketosteroids of type **6**. An additional O atom is present between C-6 and C-7. For this reason, the $^{13}C\{^1H\}$ NMR spectrum of lactone **7** may be simpler to interpret by comparing it with that of Δ^2-6-ketosteroid **6** taking into account the effect of the additional O atom. Spectra of the 6-ketosteroids and the corresponding lactones were analyzed by comparing them with those of brassinosteroids [5].

We used literature data [5] to assign signals in the spectrum of Δ^2-lactone **7**. Comparison with the data in Table 1 showed that converting the 6-ketone to the corresponding lactone produces several changes in the $^{13}C\{^1H\}$ spectrum. The chemical shift of C-6 (176.4 ppm) draws attention. This value is typical of lactones. Furthermore, introducing the O atom between C-6 and C-7 causes a downfield shift for the signals of C-7, C-8, C-9, and C-14.

TABLE 1. ^{13}C {^{1}H} NMR Spectra (δ, ppm) for Compounds **1-10**

Atom	1a	1b	2	3a	3b	4a	4b	5	6	7	8a	8b	9	10
C-1	37.2	37.3	37.5	33.5	33.5	39.2	39.3	40.3	39.4	39.4	32.9	29.4	37.4	41.9
C-2	31.6	31.6	32.0	25.9	26.0	33.4	33.5	33.5	124.6	123.4	128.4	123.4	67.6	70.8
C-3	71.5	71.8	71.8	34.8	34.8	59.0	59.1	51.0	125.0	123.5	123.3	119.5	68.2	70.2
C-4	42.2	42.3	42.8	11.7	11.7	32.4	32.4	34.0	21.8	25.9	132.8	127.2	34.5	34.0
C-5	140.8	140.8	141.3	46.3	46.3	50.6	50.6	141.7	53.5	44.2	140.8	138.6	40.6	40.0
C-6	121.7	121.7	122.0	209.7	209.6	209.6	209.4	127.9	212.1	176.4	200.0	157.0	213.6	176.9
C-7	31.8	32.1	32.3	44.8	44.8	46.6	46.6	34.5	47.1	70.4	45.5	37.6	46.3	70.8
C-8	31.8	32.1	32.3	35.3	35.3	37.8	37.9	31.9	37.7	39.8	37.9	33.1	37.1	39.2
C-9	50.0	50.2	50.6	46.1	46.2	53.9	54.0	49.8	53.9	59.1	50.4	52.0	54.3	58.3
C-10	37.0	36.5	36.8	46.7	46.7	40.7	40.7	36.6	40.1	41.3	36.0	36.8	41.0	40.4
C-11	21.1	21.1	21.2	22.9	22.9	21.3	21.4	21.0	21.2	22.5	21.3	21.4	21.0	22.5
C-12	39.7	39.8	40.0	39.8	39.7	39.5	39.5	39.6	39.4	39.7	39.6	39.6	39.5	40.0
C-13	42.2	42.3	42.8	42.7	42.7	43.0	43.0	42.3	42.7	42.6	42.5	42.7	42.9	41.9
C-14	56.0	56.8	57.4	57.0	57.1	56.7	56.8	56.4	56.9	51.5	56.7	56.4	56.8	51.7
C-15	24.2	24.3	24.5	24.1	24.2	23.9	24.0	24.2	24.1	24.9	24.1	24.2	23.9	24.8
C-16	27.9	28.3	29.1	28.3	28.9	28.0	28.0	28.2	28.8	27.9	28.0	28.2	28.1	28.1
C-17	56.1	56.1	56.5	56.0	56.0	56.2	56.1	56.1	56.0	56.0	56.1	56.0	56.2	56.2
C-18	11.8	12.0	12.3	12.0	12.3	12.0	12.0	12.0	12.2	11.8	11.9	11.9	11.9	12.1
C-19	19.4	19.1	19.6	19.7	19.7	13.1	13.1	19.4	13.5	16.3	17.0	16.8	23.5	17.9
C-20	35.7	36.2	40.8	36.2	40.5	35.7	36.1	36.2	40.4	35.7	35.8	35.7	35.7	36.2
C-21	18.7	18.8	21.3	18.7	21.1	18.6	18.8	18.8	21.2	18.6	18.8	18.7	18.6	18.7
C-22	36.0	34.0	138.8	33.9	138.1	36.1	33.9	34.0	138.0	36.0	36.2	36.0	36.1	34.0
C-23	23.8	26.2	129.8	26.1	129.6	23.8	26.2	26.3	129.6	23.8	23.9	23.9	23.8	26.3
C-24	39.5	45.2	51.6	45.8	51.3	39.5	45.9	45.9	51.3	39.8	39.5	39.5	39.5	46.2
C-25	28.2	29.2	32.3	29.3	31.9	28.0	29.3	29.3	31.9	28.0	28.0	28.0	28.1	29.4
C-26	22.4	18.9	21.4	19.0	21.3	22.5	19.1	19.1	21.2	22.5	22.6	22.6	22.5	19.0
C-27	22.7	19.1	19.3	19.8	18.4	22.8	19.8	19.8	19.0	22.8	22.8	22.8	22.8	19.8
C-28		23.1	25.6	23.1	25.5		23.2	23.2	25.4					23.3
C-29		11.9	12.4	12.0	12.3		12.0	11.8	12.2					12.1
CH_3CO								20.8					21.2	
													21.6	
CH_3CO								168.8					170.1	
													170.2	

Cholesta-2,4-dien-6-one (**8a**) has two double bonds conjugated to the 6-ketone [19]. Therefore, signals of four vinylic C atoms and the carbonyl C-6 (200.0 ppm) appear in the ^{13}C {^{1}H} NMR spectrum of compound **8a**. They were assigned (Table 1) by analyzing spectra of analogous conjugated ketosteroids [1]. Analogous signals are also present in the spectrum of the oxime (**8b**), which was prepared as usual from cholesta-2,4-dien-6-one. The significant upfield shift in the spectrum of oxime **8b** for the signal of C-6 and the vinylic C atoms compared with the positions of these same signals in the spectrum of 2,4-dien-6-ketone **8a** is interesting. This shift is surely caused by the smaller electronegativity of the oxime N atom compared with the ketone O atom. It should be noted that the signals of atoms in rings *C* and *D* and the side chain of compounds **8a** and **8b** can be assigned with little difficulty. Table 1 shows that they agree well with the analogous values in the spectrum of cholesterol.

One peculiarity in the structure of 2β,3β-diacetoxy-6-ketone (**9**) is the presence of *cis-A/B* fusion. This causes a significant downfield shift (to 23.5 ppm) for the signal of C-19 in the ^{13}C {^{1}H} spectrum. We note that this effect was observed earlier in a series of *cis-A/B*-6-ketosteroids [1, 6], including ecdysteroids [2, 3]. This shift is considered to be due to steric interaction of the 19-methyl group with the H atom in the 5β position. Individual signals in the spectrum of compound **9** were assigned mainly by comparing them with analogous signals in the spectrum of (22S,23S)-2β,3β,22,23-tetrahydroxy-5β-stigmastan-6-one [8]. Naturally we took into account the changes in the chemical shifts caused by the presence of the 2β- and 3β-acetoxy groups in molecule of steroid **9**.

The main features of the ^{13}C {^{1}H} NMR spectrum of 2β,3α-dihydroxylactone **10** [14] agree in principle with the analogous features in the spectrum of Δ^2-lactone **7** (Table 1). The chemical shifts of C-2 and C-3, which are vinylic in compound **7** and carbinols in compound **10**, show the greatest differences. Furthermore, the presence of the 2,3-diol in steroid **10** causes the signals of C-1 and C-4 to shift to downfield owing to the β-effect.

The authors thank V. N. Zhabinskii, T. N. Netesova, and E. V. Batura for help with the study.

EXPERIMENTAL

All ^{13}C NMR spectra were obtained from $CDCl_3$ (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 TMS internal standard. We used PGD (^{13}C {^{1}H}), 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, and number of scans ~100 for ^{13}C {^{1}H} and ~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.

REFERENCES

1. J. W. Blunt and J. B. Stothers, *Org. Magn. Reson.*, **9**, 439 (1977).
2. A. A. Akhrem and N. V. Kovganko, *Ecdysteroids: Chemistry and Biological Activity* [in Russian], Nauka i Tekhnika, Minsk (1989), p. 327.
3. R. Lafont and I. Wilson, *The Ecdysone Handbook*, 2nd Ed., The Chromatographic Society, Nottingham (1996).
4. A. Porzel, V. Marquardt, G. Adam, G. Massiot, and D. Zeigan, *Magn. Reson. Chem.*, **30**, 651 (1992).
5. T. Ando, M. Aburatani, N. Koseki, S. Asakawa, T. Mouri, and H. Abe, *Magn. Reson. Chem.*, **31**, 94 (1993).
6. W. B. Smith, *Org. Magn. Reson.*, **9**, 644 (1977).
7. F. Werner, *Synthese de molecules hybrides brassinosteroides - ecdysteroides, inhibiteurs potentiels du mode d'action des ecdysteroides*, Doctoral Thesis, Strasbourg (1996).
8. C. Brosa, S. Nusimovich, and R. Peracoula, *Steroids*, **59**, 463 (1994).
9. G. Popjak, J. Edmond, F. A. L. Anet, and N. R. Easton, Jr., *J. Am. Chem. Soc.*, **99**, 931 (1977).
10. J. L. C. Wright, A. G. McInnes, S. Shimizu, D. G. Smith, J. A. Walter, D. Inler, and W. Khalil, *Can. J. Chem.*, **56**, 1898 (1978).
11. S. Seo, U. Sankawa, H. Seto, A. Uomori, Y. Yoshimura, Y. Ebizuka, H. Noguchi, and K. Takeda, *J. Chem. Soc., Chem. Commun.*, 1139 (1986).
12. S. Seo, A. Uomori, Y. Yoshimura, K. Takeda, H. Seto, Y. Ebizuka, H. Noguchi, and U. Sankawa, *J. Chem. Soc., Perkin Trans. 1*, 2407 (1988).
13. M. Tandon, Y. N. Shukla, and R. S. Thakur, *Phytochemistry*, **29**, 2957 (1990).
14. N. V. Kovganko and Zh. N. Kashkan, *Zh. Org. Khim.*, **26**, 2545 (1990).
15. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, V. N. Zhabinskii, and N. V. Kovganko, *Dokl. Akad. Nauk SSSR*, **275**, 1089 (1984).
16. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, N. V. Kovganko, and V. N. Zhabinskii, *Zh. Org. Khim.*, **23**, 762 (1987).
17. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, and N. V. Kovganko, *Dokl. Akad. Nauk BSSR*, **25**, 615 (1981).
18. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, and N. V. Kovganko, *Dokl. Akad. Nauk SSSR*, **269**, 366 (1983).
19. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, and N. V. Kovganko, *Vestsi Akad. Navuk BSSR, Ser. Khim. Navuk*, **2**, 65 (1983).