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# $^{13}$C NMR OF 2,3,14-TRISUBSTITUTED $\Delta^{4,7}$-6-KETOSTEROIDS AND RELATED COMPOUNDS

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*$^{13}$C NMR spectra have been studied and signals of C atoms assigned for steroids **3-5** and **7-10**, which are close structural analogs of the natural ecdysteroids 4-dehydroecdysterone **1**, diaulusterol A **2**, and 5$\alpha$,20-dihydroxyecdysone **6**.*

**Key words:** $\Delta^{4,7}$-6-ketosteroids, $^{13}$C NMR spectra.

Natural ecdysteroids include compounds that have a cyclic portion and typical functional groups: 2,3-diol, 14$\alpha$-hydroxy-$\Delta^7$-6-ketone, and additional $\Delta^4$-bonds. These compounds include 4-dehydroecdysterone (**1**) [1], diaulusterol A (**2**) [2], and phytoecdysteroids of the alga *Laurentia pinnata*, for example, acetylpinnasterol [3-4]. We developed [5-6] chemical syntheses of steroids containing a 2$\beta$,3$\beta$,14$\alpha$-trihydroxy-$\Delta^{4,7}$-6-ketone that are related to natrual ecdysteroids **1** and **2**. Several spectral methods, including of course $^{13}$C NMR, were required to establish the structures of these complicated compounds.

The present article reports $^{13}$C NMR spectra of 2,3-disubstituted $\Delta^{4,7}$-6-ketosteroids that we synthesized previously [5, 6] and of the starting materials, i.e., derivatives of 2,3,5-trihydroxy-$\Delta^7$-6-ketosteroids and structurally related compounds. This is a continuation of research on the $^{13}$C NMR spectra of steroids prepared via chemical synthesis [7-9]. The main goal of our research is to find reliable criteria for establishing the structures of this type of steroids using $^{13}$C NMR spectra.

Table 1 presents data for the $^{13}$C NMR of the studied steroids. Signals in the $^{13}$C NMR spectra were assigned to resonances of particular atoms in the steroids using mainly chemical shifts and multiplicities determined using the DEPT method. It should be noted that all discussed compounds were prepared from cholesterol and $\beta$-sitosterol. Therefore, signals of C atoms in rings *C* and *D* and side chains were assigned by comparing their spectra with those of the corresponding cholestane and stigmastane steroids with the same structural fragments that we studied previously [7-9]. Comparison of the analyzed spectra with those in the literature for structurally similar compounds, 4-dehydroecdysterone (**1**) [1], diaulusterol A (**2**) [2], and 5$\alpha$,20-dihydroxyecdysone (**6**) [10], the data for which appear in Table 1, was very helpful in identifying the signals of C atoms in rings *A* and *B*.

The $^{13}$C NMR spectra of **1-10** have certain common characteristic features, which is explained by the fact that they have the same structural elements: $\Delta^7$-6-ketones and oxygen-containing substituents on C-2 and C-3. The latter feature causes the signals for C-2 and C-3 to be located usually at weak field with $\delta$ 65-70 ppm. The presence of the $\Delta^7$-6-ketone in **1-10** results in the C atoms having chemical shifts $\delta$ 190-200 (C-6), 120-125 (C-7), and 160-170 ppm (C-8).

Table 1 shows that the presence in **1-5** of an additional 4(5)-double bond is easily proven using the positions of the signals for C-4 ($\delta$ 125-130 ppm) and C-5 ($\delta$ 146-148 ppm). These signals are well resolved in the spectra and are easily assigned. The presence of signals for C-6 (189-193 ppm), C-7 (123-125 ppm), and C-8 (160-170 ppm) proves that the molecules contain a $\Delta^7$-6-ketone. Comparison of the spectra of **1-5** with those in the literature [1, 11-13] for typical ecdysteroids that have, as a rule, a 14$\alpha$-hydroxy-$\Delta^7$-6-ketone and *cis-A/B*-fusion, is useful. Thus, it can be concluded by comparison with the published [1] spectra of 4-dehydroecdysterone (**1**) and 20-hydroxyecdysone that conjugation of the $\Delta^7$-6-ketone and the $\Delta^4$-bond shifts the signal of C-6 to strong field by 13.6 ppm; the signals of C-7 and C-8, to weak field by 2.3 and 1.6 ppm, respectively. The signal for allylic C-9 (43.5-47.8 ppm depending on the substituent on C-14) is characteristic of $\Delta^{4,7}$-6-ketosteroids **1-5**. Considering that this signal in spectra of ecdysteroids that do not have a 4(5)-double bond usually appears

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

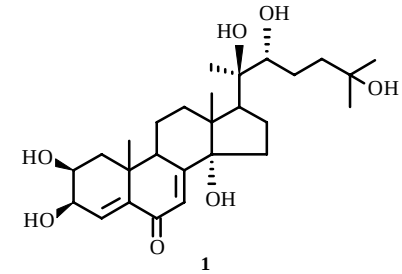
| Atom | **1** [1] | **2** [2] | **3** | **4a** | **4b** | **4c** | **5** | **6** [10] | **7a** | **7b** | **8** | **9** | **10a** | **10b** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C-1 | 38.1 | | 37.6 | 35.4 | 35.2 | 35.7 | 35.7 | 35.8 | 27.7 | 27.7 | 30.4 | 33.7 | 122.8 | 121.6 |
| C-2 | 68.2 | 66.7 | 67.2 | 67.2 | 67.1 | 67.8 | 67.8 | 69.6 | 68.9 | 68.9 | 68.2 | 82.3 | 67.6 | 67.0 |
| C-3 | 67.5 | 64.4 | 66.7 | 66.8 | 66.7 | 66.8 | 67.1 | 67.7 | 69.4 | 69.4 | 69.0 | 76.9 | 68.6 | 67.2 |
| C-4 | 130.2 | 128.1 | 129.2 | 124.7 | 127.5 | 127.2 | 124.6 | 34.4 | 34.8 | 34.7 | 31.6 | 39.0 | 32.6 | 31.8 |
| C-5 | 146.7 | 146.2 | 147.5 | 146.4 | 146.5 | 146.0 | 147.7 | 79.6 | 77.3 | 77.1 | 75.6 | 87.9 | 48.5 | 47.7 |
| C-6 | 192.8 | 188.7 | 192.1 | 189.4 | 189.0 | 188.7 | 189.4 | 200.9 | 197.6 | 197.7 | 196.2 | 192.0 | 201.0 | 200.3 |
| C-7 | 124.4 | 123.6 | 123.5 | 123.6 | 124.6 | 125.0 | 124.3 | 119.7 | 119.8 | 119.7 | 119.9 | 121.9 | 119.8 | 119.8 |
| C-8 | 169.5 | 166.9 | 168.8 | 166.4 | 162.2 | 160.7 | 167.2 | 166.7 | 165.1 | 164.9 | 165.0 | 167.0 | 163.8 | 163.6 |
| C-9 | 44.6 | | 44.0 | 43.7 | 43.5 | 43.8 | 47.8 | 38.1 | 44.4 | 44.2 | 44.2 | 44.7 | 48.1 | 71.9 |
| C-10 | 40.3 | | 39.3 | 38.8 | 39.0 | 38.7 | 39.2 | 44.6 | 40.2 | 40.2 | 43.0 | 47.8 | 146.5 | 150.4 |
| C-11 | 21.8 | | 21.0 | 20.6 | 20.7 | 20.6 | 23.7 | 21.8 | 21.9 | 21.8 | 21.7 | 21.9 | 33.1 | 35.8 |
| C-12 | 32.3 | | 30.8 | 30.4 | 29.8 | 29.8 | 39.5 | 31.3 | 38.8 | 38.8 | 38.9 | 38.8 | 40.7 | 37.0 |
| C-13 | 48.6 | | 46.9 | 46.6 | 48.0 | 48.9 | 45.4 | 47.9 | 44.9 | 44.8 | 45.0 | 44.9 | 48.7 | 48.2 |
| C-14 | 85.2 | | 84.8 | 84.9 | 96.4 | 98.2 | 56.8 | 83.9 | 55.6 | 55.6 | 55.8 | 56.0 | 57.0 | 50.1 |
| C-15 | 31.8 | | 31.4 | 31.6 | 24.6 | 25.7 | 22.6 | 31.8 | 22.5 | 22.5 | 22.5 | 22.5 | 22.0 | 21.4 |
| C-16 | 21.4 | | 26.9 | 26.5 | 26.6 | 26.7 | 28.2 | 21.4 | 28.0 | 28.0 | 27.7 | 27.7 | 28.6 | 27.9 |
| C-17 | 50.6 | | 50.9 | 50.5 | 50.9 | 50.9 | 56.8 | 49.8 | 56.3 | 56.3 | 56.3 | 56.0 | 56.6 | 56.2 |
| C-18 | 18.0 | 12.5 | 16.2 | 15.8 | 16.6 | 16.8 | 13.1 | 17.6 | 12.5 | 12.4 | 12.5 | 12.5 | 13.5 | 12.3 |
| C-19 | 22.6 | 21.1 | 22.4 | 22.1 | 22.1 | 22.0 | 23.1 | 16.9 | 18.8 | 18.8 | 17.0 | 15.9 | 30.4 | 29.2 |
| C-20 | 78.0 | | 36.3 | 36.3 | 35.9 | 36.0 | 36.9 | 76.8 | 36.0 | 36.4 | 36.0 | 36.3 | 36.9 | 36.3 |
| C-21 | 21.0 | 18.7 | 20.0 | 20.2 | 19.9 | 17.6 | 19.6 | 21.1 | 18.7 | 18.8 | 18.8 | 18.9 | 17.7 | 18.8 |
| C-22 | 78.4 | | 34.4 | 34.0 | 34.0 | 33.9 | 34.3 | 77.4 | 36.0 | 33.7 | 36.0 | 33.7 | 34.4 | 33.8 |
| C-23 | 27.3 | | 26.7 | 26.4 | 26.4 | 26.5a | 26.8 | 27.2 | 23.9 | 26.2 | 23.9 | 26.2 | 26.8 | 26.1 |
| C-24 | 42.4 | | 46.2 | 45.8 | 45.8 | 45.8 | 46.4 | 42.3 | 39.4 | 45.7 | 39.4 | 45.8 | 46.5 | 45.9 |
| C-25 | 71.3 | 83.6 | 29.5 | 29.2 | 29.2 | 29.2 | 29.8 | 69.6 | 28.0 | 29.1 | 28.0 | 29.2 | 29.8 | 29.8 |
| C-26 | 28.9 | 26.1 | 19.2 | 19.0 | 19.0 | 19.0 | 19.5 | 29.6 | 22.5 | 19.0 | 22.5 | 19.0 | 19.5 | 19.0 |
| C-27 | 29.7 | 26.2 | 19.2 | 19.9 | 19.0 | 19.9 | 20.4 | 29.8 | 22.8 | 19.8 | 22.8 | 19.8 | 20.5 | 19.8 |
| C-28 | | 172.4 | 23.4 | 23.1 | 23.1 | 23.1 | 23.2 | | | 23.0 | | 23.1 | 23.7 | 23.1 |
| C-29 | | 42.1 | 12.1 | 12.0 | 12.0 | 12.0 | 12.6 | | | 11.9 | | 12.0 | 12.6 | 12.0 |
| C-30 | | 65.2 | | | | | | | | | | | | |
| C-31 | | 22.3 | | | | | | | | | | | | |
| $\underline{C}H_3CO$ | | | | 20.9 | 20.8 | 20.9 | 21.5 | | | 21.0 | 20.9 | 20.2 | 21.7 | 21.0 |
| | | | | 21.1 | 21.1 | 20.9 | 21.7 | | | 21.2 | 21.1 | | 21.7 | 21.0 |
| | | | | | | 21.1 | | | | | | | | |
| $CH_3\underline{C}O$ | | | | 170.3 | 170.6 | 167.7 | 170.9 | | 170.3 | 170.2 | 168.9 | 171.1 | 170.8 | 170.8 |
| | | | | 170.4 | 170.7 | 170.3 | 171.0 | | 170.7 | 170.6 | 170.2 | | 171.2 | 171.2 |
| | | | | | | 170.3 | | | | | | | | |
| Solvent | $CD_3OD$ | $CDCl_3$ | $CDCl_3$-$CD_3OD$ (2:1) | $CDCl_3$ | $CDCl_3$ | $CDCl_3$ | $CDCl_3$ | $C_5D_5N$ | $CDCl_3$ | $CDCl_3$ | $CDCl_3$ | $CDCl_3$ | $CDCl_3$ | $CDCl_3$ |

at 34-35 ppm, the presence of such a large shift is useful for proving the structure of $\Delta^{4,7}$-6-ketosteroids.

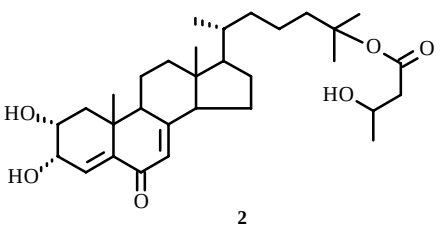
Table 1 shows that the presence on C-2 and C-3 of hydroxy or acetoxy groups causes the $^{13}C$ NMR signals of these atoms to appear at 64-69 ppm. A comparison of the spectra of a 2$\alpha$,3$\alpha$-diol (**2**) and a 2$\beta$,3$\beta$-diol (**3**) indicates that the C-2 and C-3 signals in the first instance occur at stronger field. However, in our opinion, these differences are not significant enough to use them to determine the stereochemistry of 2,3-diols from $^{13}C$ NMR spectra.

TABLE 2. Effects of C-14 Substituents (δ, ppm) on Chemical Shifts of Neighboring C Atoms in $^{13}C$ NMR Spectra of **4a-c**

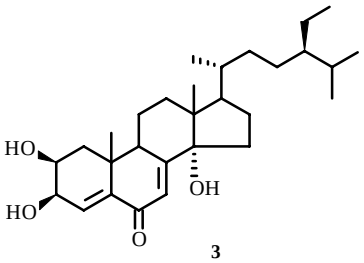
| Substituent | α-Effect | β-Effect | | | γ-Effect | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | C-14 | C-8 | C-13 | C-15 | C-7 | C-9 | C-12 | C-16 | C-17 | C-18 |
| α-OH | +28.1 | -0.8 | +1.2 | +9.0 | -0.7 | -4.1 | -8.1 | -1.7 | -6.3 | +2.7 |
| α-OOH | +39.6 | -5.0 | +2.6 | +2.0 | +0.3 | -4.3 | -10.3 | -1.6 | -5.9 | +3.5 |
| α-OAc | +41.4 | -6.5 | +3.5 | +3.1 | +0.7 | -4.0 | -9.7 | -1.5 | -5.9 | +3.7 |



**1**



**2**



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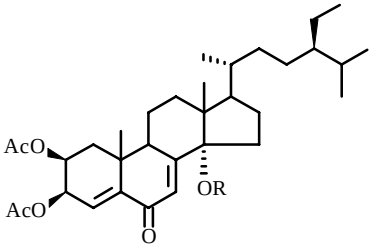
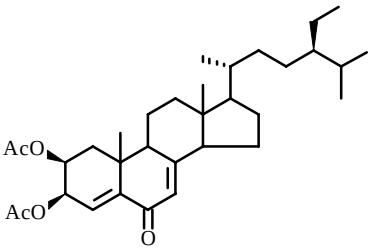


**4a:** R = H
**b:** R = OH
**c:** R = Ac



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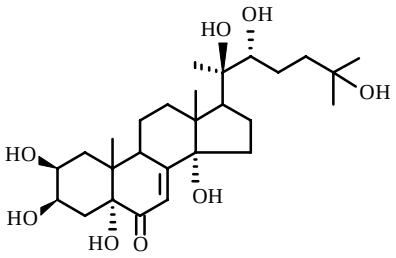
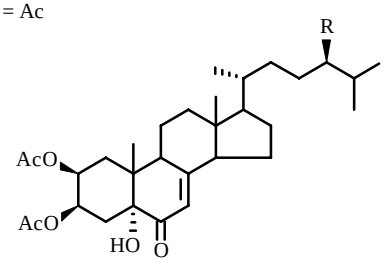
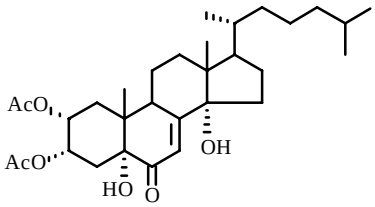


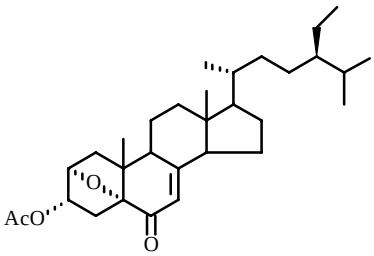
**6**



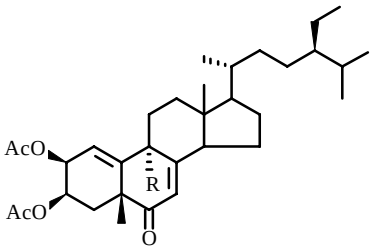
**7a:** R = H
**b:** R = Et



**8**



**9**



**10a:** R = H
**b:** R = OH

Proving the presence of a 14α-hydroxy is an important problem that constantly arises in elucidating the structure of ecdysteroids and related compounds. The presence of a 14α-hydroxy in $\Delta^7$-6-ketosteroids can be confirmed using $^1H$ NMR spectra only by indirect means, for example, by a shift to weak field of the H-9α signal [11]. In this respect, $^{13}C$ NMR spectra have definite advantages. $^{13}C$ NMR spectra can be used to establish the presence in the 14-position of not only hydroxyls but also other electronegative substituents, for example, hydroperoxide or acetate groups. These functional groups cause

characteristic changes in the chemical shifts of nearby C atoms. Such changes are considered $\alpha$-, $\beta$-, and $\gamma$-effects of the substituents in $^{13}$C NMR spectroscopy [14]. The $\alpha$- and $\beta$-effects are most important for proving the structure. The effects of C-14 substituents in $\Delta^{4,7}$-6-ketosteroids were determined quantitatively by comparing the main spectral parameters of 14-substituted steroids **4a-c** with those of **5**, which has no such substituent. Table 2 gives the results and shows that, as expected, C-14 experiences the greatest shift to weak field from the $\alpha$-effect. The chemical shift of C-14 in the spectrum of **4b** (96.4 ppm) agrees with the published value [15] for a compound of analogous structure. The shift to weak field by 13.3 ppm for C-14 in the spectrum of **4c** compared with the signal in the spectrum of **4a** (so-called acetylation effect) is most probably caused by the axial orientation of the acetoxy group [14]. Further analysis of Table 1 reveals a shift to strong field of the signals for C-9, C-12, and C-17 in **4a-c** compared with their positions in the spectrum of **5**. This is certainly caused by a $\gamma$-*gauche*-effect of the C-14 substituents. The presence (or absence) of this shift can be used to establish the structure of new compounds of this type because the signals of these atoms are easily identified in the $^{13}$C NMR spectrum.

The main parameters in the $^{13}$C NMR spectra of 5$\alpha$-hydroxy-$\Delta^7$-6-ketosteroids **7-8** are in general similar to those of 5$\alpha$,20-dihydroxyecdysone **6** [10] and 2,3,5-trihydroxy-6-ketosteroids [16]. The C-1 and C-4 signals were somewhat difficult to assign.

In our opinion, the C-4 signal, which has two neighboring hydroxyls, should be located at weaker field than the C-1 signal. This can be seen in Table 1. A characteristic feature of the spectra of **7** and **8** that confirms they contain a 5$\alpha$-hydroxyl is the presence of signals of quaternary C-5 at weak field (75-77 ppm). The C-5 signal of the 2$\alpha$,3$\alpha$-diacetoxysteroid **8** is observed at stronger field compared with the position of such signals in the spectra of 2$\beta$,3$\beta$-diacetoxysteroids **7a-b**. This same phenomenon caused by a $\gamma$-*gauche*-effect of an axial 3$\alpha$-substituent is observed in the spectra of brassinosteroids [16, 17]. This makes it possible to determine the configuration of C-3, of course if the *A/B* fusion is known, from the value of the C-5 chemical shift.

Electronegative substituents occur on C-2, C-3, and C-5 in 2$\alpha$,5$\alpha$-epoxy-3$\alpha$-acetoxy-$\Delta^7$-6-ketosteroid **9**. For this reason, these atoms are observed at weak field at 82.3, 76.9, and 87.9 ppm, respectively. The chemical shifts of C-2, C-3, and C-5 typically are greater than those of the corresponding atoms in the spectra of 2,3-diacetoxy-5$\alpha$-hydroxy-$\Delta^7$-6-ketosteroids **7** and **8**. This enables these compounds to be distinguished by the $^{13}$C NMR spectra. The C-1 and C-10 signals in the spectrum of **10a**, which is a Westphalen—Lettre rearrangement product, have chemical shifts of 122.8 and 146.5 ppm, respectively. This indicates that there is a double bond between them. Furthermore, the signal of the 19-methyl is shifted significantly to weak field (30.4 ppm) in this spectrum. This is surely caused by its location on C-5 and very close to the 6-ketone. Another feature of the spectrum of **10a** is that fact that the C-11 signal appears at 33.1 ppm. Such a significant shift to weak field compared with the position of signals of analogous atoms in spectra of **1-9** is caused by the lack of a methyl on C-10 in **10a**. Thus, it can be seen that the situation is analogous for 19-norsteroids, in which the C-11 signal appears at weak field (26-27 ppm) [14].

There is an additional 9$\alpha$-hydroxy in **10b** compared with **10a**. Its presence causes several characteristic changes in the $^{13}$C NMR spectrum. In particular, the C-9 signal in the spectrum of **10b** is significantly shifted to weak field compared with its position in the spectrum of **10a** by the $\alpha$-effect of the 9$\alpha$-hydroxy. The $\beta$-effect of this same hydroxyl also shifts C-10 and C-11 to weak field. A shift to strong field from the $\gamma$-effect of the 9$\alpha$-hydroxy is also noticeable for C-12 and C-14 in the spectrum of **10b** compared with their positions in the spectrum of **10a**. Analogous trends are observed in the spectra of 3$\beta$,5$\alpha$,9$\alpha$-trihydroxy-$\Delta^7$-6-ketosteroids that were recently found in fungi [18, 19].

Thus, application of $^{13}$C NMR can rather simply prove the presence of the principal structural elements in 2,3-disubstituted derivatives of $\Delta^{4,7}$-6-ketosteroids and 5$\alpha$-hydroxy$\Delta^7$-6-ketosteroids.

## EXPERIMENTAL

$^{13}$C NMR spectra were recorded on a Bruker AC-200 spectrometer at 50.32 MHz with 40-50 mg in 0.5 mL of solution. Chemical shifts are reported on the $\delta$ scale relative to TMS internal standard. Conditions have been reported in detail [9]. Compounds **3-5** and **7-10** were synthesized by the literature methods [5, 6].

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