135. Dissimilar Behaviour of 3β,16α-Dihydroxy-5α-androstan-17-one Diacetate under Basic and Acidic Conditions

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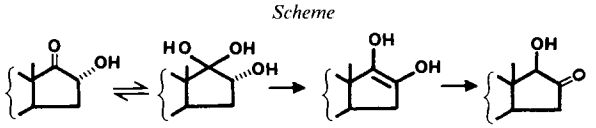
The base-catalysed rearrangement of 3β,16α-dihydroxy-5α-androstan-17-one diacetate (**1**) in (D_6)benzene/CD_3OD to 3β,17β-dihydroxy-5α-androstan-16-one (**3**) is followed by ^{13}C-NMR spectroscopy. By the same procedure, it is determined that in (D_6)benzene/CD_3OD, but under acid catalysis, **1** does not rearrange to **3** but yields the intermediate product 3β,16α-dihydroxy-5α-androstan-17-one 17α-methyl hemiacetal (**5**).

Introduction. – α-Hydroxy ketones rearrange under acidic or basic conditions to give isomeric hydroxy ketones [1] [2], and the rearrangement of 16-hydroxy-17-oxo steroids to their 17β-hydroxy-16-oxo isomers belong to this type of reaction. Observations on the relative stability of those steroidal ring-D hydroxy ketones have led to mechanistic studies which show that the lability of the 16β-hydroxy-17-oxo compound in both media is generated by conformational ring-D changes (rigid envelope to flexible half-chair) [3]. It has been also demonstrated that in both media, the isomerisation occurs through an intramolecular 1,2-hydride shift [4] [5].

Although 16α-hydroxy-17-oxo steroids have been shown to be more stable than their 16β-epimers, the former structures are also transformed, under basic conditions, into 17β-hydroxy-16-oxo steroids, but at a slower rate [6]. On the other hand, it has been reported that under acidic conditions, 16α-hydroxy-17-oxo steroids do not rearrange to the isomeric hydroxy ketone [6].

The simple enolisation initially proposed for the base-induced rearrangement of 16α-hydroxy-17-oxo steroids [6] has been later discarded by mass-spectroscopic analysis of ^{18}O-labelled substrates [7]. As a consequence, a new 17-hydration-dehydration mechanism was postulated for this isomerization *(Scheme)*.

Scheme



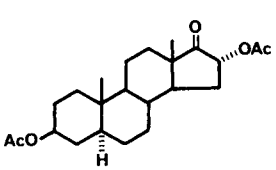
In order to obtain a definitive proof for the proposed mechanism, we now followed the course of the isomerization by ^{13}C-NMR spectroscopy. For this purpose, a catalytic amount of either base or acid was added to 3β,16α-dihydroxy-5α-androstan-17-one diacetate (**1**) in (D_6)benzene/CD_3OD 1:1. The ^{13}C-NMR spectra were analysed taking into account that resonances of specifically deuteriated C-atoms in ^{1}H broad band

decoupled ^{13}C-NMR spectra are usually not differentiated from the background due to spin-spin splitting, quadrupole broadening, and the reduction of signal-to-noise ratio for the lack of *Overhauser* effects [8].

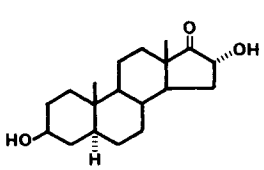
Results. – *Base Catalysis.* The ^{13}C-NMR spectrum ((D$_6$)benzene/CD$_3$OD 1:1) of **1** is given in (*Table 1* and *Fig. 1a*). After addition of a catalytic amount of KOD to the solution, the changes that took place were followed by ^{13}C-NMR (*Fig. 1b* and *1c*) and compared with the spectra of pure 3β,16α-dihydroxy-5α-androstan-17-one (**2**; *Fig. 1d*) and 3β,17β-dihydroxy-5α-androstan-16-one (**3**; *Fig. 1e*). *Fig. 1b* shows that signals from the 16α-acetoxy-17-oxo system **1**, mainly those of C(18) at 14.2 ppm and C(16) at 73.0 ppm, have disappeared while those from the 16α-hydroxy-17-oxo system **2** have appeared (C(18) at 14.4 ppm and C(16) at 71.3 ppm), and that the original signal of C(3) at 74.1 ppm is unchanged. *Fig. 1b* also presents signals from the isomerized product **3** (*cf. Fig. 1e*), except for the signal of C(17), expected to appear at 86.6 ppm. After 12 h reaction (*Fig. 1c*), compound **1** is almost completely transformed into **3**, as shown by the spectra of *Fig. 1c* and *1e* which are almost identical except for the missing signal at 86.6 ppm.

Acid Catalysis. A catalytic amount of D_2SO_4 was added to the solution of compound **1** in (D$_6$)benzene/CD$_3$OD 1:1, and the ^{13}C-NMR spectra were recorded at increasing times and compared with that of **2** (*Fig. 2*). In *Fig. 2b*, the signals attributed to C(3) and C(16) of **1** (74.1 and 73.0 ppm, resp.; *Fig. 2a*) have their intensity diminished, while the signals corresponding to the same C-atoms of **2** (*Fig. 2f*) have appeared (70.7 and 71.3 ppm, resp.). After 10 h (*Fig. 2c*), two new signals (at 74.9 and 105.6 ppm) have appeared, in addition. The evolution of the reaction to the unknown product responsible for these two signals is accompanied by the disappearance of compound **2**, as clearly indicated by *Fig. 2d* and *2e*. No signals of the rearranged product **3** are detected during the experiment (200 h).

The following facts indicate that the new compound detected (*Fig. 2e*) might be the fully methyl-deuteriated 17,17-dimethyl acetal corresponding to **4**: The SFORD spectrum indicates a *s* for the signal at 105.6 ppm, and its chemical-shift value and the reaction medium (C_6D_6/CD_3OD/D_2SO_4) would be in accordance with such an acetal. However, we prepared the protonated analogue **4** which had been previously synthesised (no



1



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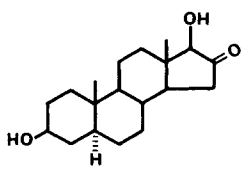

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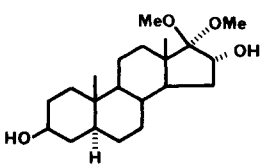

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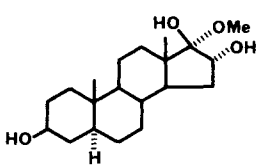

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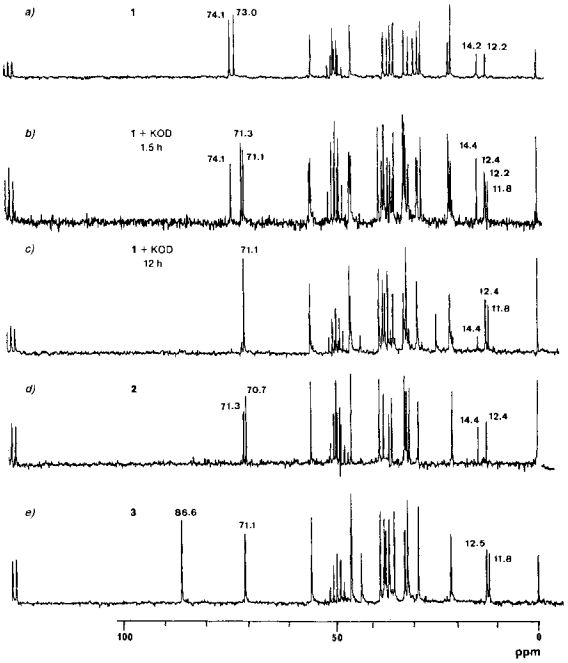

Fig. 1. *Partial* (0–120 ppm) *^{13}C-NMR spectra*
a) *of* **1**, *without KOD addition*, b) *of* **1**, *1.5 h after KOD addition*, c) *of* **1**, *12 h after KOD addition*, d) *of* **2**, *and* e) *of* **3**

^{13}C-NMR spectrum) [9] and analysed it by ^{13}C-NMR spectroscopy (see *Table 1* and *Fig. 2g*). Comparison of its ^{13}C-NMR spectrum with that of the unknown compound *(Fig. 2e)* indicates several differences.

In order to identify the unknown product, compound **1** was dissolved in benzene/CH_3OH 1:1 containing a microdrop of H_2SO_4. After 7 days, the product was isolated and the mixture separated by HPLC in compound **2** (15%) and the unknown product (85%). Analysis by ^{1}H-NMR ($CDCl_3$, (D_5)pyridine, and (D_6)benzene/CD_3OD 1:1) and by ^{13}C-NMR spectroscopy ((D_6)benzene/CD_3OD 1:1) established the hemiacetal structure **5** for the unknown product. Indeed, the CH_3O function of **5** appears as a *s* at *ca.* 3.25–3.35 (depending on the solvent) in the ^{1}H-NMR *(Table 2)* and gives rise to a signal at 50.9 ppm in the ^{13}C-NMR spectrum. In $CDCl_3$ solution, **5** shows other characteristic ^{1}H-NMR signals for a steroid system like those of CH_3(18) and CH_3(19) at 0.87 and 0.81 ppm, respectively. When this *same* solution was analysed by ^{13}C-NMR spectroscopy, signals identical to that of **2** resulted, indicating that in $CDCl_3$ solution, the postulated hemiacetal **5** had been transformed into **2**. Indeed, reexamination of the *same* solution by ^{1}H-NMR yielded a spectrum with the signals of CH_3(18) and CH_3(19) at 0.94 and 0.83 ppm, respectively, *i.e.* at the values determined for the angular CH_3 groups of **2**, and with a signal at 3.38 ppm arising from CH_3OH produced in the transformation **5**→**2**, instead of the original CH_3O signal at 3.26.

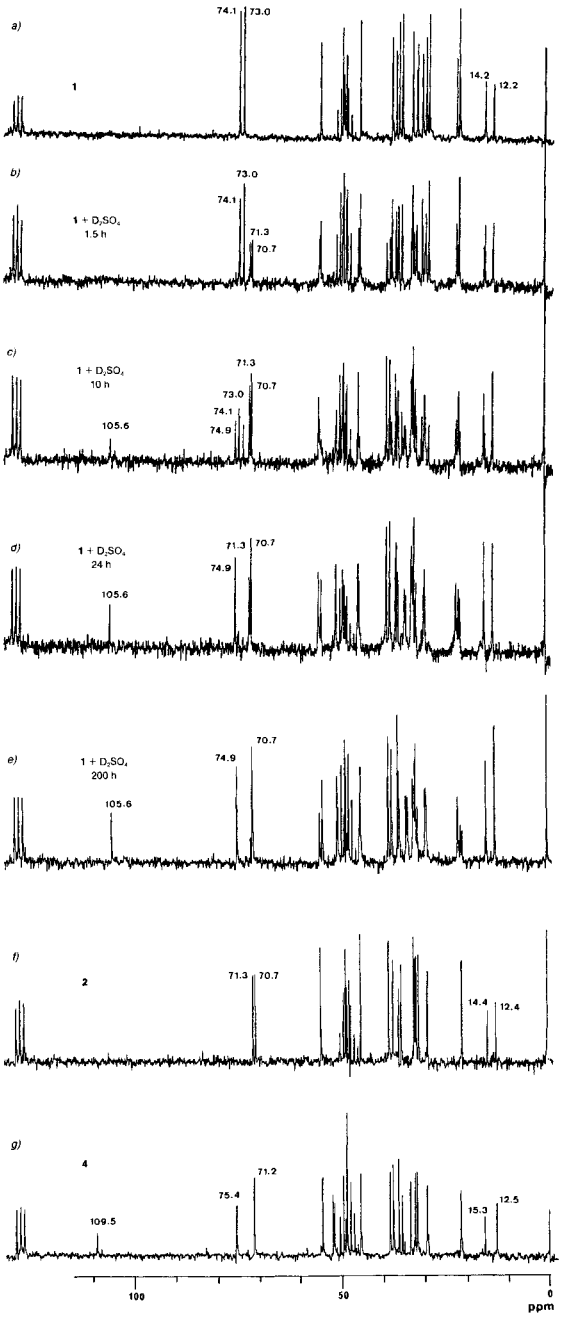

Fig. 2. *Partial* (0–120 ppm) *^{13}C-NMR spectra* a) *of* **1**, *without D_2SO_4 addition*, b) *of* **1**, *1.5 h after D_2SO_4 addition*, c) *of* **1**, *10 h after D_2SO_4 addition*, d) *of* **1**, *24 h after D_2SO_4 addition*, e) *of* **1**, *200 h after D_2SO_4 addition*, f) *of* **2**, *and* g) *of* **4**

Table 1. *^{13}C-NMR Data* ((D_6)benzene/CD_3OD 1:1, 1% TMS) *of Compounds* **1–4**

	1	**2**	**3**	**4**
C(1)	37.0	37.4	37.3	37.6
C(2)	27.9	31.6	31.6	31.7
C(3)	74.1	70.7	71.1	71.2
C(4)	34.4	38.4	38.3	38.4
C(5)	44.9	45.2	45.2[a])	45.5
C(6)	28.6	28.9	29.0	29.3
C(7)	31.9	32.2	32.2	32.1
C(8)	35.2	35.3	34.8	35.3
C(9)	54.4	54.8	54.8	54.6
C(10)	35.9	36.0	36.1	36.1
C(11)	20.5	20.6	21.0	21.1
C(12)	30.8	31.9[a])	36.9	33.3
C(13)	48.7	48.0	42.9	49.1
C(14)	49.0	48.8	45.3[a])	48.8
C(15)	29.6	31.1[a])	36.2	36.2
C(16)	73.0	71.3	217.8	75.4
C(17)	214.4	219.3	86.6	109.5
C(18)	14.2	14.4	11.8	15.3
C(19)	12.2	12.4	12.5	12.5
CH_3COO	20.5	–	–	–
	21.1	–	–	–
CH_3COO	171.2	–	–	–
	170.7	–	–	–
CH_3O	–	–	–	51.8
				52.2

[a]) Values may be interchanged.

Table 2. *Some ^{1}H-NMR Data of* **2**, **5**, *and* **2**/**5**[a]) *in $CDCl_3$ and (D_5)Pyridine.* δ Values in ppm from TMS.

	2			**2**/**5**[a])			**5**[b])
	$CDCl_3$	C_5D_5N	PIS[c])	$CDCl_3$	C_5D_5N	PIS[c])	$CDCl_3$
CH_3(18)	0.946	0.886	–0.060	0.936	0.888	–0.048	–
CH_3(19)	0.828	0.797	–0.031	0.824	0.802	–0.022	–
CH_3(18)	–	–	–	0.802	0.974	0.172	0.810
CH_3(19)	–	–	–	0.865	0.824	–0.041	0.872
CH_3O	–	–	–	3.258	3.351	0.093	3.265

[a]) Final mixture of **2** and unknown product **5** obtained by acid catalysis in protonated solvents.
[b]) Isolated unknown product **5**.
[c]) PIS = Pyridine-induced shift.

Discussion. – *Base Catalysis.* The rearrangement of the 16α-hydroxy-17-oxo steroid under study to the 17β-hydroxy-16-oxo isomer occurs easily under basic conditions. That the saponification of the AcO group at C(3) of **1** is slower than that at C(16) is shown by the persistence of the ^{13}C-NMR signal of C(3) in *Fig. 1b* when compared to *Fig. 1a*. This difference could be explained by the additional electronic deficit of the 16α-AcO due to the inductive effect of the carbonyl group at C(17). The presence in *Fig. 1b* of a signal attributable to C(16) bearing an α-OH group (at 71.3 ppm) indicates that the system

16α-hydroxy-17-oxo is more stable in basic medium than the 16β-hydroxy-17-oxo epimer which rearranges almost immediately to the 17β-hydroxy-16-oxo system [4] [5].

It is noteworthy that the rearranged compound **3** is already present in the mixture a short time after the beginning of the experience (see the signal at 11.8 ppm (C(18) in *Fig. 1b*). The absence of the signal at 86.6 ppm (C(17)) of **3** in *Fig. 1b* and *1c* could be explained by the fact that H_α–C(17) has been replaced by a D-atom on enediol formation. However, after 12 h, compound **1** has been completely converted into **3** without showing any signals arising from a triol or enediol *(Scheme)*. Thus, our results do not confirm without any doubt the operation of the proposed hydration-dehydration mechanism [7] under basic conditions.

Acid Catalysis. The spectra *(Fig. 2)* show that, under acid catalysis, the hydrolysis of both acetyl groups of **1** have comparable rates. As it has been previously reported [6], the hydroxylated product **2**, within 200 h, does not rearrange to **3**. Instead, the intermediate 17α-methyl hemiacetal **5** is formed as main product. Its C(17) configuration is established by analysis of the ^{1}H-NMR chemical shifts of CH_3(18) and CH_3(19) in $CDCl_3$ and in (D_5)pyridine and by correlating the pyridine-induced shifts (PIS) of these signals with the ones of structures having different chirality at C(17) [10–12]. The instability of **5** in $CDCl_3$ solution (→ **2**) is produced by traces of acid.

The formation of **5** from **1** inhibits the prosecution of the rearrangement to **3** since for the enediol formation (see *Scheme 1*), a β-elimination of MeOH should occur. Although this elimination should be produced between substituents oriented *trans* and quasidiaxial, it does not take place, probably because of the inappropriate medium conditions [13] and the possible H-bond formation between the CH_3O and the 16α-OH group. The existence of this H-bond has been ascertained by molecular mechanics calculation using the MM2 program [14]. On the other hand, the reaction conditions are unsuitable for promoting a *cis* dehydration reaction.

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Experimental Part

^{1}H- and ^{13}C-NMR Spectra: at 100.1 and 25.2 MHz, resp., *Varian-XL-100-15* spectrometer operated in the FT mode using a *620L-100* computer interfaced to a *Sykes* 7000 dual disk drive. Samples were spun in 5-mm tubes at 27°. ^{13}C-NMR spectra were measured over a spectral width of 5852 Hz, using 45° pulses and a pulse-repetition rate of 0.701 s. A 8-K data table was used giving spectra after *Fourier* transformation with a resolution of 1.53 Hz per point. Fully ^{1}H-decoupled spectra were the result of 4000 pulses and obtained by irradiation of the ^{1}H-NMR spectrum at a central frequency of 4 ppm with the irradiation frequency modulated by an external swept square-wave modulator. Lines were artificially broadened to *ca.* 1 Hz by exponential weighting of the FID. For the sequencial studies, **1** (70 mg) was dissolved in C_6D_6/CD_3OD 1:1 containing 1% of TMS as internal standard. After recording of the first spectrum, a microdrop (50 μl) of either KOD (40% in D_2O) or D_2SO_4 was added, and spectra were registered at indicated times.

Compounds **1**–**4** were synthesised by the procedures referred to below; their structures were confirmed by spectroscopic methods (IR, ^{1}H-NMR, MS): 3β,16α-dihydroxy-5α-androstan-17-one diacetate (**1**) [15], 3β,16α-dihydroxy-5α androstan-17-one (**2**) [16], 3β,17β-dihydroxy-5α-androstan-16-one (**3**) [16], and 3β,16α-dihydroxy-5α-androstan-17-one 17,17-dimethyl acetal (**4**) [9]. Assignments of their ^{13}C-NMR signals were carried out by comparison to related compounds and by the attached-proton-test (APT) technique.

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