SIMPLE NMR DETERMINATION OF 5α/5β CONFIGURATION OF 3-OXOSTEROIDS+

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A simple method to distinguish the 5α- from the 5β-isomers of 3-oxosteroids based on low-frequency ^{1}H NMR spectra was proposed. Additional ^{1}H and ^{13}C NMR characteristics were derived from the comparison of completely assigned spectra of the 5α- and 5β-isomers. The effect of substitution at different positions of steroid skeleton was evaluated on a series of isomeric 3-oxosteroids, prepared for this purpose.

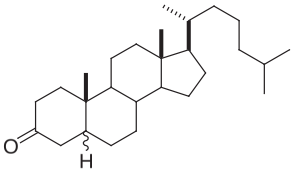
Keywords: Steroids; Ketones; Cyclohexanes; 3-Oxosteroids; 5α- and 5β-configuration; NMR spectroscopy; Absolute configuration.

A simple and reliable discrimination between 5α- and 5β-isomers in the synthesis, isolation and testing of biologically active 3-oxosteroids (mainly neurosteroids and brassinosteroids) is very important. This has been usually accomplished by means of circular dichroism (CD) spectra[2]. The Cotton effect of 5α-isomer is positive, while that of 5β-isomer is negative. During our syntheses of steroid derivatives, we found that ^{1}H NMR spectra of 3-oxosteroids with the 5β-configuration differ from those with 5α-configuration by the presence of a one-proton signal that appears as a triplet around 2.7 ppm. Therefore, we have decided to check this empirical rule, and to estimate its validity and limitations over a broad series of substituted 3-oxosteroids.

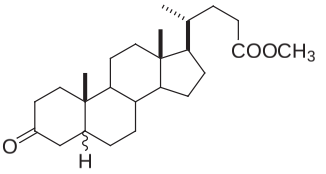
Some of the compounds were synthesized in connection with other projects[2], the other were taken from the collection of steroids in our Institute and/or from the literature. The last group of compounds was synthesized

+ Part CDXII in the series On Steroids. Part CDXI see ref.[1]

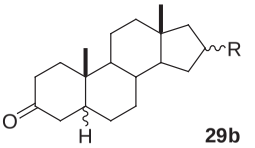
3-Oxosteroids substituted in ring D



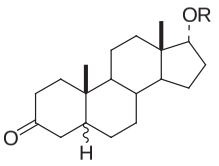
1a **1b**

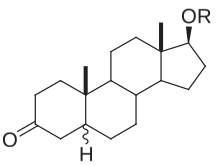


2a **2b**



29b R = α-OH
30b R = β-OH
31b R = β-OAc
32b R = β-OBzl

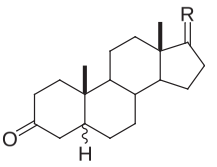




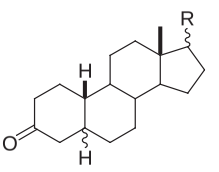
3a	**3b**	R = H
4a	**4b**	R = CHO
5a	**5b**	R = $COCH_3$
6a	**6b**	R = COC_2H_5
7a	**7b**	R = COC_3H_7
8a	**8b**	R = COC_6H_5
9a	**9b**	R = COC_6H_{13}
10a	**10b**	R = COC_7H_{15}
11a	**11b**	R = COC_9H_{19}
12a	**12b**	R = $COC_{11}H_{23}$
13a	**13b**	R = $COC_{13}H_{27}$
14a	**14b**	R = $COC_{17}H_{35}$
15a	**15b**	R = CO–⬡
		R = CS-O–⬡

16a	**16b**
17a	**17b**
18a	**18b**
19a	**19b**
20a	**20b**
21a	**21b**
22a	**22b**
23a	**23b**
24a	**24b**
25a	**25b**
26a	**26b**
27a	**27b**

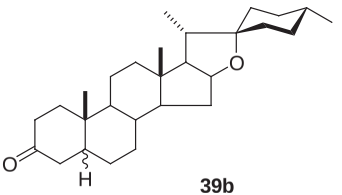
28b



33a R = H_2
34a **34b** R = O

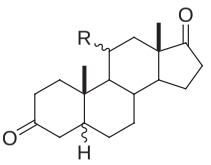


35a **35b** R = β-OH
36a **36b** R = α-C≡CH, β-OH
37b R = β-CN
38b R = =O



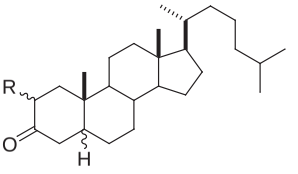
39b

3-Oxosteroids substituted in ring C



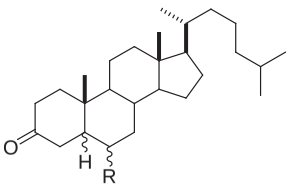
40a **40b** R = β-OH
41a **41b** R = β-CHO
42a **42b** R = α-$OCOCH_3$

3-Oxosteroids substituted in ring A

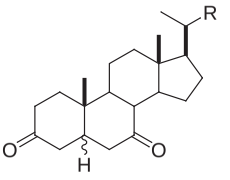


43a R = α-Br
44a **44b** R = α-OAc
45a **45b** R = β-OAc
46b R = β-OH

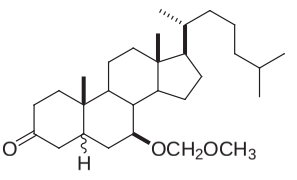
3-Oxosteroids substituted in ring B



47a R = α-OH
48a **48b** R = β-OH
49a **49b** R = =O



50a R = $CH_2CH_2CH_2CH(CH_3)_2$
51a R = $OCOC_6H_5$
52b R = $CH_2CH_2COOCH_3$



53a **53b**

FIG. 1

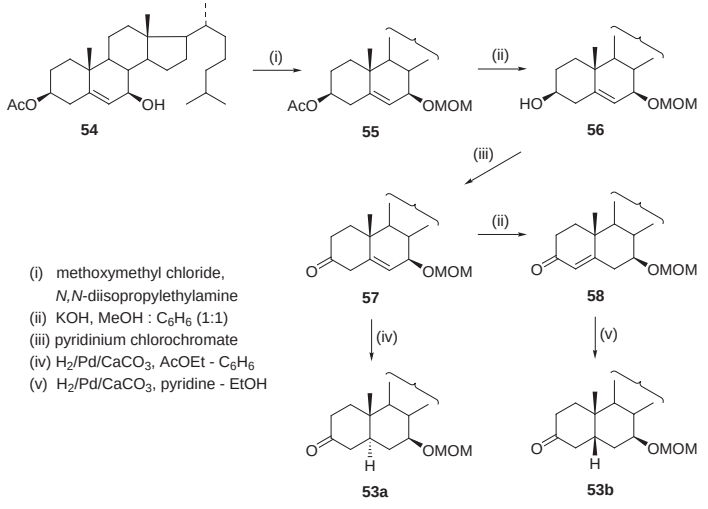
3-Oxosteroids evaluated in NMR determination of 5α- and 5β-configuration (letters **a** and **b** indicate 5α- and 5β-configuration, respectively)

directly for this study and their preparation is described in this paper. All the 3-oxosteroids used are summarized in Fig. 1 (the letters **a** and **b** denote 5α- and 5β-configuration, respectively). In some cases, only one of the isomers was available.

The largest group, consisting of compounds with various lengths of the acyloxy groups in position 17, was synthesized for the study of the effect of the side chain length on the hydrogenation of the double bond in Δ^4-3-oxosteroids[2] (**3a**–**27b**).

From the Institute collection of steroid derivatives, we used 5α-cholestan-3-one (**1a**) and 5β-cholestan-3-one[3,4] (**1b**), 2β-bromocholestan-3-one[5] (**43a**), cholestane-3,7-dione[6] (**50a**), methyl-3,7-dioxocholanate[7] (**52b**) and 3,7-dioxopregnane derivative[8] **51a**.

Cholestane derivatives substituted in position 7 (**53a**, **53b**) were synthesized from cholest-5ene-3β,7β-diol, 3-acetate (**54**), in which the free 7β-hydroxy group was converted to the methoxymethyl ether **55** by the reaction with methoxymethyl chloride. Following the hydrolysis of the acetate group in position 3, alcohol **56** was oxidized to ketone **57** under an argon atmosphere. Ketone **57** was then hydrogenated in ethyl acetate–ethanol on $Pd/CaCO_3$, yielding a compound with a *trans*-annellation of A/B rings identified as 7β-methoxy-5α-cholestan-3-one[9] (**53a**). The double bond in ketone **57** was shifted to position 4 with KOH in methanol, affording a more stable conjugated Δ^4-3-oxo-derivative **58**, which was hydrogenated on the same catalyst in pyridine–ethanol (1 : 1) to give 7β-(methoxymethyl)-5β-cholestan-3-one (**53b**) (Scheme 1).



SCHEME 1

6β-Hydroxycholestan-3-one derivatives **48a** and **48b** were prepared by catalytic hydrogenation of 6β-hydroxycholest-4-en-3-one (**59**) on a Pd catalyst in a mixture of ethyl acetate and ethanol. The main reaction product with the 5α-configuration and a minor one with the 5β-configuration were obtained by chromatographic separation. Oxidation of alcohols **48a** and **48b** with the Jones reagent in acetone afforded isomeric 3,6-diones **49a** and **49b** (Scheme 2).

Diketone **51a** was obtained as a side-product during the inversion of configuration of 3β-tosylate **60** in position 3 (Scheme 3).

Procedures[10] described earlier were used for the preparation of 2α-acetoxy-5α-cholestan-3-one (**44a**) and 2β-acetoxy-5α-cholestan-3-one (**45a**). Estrane derivatives **35a** and **35b** were prepared from the Δ^4-3-oxo-derivative **61** by the hydrogenation on a Pd catalyst and the isomers were separated by chromatography (Scheme 4).

NMR DISCUSSION

The ^{1}H NMR spectra of the 5β-isomers of 3-oxosteroids (without any other substitutents in rings A and B) display a characteristic signal of the axial 4α hydrogen near δ 2.70 as a "pseudo-triplet", with splitting 13–15 Hz. Our study of the series of 3-oxosteroids showed that substitution in rings D and C has a negligible effect on the chemical shift of H-4α (Table I). The signal of this proton is shifted out of the "steroid envelope" and is easily observable even in low-frequency NMR spectra (*e.g.* at 200 MHz; see compound **1b**, Fig. 2a).

On the other hand, in the 5α-isomers of 3-oxosteroids, the axial proton in position 4 (H-4β) gives a similar "triplet" but it appears at higher fields (δ < 2.40; see Table I). Its identification in low-frequency NMR spectra is usually difficult due to the overlap with other signals (H-2α, H-2β, H-4α) in this region, and requires measurement at higher frequencies and detailed analysis (*e.g.* by 2D-COSY experiment). Nevertheless, the absence of the triplet of the axial proton H-4α around 2.70 ppm indicates the 5α-configuration in the corresponding 3-oxosteroid (see compound **1a**, Fig. 2b).

A typical situation is illustrated in the ^{1}H NMR spectra of 5α- and 5β-cholestan-3-one (**1a**, **1b**) measured at 200 and 500 MHz (Fig. 2). Figure 2 shows only the low-field part of the spectra (δ > 2.00), which also contains the equatorial protons in positions 1 and 12, in addition to the signals of the four protons adjacent to 3-oxo group. The chemical shifts and coupling constants of the protons in ring A are shown in partial structural formulas. With a good resolution, it is possible to observe long-range couplings

through the 3-oxo group between two equatorial (^4J(H-2eq,H-4eq) = 2.3 Hz) and a smaller one between the axial protons (^4J(H-2ax,4ax) = 0.8 Hz) that may lead to an additional fine splitting or broadening of the lines of the H-4 axial pseudo-triplet.

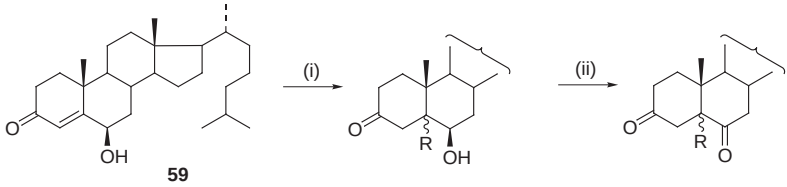
Our results suggest (see Table I) that this simple rule for distinguishing the 5α- from the 5β-isomers can be safely used for 3-oxosteroids with substituents in rings D and C (including 19-nor-3-oxosteroids). Complications arise in 3-oxosteroids substituted in ring B, if the substituent comes closer to H-4ax, at least in the case of the 5β-isomer. While the rule still works for 7β-OR derivatives **53a** and **53b**, it fails for the isomeric pairs with 7-oxo or 6β-OR substitution (**49**, **48**) (see Table I). Substitution in ring A leads, as may be expected, to much larger effects on the H-4ax chemical shift and the above simple rule cannot be applied.

Additional NMR characteristics of the 5α- and 5β-isomers of 3-oxosteroids could be derived from the comparison of completely assigned ^{1}H and ^{13}C NMR spectra (^{1}H at 500 MHz; ^{13}C at 125.7 MHz) of the **1a**, **1b** isomeric pair and others. Significant chemical shift differences of protons H-4β, H-7α, H-9α and carbon atoms C-7, C-9, C-19 (Table II) can be explained by a different number of γ-gauche interactions operating in *trans*- and *cis*-annellated A/B rings of the 5α- and 5β-isomers. It is noteworthy that the proton signals of Me-19 have nearly the same chemical shifts for the 5α- and 5β-isomers (*e.g.* δ 1.01 and 1.02 in **1a** and **1b**, respectively), while the positions of their carbon signals differ dramatically (δ 11.46 and 22.67 in **1a** and **1b**, respectively). At a very good resolution, a long-range coupling between Me-19 and the axial H-1α can be observed in the 5α-isomer (*e.g.* 0.6 Hz in **1a**) while it is absent in the 5β-isomer. Under a proper distribution of the signals in ^{1}H NMR spectra, the 5α- and 5β-isomers of 3-oxosteroids can be distinguished on the basis of interproton NOEs specific for individual isomers (Fig. 3).

The characteristics based on the ^{13}C chemical shifts of Me-19, C-7 and C-9 must be used with care when the C ring is substituted (the complete ^{13}C NMR data for the compounds measured in this study are given in Table III). Also, the typical values of the proton chemical shifts of H-7α and H-9 can be influenced significantly by the substitution in positions 11 and 12 of ring C. Substituents in ring B or A may change the chemical shifts of the above discussed protons and carbons dramatically and the characteristic values given in Table II cannot be used for the analysis of similar 3-oxosteroids.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Analytical samples were dried over phosphorus pentoxide at 50 °C/100 Pa. Optical rotations were measured at 25 °C on a Perkin–Elmer 141 MC polarimeter and are given in 10^{-1} deg cm^2 g^{-1}. Infrared spectra (wavenumbers in cm^{-1}) were recorded on a Bruker IFS 88 spectrometer in chloroform. ^{1}H NMR spectra were measured on a Varian UNITY-200 (^{1}H at 200 MHz) or UNITY-500 (^{1}H at 500 MHz) spectrometer and ^{13}C NMR spectra (APT sequence) on a Varian

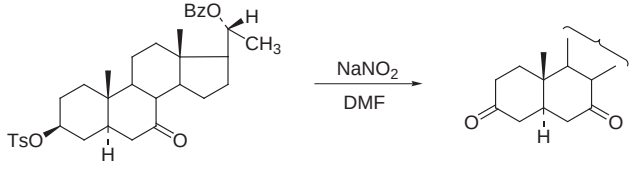


59 **48a**, R = α-H; **48b**, R = β-H **49a**, R = α-H; **49b**, R = β-H

(i) H_2/Pd/$CaCO_3$, AcOEt - EtOH (8 : 3); (ii) Jones reagent

SCHEME 2

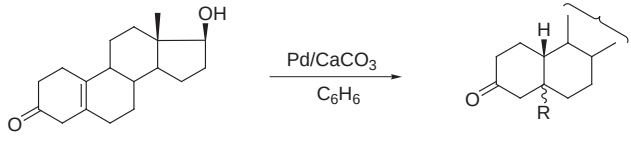
UNITY-500 (at 125.7 MHz) in $CDCl_3$ at 20 °C. Chemical shifts (δ-scale) were referenced to internal tetramethylsilane (^{1}H) or $CDCl_3$ (^{13}C; δ($CDCl_3$) 77.0). Coupling constants (*J*) and



60 **51a**

SCHEME 3

width of multiplets (*W*) are given in Hz. COSY spectra were used for the assignment of the proton coupling patterns and HMQC spectra for the correlation of the directly bonded hydrogen and carbon atoms. Thin layer chromatography (ICN Silica G, TLC-60 A) was used to check the purity of the compounds synthesized. Column chromatography was carried out on silica gel for thin layer chromatography (Silpearl, Kavalier, Czech Republic), using a slight overpressure. Whenever aqueous solutions of hydrochloric acid, potassium hydrogen-



61 **35a**, R = α-H; **35b**, R = β-H

SCHEME 4

carbonate and potassium carbonate are used, their concentration is always 5%. Room temperature was in the range 20–22 °C.

7β-(Methoxymethoxy)cholest-5-en-3β-yl Acetate (**55**)

To a solution of 7β-hydroxycholest-5-en-3β-yl acetate (**54**; 1 g, 2.25 mmol) in dichloromethane (10 ml), *N*,*N*-diisopropylethylamine (1.5 ml, 8.6 mmol) and methoxymethyl chloride[18] (0.5 ml, 8.6 mmol) were added and the mixture was stirred under argon at room temperature. After 4 h, ether (50 ml) and water (50 ml) were added. After layer separation, the water layer was extracted with ether and the combined organic layers were washed with aqueous citric acid (5%) and a solution of sodium hydrogencarbonate, and dried over anhy-

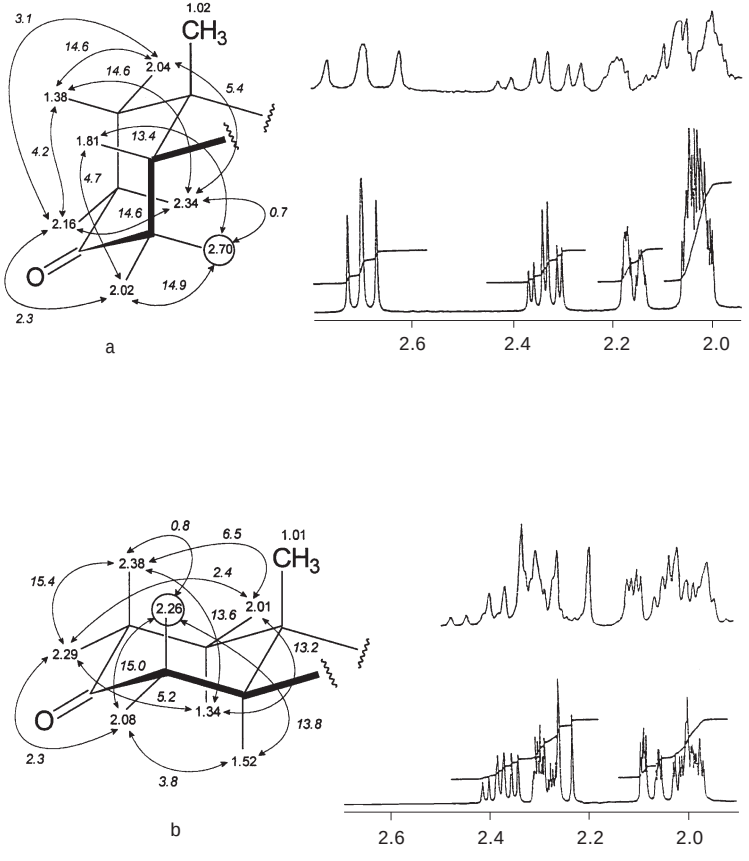


FIG. 2

Low-field part of the ^{1}H NMR spectra of 5α- and 5β-cholestan-3-ones (**1a**, **1b**) at 200 and 500 MHz frequencies. Chemical shifts and coupling constants of protons in ring A (indicated with arrows) are shown in formulas a (compound **1b**) and b (compound **1a**)

TABLE I
Chemical shifts of axial H-4 in 5α- and 5β-isomers of 3-oxosteroids

Isomers	Chemical shift									
	Substitution in ring D									
	1[a]	**2**[a]	**3**[a]	**4**[a,b]	**5**[a,b]	**6**[a,b]	**7**[a,b]	**8**[a]	**9**[a,b]	**10**[a,b]
5α-	2.26	<2.40	2.27	2.27	2.32	2.28	2.28	2.28	2.27	2.30
5β-	2.70	2.70	2.70	2.70	2.72	2.72	2.72	2.75	2.72	2.70
	11[a,b]	**12**[a,b]	**16**[a,c]	**17**[a,b]	**13**[a,b]	**14**[a,b]	**15**[d]	**18**[a,c]	**19**[a,b]	**20**[a,b]
5α-	<2.40	<2.40	2.30	<2.40	<2.40	<2.40	<2.40	2.27	2.26	2.27
5β-	2.72	2.72	2.68	2.68	2.72	2.70	2.74	2.69	2.69	2.69
	21[a,b]	**22**[a,b]	**23**[a,b]	**24**[a,b]	**25**[a,b]	**26**[a,b]	**27**[a,b]	**28**[c]	**29**[c]	**30**[c]
5α-	~2.30	2.26	2.30	<2.40	<2.40	<2.40	<2.40			
5β-	2.71	2.69	2.70	2.70	2.70	2.70	2.70	2.68	2.67	2.67
	31[c]	**32**[c]	**33**[c]	**34**[f]	**35**[a]	**36**[e]	**37**[g]	**38**[g]	**39**[h]	
5α-			2.24	<2.40	2.10	2.27				
5β-	2.69	2.70		2.68	2.59	2.82	2.57	2.59	2.69	
	Substitution in ring C				**Substitution in ring B**					
	40[a,b]	**41**[a,b]	**42**[a,b]	**47**[e]	**48**[a]	**49**[a]	**50**[a]	**51**[a]	**52**[a]	**53**[a]
5α-	<2.50	<2.40	2.28	2.22	2.81	~2.59	2.37	2.36		2.27
5β-	2.64	2.61	2.67		2.32	2.65			~2.23	2.51
	Substitution in ring A									
	43[a]	**44**[a,i]	**45**[a,i]	**46**[i]						
5α-	2.43	2.42	2.39							
5β-		2.1–2.9	2.1–2.9	2.1–2.9						

[a] This paper; [b] ref.[2]; [c] ref.[11]; [d] ref.[12]; [e] ref.[13]; [f] ref.[14]; [g] ref.[15]; [h] ref.[16]; [i] ref.[17]

drous magnesium sulfate. The solvents were evaporated and the product crystallized from ether to afford compound **55** (130 mg, 72%), m.p. 102–104 °C (light petroleum–ether), $[\alpha]_D$ –57 (*c* 0.2). IR: 1 706 (C=O); 1 144, 1041, 1 097, 912 (C–O–C–O–C). ^{1}H NMR (200 MHz): 5.47 s, 1 H (H-6); 4.69 d and 4.61 d, 2 H, J_{gem} = 6.7 (C(7)-OCH_2O); 4.60 m, 1 H (H-3); 3.66 td, 1 H, *J* = 8.2, *J* = 2 (H-7); 3.38 s, 3 H (OCH_3); 2.04 s, 3 H (3 × H CH_3COO); 1.07 s, 3 H (3 × H-19); 0.92 d, 3 H, *J* = 6.4 (3 × H-21); 0.84 d, 6 H, *J* = 6.7 (3 × H-26 + H-27); 0.68 s, 3 H (3 × H-18). For $C_{31}H_{52}O_4$ (488.8) calculated: 78.18% C, 10.72% H; found: 78.47% C, 10.98% H.

7β-(Methoxymethoxy)cholest-5-en-3β-ol (**56**)

A solution of 7β-(methoxymethoxy)cholest-5-en-3β-yl acetate (**55**; 800 mg, 1.64 mmol) and potassium hydroxide (800 mg, 14.3 mmol) in a mixture of benzene (40 ml) and methanol (40 ml) was stirred for 2 h at room temperature. The reaction mixture was then diluted with water, extracted with ethyl acetate and the extract was washed with a solution of potassium carbonate, citric acid, water, and dried over anhydrous magnesium sulfate. Crystallization from ether afforded 640 mg (87%) of alcohol **56**, m.p. 170–171 °C. $[\alpha]_D$ –12 (*c* 0.2). IR: 1 695 (C=C); 1 144, 1 041, 1 097, 912 (C–O–C–O–C). ^{1}H NMR (200 MHz): 5.47 s, 1 H (H-6); 4.69 d and 4.61 d, 2 H, J_{gem} = 6.7 (C(7)-OCH_2O); 3.66 td, 1 H, *J* = 8.2, *J* = 2 (H-7); 3.52 m, 1 H (H-3); 3.38 s, 3 H (OCH_3); 1.07 s, 3 H (3 × H-19); 0.92 d, 3 H, *J* = 6.4 (3 × H-21); 0.84 d, 6 H, *J* = 6.7 (3 × H-26 + H-27); 0.68 s, 3 H (3 × H-18). For $C_{29}H_{50}O_3$ (446.7) calculated: 77.97% C, 11.28% H; found: 78.16% C, 11.36% H.

7β-(Methoxymethyl)cholest-5-en-3-one (**57**)

A solution of alcohol **56** (200 mg, 0.45 mmol) in benzene (5 ml) was stirred with pyridinium chlorochromate on aluminium oxide[19] (200 mg, 0.2 mmol) under argon at room temperature. After 48 h, inorganic solids were filtered off and the filtrate was evaporated. Crystallization from ether afforded 57 mg (95%) of compound **57**, m.p. 69–72 °C (ether–light petroleum), $[\alpha]_D$ +5 (*c* 0.3). IR: 1 699 (C=O); 1 144, 1 095, 1 043, 912 (C–O–C–O–C). ^{1}H NMR (200 MHz): 5.43 t, 1 H (H-6); 4.70 d and 4.62 d, 2 H, J_{gem} = 6.7 (C(7)-OCH_2O); 3.66 td, 1 H, *J* = 8.2, *J* = 2 (H-7); 3.38 s, 3 H (CH_3O); 1.22 s, 3 H (3 × H-19); 0.92 d, 3 H, *J* = 6.4 (3 × H-21); 0.86 d, 6 H, *J* = 6.7 (3 × H-26 + H-27); 0.72 s, 3 H (3 × H-18). For $C_{29}H_{48}O_3$ (444.7) calculated: 78.33% C, 10.88% H; found: 78.54% C, 10.92% H.

7β-(Methoxymethoxy)-5α-cholestan-3-one (**53a**)

A solution of ketone **57** (50 mg, 0.11 mmol) in ethanol (8 ml) and ethyl acetate (3 ml) was stirred in the presence of palladium on calcium carbonate (5%, 20 mg) under hydrogen at room temperature. After 7 h, the catalyst was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in acetone (2 ml) and the Jones reagent was added dropwise until the yellowish colour persisted for 5 min. Methanol was then added, followed by water after 15 min. The solvents were partially evaporated *in vacuo* (to 1/3 of the volume). Another portion of water was added and the product was extracted with ethyl acetate. The extract was washed with a solution of citric acid, sodium hydrogencarbonate, water and dried over anhydrous magnesium sulfate. Preparative TLC of the residue (46 mg, 92%) on silica gel (light petroleum–ether, 4 : 1) yielded ketone **53a** (34 mg, 68%), m.p. 114–118 °C (benzene–ether), $[\alpha]_D$ –60 (*c* 1.8). IR: 1 718 (C=O); 1 144, 1 095, 1 043, 912 (C–O–C–O–C).

TABLE II
NMR characteristics of 5α- and 5β-isomers of 3-oxosteroids

Parameter	Spectrum	
	^{1}H NMR	
	5α-	**5β-**
H-4 (axial)	<2.40 ppm (H-4β)	≈2.70 ppm (H-4α)
J(4ax,4eq) ≈ J(4ax,5)	13–15 Hz	13–15 Hz
J(19,1α)	≈0.5 Hz	≈0
H-7α	≈0.92 ppm	≈1.11 ppm
H-9α	≈0.75 ppm	≈1.51 ppm
Specific NOEs	Me-19/H-2β	Me-19/H-1α
	Me-19/H-4β	Me-19/H-5
	H-5/H-7α	H-2α/H-9
		H-4α/H-7
	^{13}C NMR	
	5α-	**5β-**
Me-19	≈11.4 ppm	≈22.6 ppm
C-7	≈31.2 ppm	≈26.4 ppm
C-9	≈53.7 ppm	≈40.8 ppm

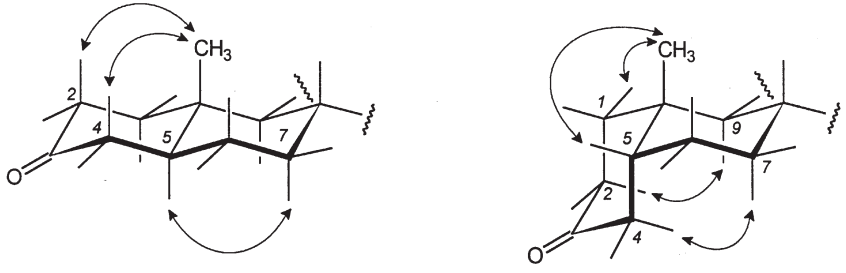


FIG. 3
Conformations of rings A and B in the 5α- and 5β-isomers of 3-oxosteroids. Specific NOEs for both isomers are indicated with arrows

^{1}H NMR (200 MHz): 4.63 s, 2 H (C(7)-OCH_2O); 3.38 s, 3 H (CH_3O); 3.34 m, 1 H (H-7); 1.05 s, 3 H (3 × H-19); 1.02 d, 3 H, *J* = 6.4 (3 × H-21); 0.94 d, 6 H, *J* = 6.7 (3 × H-26 + H-27); 0.66 s, 3 H (3 × H-18). For $C_{29}H_{50}O_3$ (446.7) calculated: 77.97% C, 11.28% H; found: 77.96% C, 11.29% H.

7β-(Methoxymethoxy)cholest-4-en-3-one (**58**)

Compound **57** (200 mg) and potassium hydroxide (100 mg, 3.6 mmol) were dissolved in methanol (10 ml) and the solution was stirred at the room temperature for 2 h. The reaction mixture was diluted with water and extracted with ethyl acetate. The extract was washed

TABLE III
Carbon-13 NMR data of 3-oxosteroids in $CDCl_3$

Carbon	1a	1b	3a	3b	4a[a]	4b[b]	6a[c]	6b[d]	7a[e]	7b[e]	9a[f]	9b[g]
1	38.56	37.23*	38.58	37.11*	38.56	37.16	38.59	37.20	38.60	37.21	38.59	37.13
2	38.20	37.03*	38.13	37.06*	38.13	37.03	38.16	37.02	38.17	37.02	38.16	36.98
3	212.20	213.48	212.10	213.35	211.89	213.07	212.04	213.19	?	213.24	211.97	213.16
4	44.74	42.38	44.65	42.33	44.64	42.29	44.68	42.33	44.68	42.33	44.68	42.26
5	46.70	44.36	46.60	44.30	46.61	44.22	46.68	44.22	46.68	44.22	46.68	44.17
6	28.97	25.80	28.89	26.04	28.83	24.63	28.88	24.65	?	24.66	28.88	24.60
7	31.72	26.64	31.36	26.46	31.80	26.49	31.76	26.54	31.78	26.54	31.77	26.49
8	35.39	35.54	35.61	35.65	35.56	35.70	35.60	35.74	35.60	35.74	35.60	35.70
9	53.80	40.76	53.58	40.51	53.48	40.56	53.53	40.60	53.53	40.61	53.54	40.57
10	35.64	34.90	35.70	34.89	37.03	34.96	37.03	34.98	?	34.98	35.74	34.93
11	21.45	21.21	20.93	20.65	20.82	20.60	20.85	20.60	20.85	20.61	20.85	20.56
12	39.90	40.10	32.34*	32.35	31.96	32.00	31.98	31.94	31.98	31.96	31.98	31.93
13	42.59	42.73	45.28	45.32	44.71	44.83	44.77	44.85	?	44.81	44.72	44.77
14	56.26*	56.48	48.53	48.66	49.82	50.03	49.92	50.12	49.95	50.15	49.94	50.10
15	24.23	24.19	24.59	24.47	24.57	26.07	24.67	26.10	24.67	26.11	24.66	26.06
16	28.23	28.28	32.01*	31.50	30.01	30.06	30.05	30.14	30.12	30.21	30.09	30.13
17	56.28*	56.33	79.88	79.74	81.83	81.87	81.56	81.59	81.52	81.55	81.52	81.52
18	12.06	12.06	17.03	16.96	16.58	16.58	16.69	16.69	16.69	16.69	16.68	16.64
19	11.46	22.67	11.46	22.56	11.47	22.63	11.48	22.64	11.48	22.64	11.48	22.59
20	35.78	35.77	–	–	–	–	–	–	–	–	–	–
21	18.66	18.68	–	–	–	–	–	–	–	–	–	–
22	36.14	36.14	–	–	–	–	–	–	–	–	–	–
23	23.82	23.82	–	–	–	–	–	–	–	–	–	–
24	39.50	39.50	–	–	–	–	–	–	–	–	–	–
25	28.00	28.00	–	–	–	–	–	–	–	–	–	–
26	22.81	22.81	–	–	–	–	–	–	–	–	–	–
27	22.56	22.56	–	–	–	–	–	–	–	–	–	–

with aqueous citric acid, water, and sodium hydrogencarbonate solution and dried over magnesium sulfate. Chromatography on silica gel afforded 30 mg (22%) of compound **58**, m.p. 86–89 °C (ether–light petroleum), $[\alpha]_D$ +5 (*c* 0.3). IR: 1 660, 1 630 (C=C–C=O); 1 144, 1 095, 1 043, 912 (C–O–C–O–C). ^{1}H NMR (200 MHz): 4.70 d and 4.62 d, 2 H, J_{gem} = 6.7 (C(7)-OCH_2O); 3.61 s, 1 H (H-7); 3.38 s, 3 H (CH_3O); 3.22 m, 1 H (H-3); 1.21 s, 3 H (3 × H-19); 0.93 d, 3 H, *J* = 6.4 (3 × H-21); 0.89 d, 6 H, *J* = 6.7 (3 × H-26 + H-27); 0.72 s, 3 H (3 × H-18). For $C_{29}H_{48}O_3$ (444.7) calculated: 78.33% C, 10.88% H; found: 78.54% C, 10.92% H.

7β-(Methoxymethoxy)-5β-cholestan-3-one (**53b**)

A solution of ketone **58** (40 mg, 0.09 mmol) in ethanol (2 ml) and pyridine (2 ml) was stirred under hydrogen in the presence of palladium on calcium carbonate (10 mg, 5%) at room temperature. After 17 h, the catalyst was filtered off and the filtrate was evaporated *in vacuo*. Preparative chromatography on a thin layer of silica gel (light petroleum–ether, 3 : 20) afforded 15 mg (38%) of oily compound **53b**, $[\alpha]_D$ +5 (*c* 0.3). IR: 1 719 (C=O); 1 144, 1 095, 1 043, 912 (C–O–C–O–C). ^{1}H NMR (200 MHz): 4.70 d and 4.62 d, 2 H, J_{gem} = 6.7 (C(7)-OCH_2O); 3.36 m, 1 H (H-7); 3.35 s, 3 H (CH_3O); 1.05 s, 3 H (3 × H-19); 0.93 d, *J* = 6.5,

TABLE III
(*Continued*)

Carbon	**17b**[h]	**18a**[i]	**18b**[j]	**19a**[k]	**19b**[l]	**20a**[m]	**20b**[n]	**22a**[o]	**22b**[p]	**35a**	**35b**
1	37.16*	38.45	37.10*	38.49	37.14	38.49	37.17	38.50	37.17	30.55	27.71
2	37.02*	38.06	36.97*	38.10	37.02	38.11	37.04	38.11	37.05	41.31	36.40
3	212.97	211.92	213.03	211.87	213.02	211.89	213.06	211.88	213.12	211.90	212.95
4	42.30	44.60	42.25	44.65	42.28	44.65	42.32	44.65	42.32	48.65	42.90
5	44.23	46.57	44.21	46.61	44.26	46.61	44.29	46.61	44.30	43.68	38.32
6	25.36	28.71	25.30	28.77	25.35	28.78	25.38	28.78	25.39	33.85	30.52
7	26.45	31.17	26.40	31.23	26.44	31.23	26.47	31.23	26.48	30.19	25.02
8	35.35	35.13	35.29	35.19	35.34	35.19	35.37	35.19	35.38	41.05	41.52
9	40.82	53.69	40.75	53.73	40.80	53.74	40.83	53.74	40.84	47.82	38.45
10	34.98	35.67	34.91	35.72	34.95	35.72	34.98	35.72	34.99	45.79	39.77
11	20.65	20.86	20.60	20.91	20.65	20.92	20.68	20.92	20.69	25.73	25.49
12	36.94*	36.79	36.95*	36.88	37.02	36.87	37.04	36.87	37.05	36.59	36.67
13	42.85	42.57	42.68	42.77	42.78	42.68	42.81	42.68	42.81	43.08	43.20
14	50.72	50.53	50.69	50.62	50.74	50.59	50.77	50.59	50.77	49.97	50.02
15	23.52	23.47	23.42	23.55	23.48	23.55	23.52	23.55	23.53	23.19	23.18
16	27.59	27.48	27.51	27.57	27.60	27.57	27.62	27.56	27.62	30.48	30.52
17	82.60	82.70	82.64	82.10	82.41	82.41	82.37	82.41	82.39	81.93	81.93
18	12.14	12.06	12.03	12.16	12.08	12.15	12.14	12.15	12.15	11.03	11.07
19	22.63	11.40	22.57	11.46	22.61	11.47	22.64	11.47	22.64	–	–

TABLE III
(*Continued*)

Carbon	42b[q]	42b[r]	43a	44a[s]	45a[t]	48a	48b	49a	49b	50a	51a[u]	52b[v]	53a[w]	53b[x]
1	38.26*	38.33*	43.95	43.59	42.22	39.80*	37.32*	36.99	36.50	37.56	37.55	35.44	38.22*	37.05*
2	38.19*	37.82*	54.59	74.47	74.43	38.20	36.49*	38.11	35.80	36.89	38.03	?	38.01*	36.35*
3	210.86	212.16	201.22	204.22	207.01	212.65	211.99	211.26	210.92	210.61	210.26	211.12*	211.42	211.99
4	44.91	42.52	51.73	44.86	43.50	42.67	42.74	41.25	42.21	44.14	44.12	45.00	44.18	43.11
5	46.84	44.34	47.50	47.87	41.79	48.87	49.96	57.52	59.77	47.80	47.73	49.55[+]	51.99	44.28
6	29.04	24.59	28.42	28.42	28.34	70.85	71.80	209.11	208.68	45.71	45.62	42.92	36.29	34.39
7	30.34	26.46	31.47	31.59	31.30	39.83	34.18	46.62	39.55	209.75	209.54	210.21*	82.94	79.60
8	34.32	34.37	34.90	34.72	35.25	30.20	30.46	38.04	36.74	49.70	49.55	47.75	41.23	39.61
9	56.24	45.33	53.62	53.84	54.79	56.26[+]	41.67	53.49	40.98	54.14	54.20	42.85	43.73	41.39
10	37.32	36.03	38.99	37.22	36.09	35.62	34.63	37.38	38.32	36.01	36.00	36.74	34.91	34.26
11	70.48	70.77	21.52	21.63	21.52	21.25	20.95	21.67	21.34	22.18	22.17	22.11	21.85	21.81
12	38.93*	39.29*	39.69	39.75	39.83	39.85*	39.96	39.39	39.92	38.60	38.03	38.85	39.82	40.03
13	47.45	47.59	42.57	42.60	42.62	42.06	42.03	43.01	43.06	42.45	42.38	42.66	43.61	43.77
14	49.78	50.02	56.06	56.10	56.12	53.74[+]	56.30	56.61	56.87	48.76	48.28	48.84[+]	55.71[+]	55.92[+]
15	21.76	21.74	24.19	24.19	24.13	24.27	24.19	24.00	23.96*	24.93	25.01	24.76	26.50	26.43
16	35.70	35.70	28.19	28.21	28.19	28.20	28.23	28.02	28.07	28.34	25.66	28.23	28.61	28.61
17	218.52	218.38	56.17	56.22	56.20	55.95[j]	56.24	56.12	56.16	54.97	54.20	54.79	55.80[+]	55.83[+]
18	14.29	14.36	12.06*	12.06	12.01	12.10	12.07	12.01	11.99	12.03	12.64	12.07	12.21	12.21
19	11.94	22.99	12.12*	12.76	14.35	14.96	24.60	12.56	22.48	10.97	10.97	22.42	11.53	22.65
20	–	–	35.76	35.76	35.74	35.77	35.75	35.68	35.68	35.61	73.23	35.20	35.63	35.64
21	–	–	18.65	18.66	18.66	18.67	18.68	18.63	18.66	18.74	20.02	18.36	18.79	18.82
22	–	–	36.11	36.13	36.12	36.13	36.12	36.06	36.07	36.09	–	31.03	36.14	36.16
23	–	–	23.80	23.81	23.80	23.82	23.81	23.80	23.89*	23.73	–	30.95	23.77	23.80
24	–	–	39.49	39.50	39.48	39.49	39.47	39.45	39.45	39.43	–	174.60	39.48	39.49
25	–	–	28.00	28.01	27.99	28.00	27.98	27.99	28.00	27.95	–	–	27.99	28.01
26	–	–	22.81	22.80	22.79	22.81	22.80	22.79	22.79	22.76	–	–	22.79	22.80
27	–	–	22.55	22.55	22.54	22.55	22.54	22.54	22.54	22.51	–	–	22.54	22.55

Additional signals: [a] CHO: 160.73; [b] CHO: 160.71; [c] COC_2H_5: 174.05, 27.91, 9.31; [d] COC_2H_5: 174.06, 27.88, 9.03; [e] COC_3H_7: 173.27, 36.56, 18.58, 13.72; [f] COC_6H_{13}: 173.42, 34.65, 31.46, 28.84, 25.04, 22.51, 14.03; [g] COC_6H_{13}: 173.39, 34.58, 31.43, 28.79, 25.00, 22.47, 13.98; [h] CHO: 161.16; [i] $COCH_3$: 171.20, 21.11; [j] $COCH_3$: 171.13, 21.09; [k] COC_2H_5: 176.10, 27.80, 9.20; [l] COC_2H_5: 174.48, 27.78, 9.23; [m] COC_3H_7: 173.74, 36.50, 18.58, 13.68; [n] COC_3H_7: 173.72, 36.48, 18.58, 13.67; [o] COC_6H_{13}: 173.92, 34.60, 31.46, 28.80, 25.08, 22.49, 14.02; [p] COC_6H_{13}: 173.93, 34.60, 31.46, 28.80, 25.08, 22.49, 14.02; [q] $OCOCH_3$: 170.01, 21.77; [r] $OCOCH_3$: 169.78, 21.39; [s] $COCH_3$: 170.13, 20.78; [t] $COCH_3$: 169.97, 20.85; [u] COC_6H_5: 165.66, 130.70, 128.63, 129.61, 132.78; [v] OCH_3: 51.49; [w] OCH_2OCH_3: 97.32, 55.26; [x] OCH_2OCH_3: 97.37, 55.33. *,[+] The assignment of the signals with the same symbols may be interchanged; ? the positions of the signals were not determined.

3 H (3 × H-21); 0.86 d, 6 H, *J* = 6.4 (3 × H-26 + H-27); 0.71 s, 3 H (3 × H-18). For $C_{29}H_{50}O_3$ (446.7) calculated: 77.97% C, 11.28% H; found: 78.14% C, 11.22% H.

6β-Hydroxy-5α-cholestan-3-one (**48a**)

Palladium on calcium carbonate (60 mg, 5%) was added to a solution of 6β-hydroxycholest-4-en-3-one (**59**; 140 mg, 0.35 mmol) in ethyl acetate (4 ml) and ethanol (1.5 ml) and the mixture was stirred under slight overpressure of hydrogen at room temperature for 4 h. The catalyst was filtered off and washed with ethanol. Preparative TLC of the residue afforded 6β-hydroxy-5α-cholestan-3-one (**48a**; 10 mg, 7%), m.p. 189–191 °C (ref.[21]: m.p. 189–191 °C).

6β-Hydroxy-5β-cholestan-3-one (**48b**)

A preparative TLC of the products from the above experiment afforded lipophilic 6β-hydroxy-5β-cholestan-3-one (**48b**; 112 mg, 80%), m.p. 116–119 °C (ref.[20]: m.p. 116–117 °C).

5α-Cholestane-3,6-dione (**49a**)

The Jones reagent was added dropwise to a solution of hydroxyketone **48a** (55 mg, 0.14 mmol) until the yellowish colour persisted for at least 5 min. Methanol (0.5 ml) was then added, and, after another 15 min, the reaction mixture was diluted with water, the solid isolated by filtration and dried in the air. The yield of diketone **49a** was 52 mg (90%), m.p. 169–172 °C, $[\alpha]_D$ +4 (*c* 0.3) (ref.[21]: m.p. 168–170 °C, $[\alpha]_D$ +10 (*c* 0.9)).

5β-Cholestane-3,6-dione (**49b**)

The same procedure as described in the previous experiment was used for the oxidation of hydroxyketone **48b** (20 mg, 0.05 mmol). The yield of product **49b** was 18 mg (89%), m.p. 184–185 °C (ref.[22]: m.p. 175–179 °C, $[\alpha]_D$ −79 (*c* 0.3)).

(20*R*)-3,7-Dioxo-5α-pregnan-20-yl Benzoate (**51a**)

Compound **51a** was obtained as a by-product from the reaction[8] of 3β-tosylate **60** with sodium nitrite in DMF. The yield 9%, m.p. 236–239 °C, $[\alpha]_D$ −57 (*c* 0.8). IR: 1 707 (C=O); 1 284 (C–O). For $C_{28}H_{36}O_4$ (436.6) calculated: 77.03% C, 8.31% H; found: 76.82% C, 8.22% H.

3-Oxo-5α-cholestan-2α-yl Acetate (**44a**) and 3-Oxo-5α-cholestan-2β-yl Acetate (**45a**)

Compounds **44a** and **45a** were synthesized according to the procedure described in ref.[23]. The melting points and optical rotations were in agreement with literature values. 3-Oxo-5α-cholestan-2α-yl acetate (**44a**): m.p. 144–146 °C, $[\alpha]_D$ +92 (*c* 0.5); 3-oxo-5α-cholestan-2β-yl acetate (**45a**): m.p 124–125 °C, $[\alpha]_D$ +57 (*c* 0.4).

17β-Hydroxy-5α-estran-3-one (**35a**) and 17β-Hydroxy-5α-estran-3-one (**35b**)

A solution of ketone **61** (320 mg, 1.17 mmol) in benzene (25 ml) was stirred in the presence of palladium on calcium carbonate (5%, 40 mg) under hydrogen at room temperature. After

6 h, the catalyst was filtered off and the filtrate was evaporated *in vacuo*. Chromatography of the residue (320 mg) on silica gel (light petroleum–ether, 9 : 1) yielded ketone **35a** (74%), m.p. 109–111 °C, $[\alpha]_D$ +33 (*c* 0.8) (ref.[24]: m.p. 111 °C, $[\alpha]_D$ +31), and ketone **35b** (7%), m.p. 132–134 °C, $[\alpha]_D$ +53 (*c* 0.8) (ref.[24]: m.p. 133–133.5 °C, $[\alpha]_D$ +52.8).

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