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Tetracyclic Triterpenes. XVII. The Inverted Conformation of Ring A and B in Steroids: 3-Substituted-4-oxo-14α-methyl-5β-cholest-8-enes*

by R. Joachimiak, J. Martynow and Z. Paryzek*****

Faculty of Chemistry, Adam Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland

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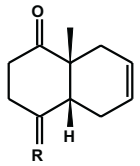
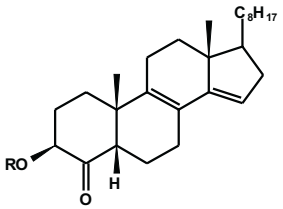
Full assignment of ^{13}C NMR shifts for a series of Δ^8-5α- and 5β-steroids is presented. These data along with some characteristic features of ^{1}H NMR and IR spectra are in accordance with inverted conformation of A and B rings in 4-oxo-5β-steroids **5** and **6** in solution. Configuration at C-3 is important for inversion of A and B ring conformation of α-methyl ketones **6** and **7**, while the intramolecular hydrogen bonding appears to be responsible for the inverted, slightly distorted chair conformation of ring A in α-hydroxy ketone **5**.

Key words: 5β-steroids, inverted conformation, ^{13}C NMR spectra

The relationship between biological activity of steroids and their structural and stereochemical variations is firmly established [1–3] as well as that the conformation of steroid molecule is crucial for effective binding to specific hormone receptor [4]. Chemical reactivity and hormonal activity of steroids in relation to their conformational mobility [5] and conformational flexibility of steroids as a function of axial or equatorial substituents in ring A have been discussed [6]. In less complex molecules, substituted decalins, conformational flexibility has been experimentally proved [7–9]. It has been concluded that cis-decalin derivative **2** in solution assumes predominantly the non-steroid [10] conformation, while compounds **1** and **3** have the normal, bile acid-like conformation.





1 R = β–OAc, H
2 R = α–OAc, H
3 R = O

4 R = p-BrC$_6$H$_4$CO

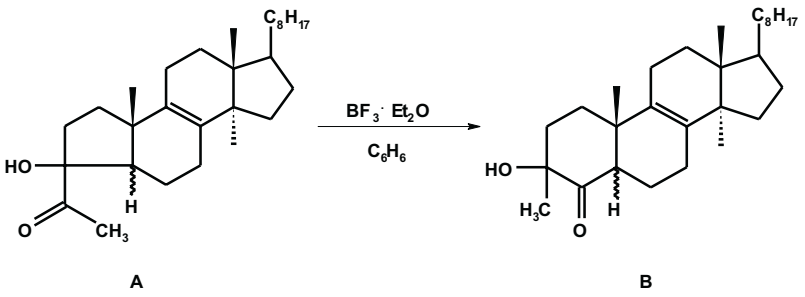
* Dedicated to Prof. M. Szafran on the occasion of his 70th birthday.

** Present address: Pharmaceutical Institute, Warsaw, Poland.
*** Author for correspondence. E-mail address: zparyzek@amu.edu.pl

The rigid structure of bile acids comprising the saturated cis-decalin fragment was the reason, why these compounds have been extensively used as building blocks of supramolecular hosts [11]. In 9-methyl-*cis*-decalin compounds like bile acids and steroids with *cis* A-B ring junction [12] rings A and B usually exist in the rigid, chair conformation. However, some conformational flexibility is gained when the Δ^8-double bond is present in the central part of the 5β-steroid molecule. This can be easily recognized by inspection of Dreiding models and analysis of computer generated structures of these steroid molecules. The data concerning the detailed structure of steroids with 8,9-double bond are scarce, due to the fact that these compounds are not easily accessible by synthesis. Recently, conformational analysis of steroidal 8,14-dienes has been reported [13,14]. It has been established that 3β-substituted cholestane derivative **4** prefers the conformation, in which ring A is an inverted chair (like **I**, Figure 1). X-ray crystallography confirmed the inverted conformation **I** of 3β,5β,17β,22E-ergosta-8,14,22-trien-3-ol benzoate in the solid state [14].

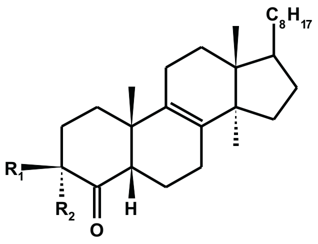
In the course of our investigation on rearrangements of A-nor steroids, a series of 14α-methyl-5α- and 5β-cholest-8-en-4-one derivatives **5–11** has been prepared [15–17]. The ketols **5, 8, 9** and **11** were synthesized from lanosterol in few steps. The acid catalyzed rearrangement of ketols **A** having 5α- and 5β-configuration (Scheme 1) followed by base-catalyzed isomerizations furnished four isomeric ketols **B** [15]. The α-methyl ketones **6, 7** and **10** were obtained from tosylhydrazone of **A** (5α-H, 3β-OH) under Bamford-Stevens conditions [17].

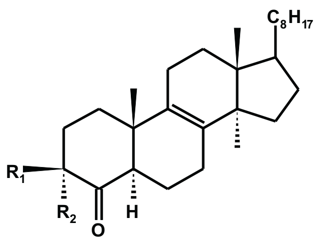
Scheme 1



A B

The structure of compounds **5–11** and configuration of important carbon atoms C(3) and C(5) was assigned on the basis of ^{1}H- and ^{13}C-NMR, IR and CD spectral data, chemical transformations and mechanistic considerations of the rearrangements under study. In this series of compounds, the 5β-isomers **5–8** are of special interest.





5 R_1=OH, R_2=CH_3
6 R_1=CH_3, R_2= H
7 R_1=H, R_2= CH_3
8 R_1=CH_3, R_2= OH

9 R_1=CH_3, R_2= OH
10 R_1=CH_3, R_2= H
11 R_1=OH, R_2= CH_3

The analysis of ^{13}C NMR spectra of compounds **5** and **6** revealed several inconsistencies (vide infra), if the compounds are assumed to have the bile acid-type conformation **N**. Therefore, on the basis of spectral data and computational studies, 5β-steroids **5–8** were analyzed in terms of the two possible conformations: **N** (normal) and **I** (inverted) shown in Figure 1.

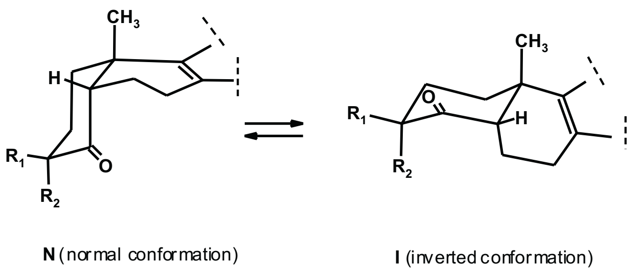


Figure 1. The conformational equilibrium of 5β-steroids **5–8**.

The position and multiplicity of the signals assigned to carbon atoms of ring A and B well correlated with the expected chemical shifts in ^{13}C NMR spectra, when the alternative conformation **I** (Figure 1) was taken into account. Additionally, supporting experimental evidence for conformation **I** or **N** was gained from analysis of ^{1}H-NMR and IR spectra of the compounds investigated.

RESULTS AND DISCUSSION

In the ^{13}C NMR spectra of compounds **5–11** (Table 1), the chemical shift assignment of ring C, D and side chain carbons was straightforward and correlated well with the published data on lanost-8-ene derivatives [18–20]. The assignment of carbon atoms C(1)–C(7) was based on their multiplicity patterns, substituent shift effects [21] as well as α-, β- and γ-effects [21].

Table 1. ^{13}C NMR chemical shifts for compounds **5–11**.

	5	**6**	**7**	**8**	**9**	**10**	**11**
C-1	30.0	32.6	36.6	33.5	31.8	36.3	34.0
C-2	35.8	29.9	31.2	36.9	36.2	32.1	38.1
C-3	74.7	41.1	44.4	75.0	74.8	44.4	75.7
C-4	215.0	216.4	212.0	214.0	213.1	214.1	215.5
C-5	55.4	58.4	54.7	50.3	50.6	55.5	51.1
C-6	25.2	25.8	22.5	22.2	17.0	17.2	17.1
C-7	22.4	24.1	17.7	17.4	24.3	24.4	24.3
C-8	137.6	135.6	140.2	140.4	132.7	132.8	132.6
C-9	129.9	131.4	127.4	126.8	135.4	135.5	135.7
C-10	40.5	40.6	41.3	42.0	42.8	43.0	43.3
C-11	21.5	21.4	21.4	21.6	22.0	22.1	22.2
C-12	30.6	30.6	30.6	30.6	30.9	31.1	30.9
C-13	44.3	44.1	44.6	44.6	44.6	44.6	44.6
C-14	50.3	50.2	50.4	50.3	50.1	50.0	49.9
C-15	30.9	30.8	31.2	31.1	30.9	30.9	30.9
C-16	28.1	28.0	28.1	28.1	28.1	28.1	28.1
C-17	50.5	50.4	50.7	50.6	50.6	50.6	50.6
C-18	15.7	15.6	15.8	15.7	15.8	15.8	15.8
C-19	26.2	24.0	28.6	28.5	18.3	18.9	18.7
C-20	36.5	36.5	36.5	36.5	36.5	36.5	36.5
C-21	18.8	18.8	18.8	18.8	18.8	18.8	18.8
C-22	36.5	36.5	36.5	36.5	36.5	36.5	36.5
C-23	24.1	24.0	24.2	24.1	24.1	24.1	24.1
C-24	39.6	39.6	39.5	39.5	39.5	39.5	39.5
C-25	28.0	28.0	28.0	28.0	28.0	28.0	28.0
C-26	22.6	22.5	22.5	22.5	22.5	22.5	22.5
C-27	22.8	22.8	22.8	22.8	22.8	22.8	22.8
C-32	24.3	24.6	24.6	24.7	24.5	24.6	24.6
3-CH_3	26.1	14.9	14.9	26.4	24.0	14.4	26.1

In compound **7**, the important signal at $\delta = 54.7$ was assigned to C(5). In the ketol **5**, however, the signal of C(5) was found at δ 55.4. This signal should be shifted upfield (in comparison with the position of C(5) in **7**), due to the pronounced γ-effect of the axial 3β-OH group, provided that ketol **5** exists in conformation **N**, the same as that assigned for **7**. Thus, the downfield shift of C(5) in **5** indicates a remarkable conformational change within rings A and B. A comparison of the position of C(5) signals in **9** and **10** reveals the typical upfield shift by 4.9 ppm in the ketol **9**. Furthermore, the chemical shifts of C(8) and C(9) in compounds **7** and **8**, which are assumed to exist in solution in conformation **N,** are very similar (C(8): $\delta = 140.2$ or 140.4; C(9): $\delta = 127.4$ or 126.8, respectively). The respective signals in **5** and **6** are shifted in opposite direction and are found at $\delta = 137.6$ or 135.6 for C(8) and at $\delta = 129.9$ or 131.4 for C(9). Additionally, the up-field shift of C(19) signal [22,23] in ^{13}C NMR spectra of **5** (δ = 26.2) and **6** ($\delta = 24.0$), when compared with those found in compound **7** ($\delta = 28.6$) and **8** (δ = 28.5), do not correlate well with conformation **N**.

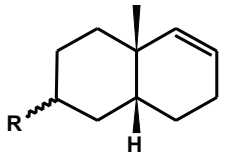
In 5β-steroids **5–8**, the position of the easily recognizable singlet of protons of the C-19 angular methyl group in ^{1}H NMR spectra was crucial for the assignment of conformation [24]. In the ketol **5** and α-methyl ketone **6**, the signal of this methyl was found at δ = 1.05 and 0.97, respectively, due to shielding caused by the anisotropic effect of C(8)–C(9) double bond and C(4) carbonyl group (assuming conformation **I**). The respective signals were substantially shifted downfield in the α-methyl ketone **7** and the ketol **8** (δ = 1.19 and 1.21, respectively), in agreement with the normal conformation **N** of these compounds. Furthermore, the 5β-proton in **5** absorbed at δ = 2.51. In conformation **N**, however, it would be expected to be much more deshielded as a result of the 1,3-*syn* diaxial interaction of this proton and the 3β-OH group. This is observed, for example, in compound **9** having 5α-H atom and 3α-OH group in the 1,3-diaxial arrangement. The 5α-H signal in **9** is shifted to δ 3.11. Also the splitting pattern of the 5β proton signal (J_1 = 7.8 and J_2 = 3.3 Hz) in **5** is in agreement with the inverted conformation of this compound in solution.

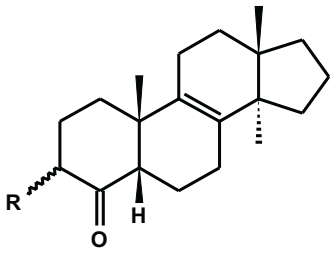
The IR spectra of α-hydroxy ketones in the region of O–H stretching vibration have been used for conformational assignment [25–28] and were very helpful in the present study. In the IR spectrum of **5** (solution in $CHCl_3$), the absence of a band that could be assigned to a free hydroxy group indicated the conformation of ring A with 3β-OH group oriented equatorially. Thus, only a band assigned to the intramolecularly bonded OH group at 3490 cm^{-1} was observed. Similarly, absorption of the associated OH group at about 3490 cm^{-1} was detected in the IR spectra of compounds **8** and **11**, having equatorial hydroxy group in the α-position to the carbonyl. The ketol **9**, in which the 3α-OH substituent in ring A of a chair conformation is axial, showed the band corresponding to the free OH group at 3590 cm^{-1}. In this compound the intramolecular hydrogen bonding could be realized only if ring A has an energetically unfavorable boat conformation.

On the basis of the spectral data presented it may be concluded that the Δ^8-unsaturated 4-oxo-5β-steroids **5** and **6** exist in solution predominantly in conformation **I** with inverted rings A and B. The conformational flexibility of these compounds results from the presence of the double bond between C(8) and C(9). Thus, the $\Delta^{8,14}$-diene system, as reported previously [13,14], is not a prerequisite for changing the equilibrium: **N** $\rightleftarrows$ **I** towards one particular conformer **I**.

The above conclusion is supported by results of computational studies of conformational equilibria. The unsaturated decalins **12–14** were chosen as simple model systems for the calculations. Using molecular mechanics (MM3) and semiempirical PM5 method (CAChe, Fujitsu) the optimized geometries for **N** and **I** conformations were calculated and the relative energies are summarized in Table 2.

These computations show that both conformers **N** and **I** of unsubstituted decalin **12** are of almost the same energy, as expected. However, methyl substitution strongly favors the **N**, steroid conformation for **14**, while the non-steroid [10] conformation **I** for **13**. In both minimum energy conformations, the methyl group of ring A is in the equatorial position. Optimizations of the geometry were also performed for model 3-substituted Δ^8-14α-methyl-5β-steroids, for calculation simplification lacking the side chain.





12 **R = H**
13 **R = β–CH_3**
14 **R = α–CH_3**

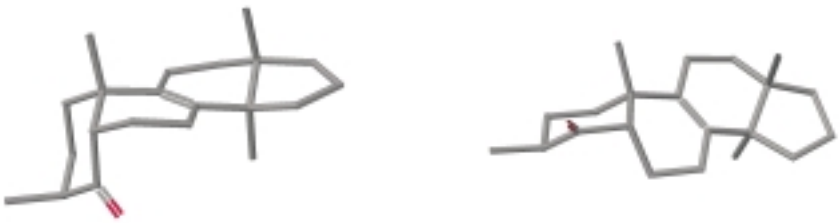
15 **R = β–CH_3**
16 **R = α–CH_3**
17 **R = β– OH**
18 **R = α– OH**
19 **R = H**

Table 2. Calculated relative energies (kcal/mol) of conformers **N** and **I** in model compounds, decalins **12–14** and steroids **15–19** (CAChe, Fujitsu).

Compound	Conformation **N**		Conformation **I**	
	MM3	PM5	MM3	PM5
12	0	0.05	0.49	0
13	1.53	1.01	0	0
14	0	0	4.74	2.47
15	1.97	1.37	0	0
16	0	0	4.47	2.15
17	2.20	1.90	0	0
18	0	0	4.45	2.67
19	0	0.36	0.73	0

These were α-methyl ketones **15** and **16** and α-hydroxy ketones **17** and **18**. The energy calculations indicate that for **17** and **18** the conformer having the equatorial hydroxyl group is of lower energy, thus indicating the remarkable stabilization resulting from the intramolecular hydrogen bonding. The relative energy calculated for **17** (ΔE = 1.90 kcal/mol) shows also that the equilibrium is almost entirely shifted towards the inverted conformation **I.** The energies calculated for conformations **N** and **I** of the model 3β-methyl ketone **15** differ by 1.37 kcal/mol, thus indicating a strong preference of the inverted conformation **I** (Figure 2). This is in accordance with all the spectral data discussed above for α-methyl ketone **6**. In contrast, similar calculation for compound **16** showed that the conformation **N** is 2.15 kcal/mol (PM5) more stable than **I.** This calculation supports the proposed conformation of the ketone **7** to be normal chair **N** with the equatorial 3α-methyl group. Interestingly, the conformation **I** is slightly preferred over **N** (ΔE = 0.36 kcal/mol, PM5) for 3-unsubstituted

ketone **19**. In ketol **5** the inverted conformation implies the unfavorable 1,3-diaxial interaction between 3α-methyl group and 6α-proton. However, due to the presence of 4-carbonyl, a small flattening of the ring A causes the relief of strain. Additionally, a remarkable stabilization by the intramolecular hydrogen bonding (vide supra) makes the flattened ring A inverted chair conformation of this compound prevailing in solution, as evidenced by the presented spectral data.



N **I**

Figure 2. CAChe-generated optimized **N** and **I** conformations of the 3β-methyl ketone **15**.

Analysis of conformational equilibria obtained from molecular modeling and spectroscopy shows that in 5β-steroids of conformation **N** (compounds **7** and **8**) ring A has the chair conformation and ring B has a conformation between 5α,6β-half-chair and 5α-sofa, while in conformation **I** (compounds **5** and **6**) ring A is the inverted chair and inverted ring B has the 5β,6α-half chair conformation. Contrary to the conclusion of Wilson *et al.* [13], the configuration of C(3) is found to be important for the position of the conformational equilibrium, at least in the case of Δ^8-unsaturated 5β-steroids with carbonyl group at C(4). This is very clearly indicated by the preference of 3β-methyl ketone **6** to exist in the inverted conformation **I** while – ketone **7**, having 3α-methyl substituent, prefers the normal chair conformation **N**. The calculations also suggest that the same is true for compounds without C(4)-carbonyl group.

EXPERIMENTAL

Previously we described the synthesis and spectral data of compounds **5–11** [15–17]. ^{1}H- and ^{13}C-NMR spectra were recorded using solutions in $CDCl_3$ on a JEOL FX 90 Q spectrometer (89.6 MHz for ^{1}H and 22.53 MHz for ^{13}C), Varian Gemini 300 VT spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C) or Bruker AM 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C). The chemical shifts (δ) are expressed in ppm relative to tetramethylsilane. SFORD, APT and DEPT techniques were used for the assignment of multiplicity of carbon signals in ^{13}C NMR spectra. IR Spectra were determinated with a FT-IR Bruker FS 113V spectrophotometer for solutions in chloroform.

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