[Chem. Pharm. Bull. 35(12)4763—4768(1987)]

Stereospecific 1,2-Hydride Shift in the Rearrangement of 16β-Hydroxy-17-oxo Steroids to 17β-Hydroxy-16-ones with Acid and Base[1)]

MITSUTERU NUMAZAWA,* MASAO NAGAOKA, and AYAKO MUTSUMI

Tohoku College of Pharmacy, 4–1 Komatsushima-4-chome, Sendai 983, Japan

(Received May 25, 1987)

When 16β-hydroxy-5α-androstan-17-one (**2a**) and its 16α-deuterio derivative (**2a**-*16-d*) were separately treated with H_2SO_4 or NaOH, compound **2a** was rearranged to the 17β-hydroxy-16-oxo isomer (**3a**) with a marked kinetic deuterium isotope effect at the 16-position ($k_H/k_D=4.5$ or 3.0). The product **3a** obtained from compound **2a**-*16-d* retained deuterium at C-17 to the extent of 16—65% while no significant loss of the isotope from the substrate was observed during the reaction. Isotope-labeling experiments showed that the intramolecular 1,2-hydride shift is principally involved in the ketol rearrangement, and that the 16-oxo function of compound **3a** enolizes preferentially toward the C-17 position rather than the C-15 position under the above conditions.

Keywords——3,16β-dihydroxy-5α-androstan-17-one; [16α-^{2}H]3β,16β-dihydroxy-5α-androstan-17-one; 3β,17β-dihydroxy-5α-androstan-16-one; ketol rearrangement; isotope effect; 1,2-hydride shift; enolization

Previous studies on the relative stability[2)] of the four isomeric 16,17-ketols of steroids of the 14α-series have demonstrated that the most unstable ketols, 16β-hydroxy-17-ones, readily isomerize to the most stable ones, 17β-hydroxy-16-ones, in basic or acidic medium. Two mechanisms are feasible for the rearrangement. One is the conventional enolization mechanism[2b,c)] where the 17-oxo function of the 16β-alcohol can give the ene-diol intermediate, and ketonization may then give either the original or rearranged ketol (mechanism A, Fig. 1). In this mechanism, the equilibrium will be strongly displaced toward the 16-ketones, which are more thermodynamically stable.[2c)] The alternative mechanism, proposed for the acid-catalyzed rearrangement by Johnson *et al.*,[3)] proceeds through a sequence involving a reversible stereospecific 1,2-hydride shift in the protonated form (mechanism B). Loss of a proton from the intermediate finally affords the 16-ketones, which predominate at equilibrium. However, to date, there is no direct evidence for this mechanism. Thus, to reach an unambiguous decision as to whether or not the alternative sequence is operative in the

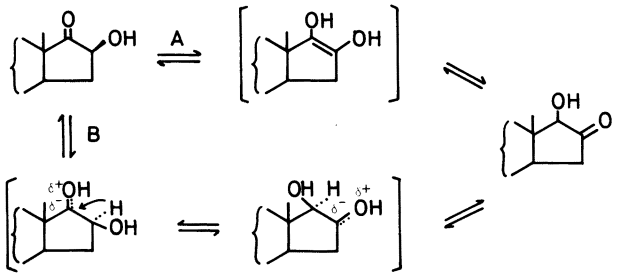


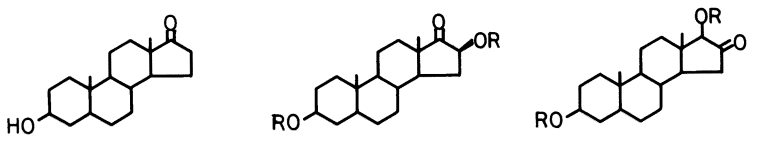
Fig. 1. Mechanisms Proposed for Rearrangement of 16β-Hydroxy-17-oxo Steroids under Basic and Acidic Conditions

rearrangement, we synthesized 16α-deuterio-16β-hydroxy-17-one **2a**-*16-d* and explored its conversion into the 17β-hydroxy-16-oxo derivative **3a** with H_2SO_4 and NaOH. The isotope-labeling experiments definitely demonstrated that stereospecific 1,2-hydride shift of the 16α-proton is actually involved in both acid- and base-catalyzed rearrangements.

Results and Discussion

Treatment of 3β-hydroxy-5α-androstan-17-one (**1**) with NaOD in D_2O–MeOD[4] gave its 16,16-dideuterated form, which was subsequently converted into [16α-^{2}H]3β,16β-dihydroxy-5α-androstan-17-one (**2a**-*16-d*)[3] *via* the 16β-acetoxy-17-oxo derivative **2b**-*16-d* essentially according to the methods reported previously.[5]

Dynamic aspects of rearrangement of the 16β-hydroxy-17-one **2a** to the 17β-hydroxy-16-oxo derivative **3a** with H_2SO_4 and NaOH were initially explored, especially in view of the deuterium isotope effect at the 16-position on the conversion of the deuterium-labeled substrate **2a**-*16-d*. The proton nuclear magnetic resonance (^{1}H-NMR) spectra of the ketols **2a** and **3a** proved to be useful for the quantitative analysis of the reaction mixtures without isolation. The signals at δ 0.93 (s, 3H) and 3.93 (m, 1H) due to the 16β-alcohol **2a**, and 0.73 (s, 3H) and 3.73 (s, 1H) due to the 17β-alcohol **3a** are those of the proton at the C-18 angular methyl and the proton at C-16 or C-17, respectively. When the 16β-alcohol **2a** and its 16-deuterio derivative **2a**-*16-d* were separately treated with 3 M H_2SO_4 in 75% MeOH at room temperature, the substrates slowly rearranged to the 17β-alcohol **3a**. As shown in Fig. 2, the rearrangement occurs with a marked kinetic deuterium isotope effect at the 16-position. The reaction time needed for the 50% conversion was about 2.5 d for compound **2a** and about 14 d



1

2a: R=H
2b: R=$COCH_3$

3a: R=H
3b: R=$COCH_3$

Chart 1

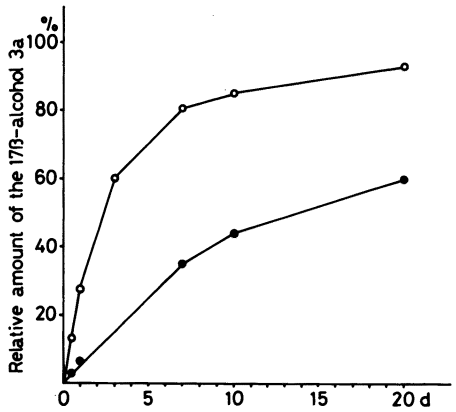


Fig. 2. Conversion of the 16β-Hydroxy-17-one **2a** (○) and Its 16α-Deuterio Derivative **2a**-*16-d* (●) to the 17β-Hydroxy-16-one **3a** with 3 M H_2SO_4

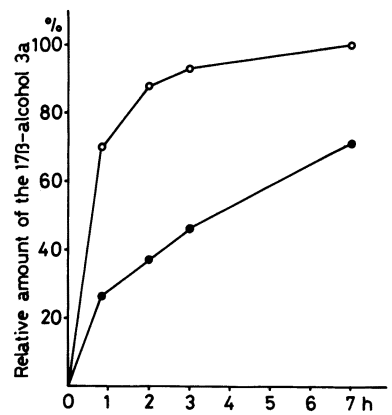


Fig. 3. Conversion of the 16β-Hydroxy-17-one **2a** (○) and Its 16α-Deuterio Derivative **2a**-*16-d* (●) to the 17β-Hydroxy-16-one **3a** with NaOH

for its deuterio derivative **2a**-*16-d*. The apparent isotope effect[6] observed in experiments using a one-day reaction time was about 4.5.

Conversion of the 16β-alcohol **2a** to the isomeric 17β-alcohol **3a** was smoothly catalyzed by 0.025 M NaOH in 75% MeOH, and 7-h reaction time was enough for almost complete rearrangement. The deuterium isotope effect at the 16-position in the conversion was about 3.0 (at 1 h) (Fig. 3). The k_H/k_D value was slightly different from that in the case of acid treatment (3.0 *vs.* 4.5). The results demonstrated that the breaking of the C–H bond at the C-16 position is involved in the rate expression[3a] of the rearrangement with both acid and base.

The deuterium contents of the recovered and rearranged ketols **2a** and **3a** obtained by treatment of the deuterated substrate **2a**-*16-d* with H_2SO_4 and NaOH were analyzed by mass (MS) and ^{1}H-NMR spectroscopies (Table I). We reasoned that the primary product **3a** produced through the 1,2-hydride shift process would retain deuterium at C-17 to the same extent as the starting material, and that the product **3a** formed through the conventional enolization mechanism would not be labeled with deuterium at C-17. These spectra showed 16—65% deuterium-labeling at the 17α-position of the ketol **3a**, while the isotope was completely retained in the recovered substrate **2a**. The isotope content of compound **3a** produced in experiments using a prolonged reaction time or drastic conditions was lower as compared to that with a short time or mild conditions. Furthermore, when the 17α-deuterio derivative of compound **3a** (57 atom%), obtained by the reaction of the 16β-alcohol **2a**-*16-d* with 0.025 M NaOH (Table I), was treated with NaOH or H_2SO_4 under the conditions employed in the rearrangement experiment, about 30 or 20% of the deuterium was lost from the labeled substrate **3a**-*17-d* (Table II). On the other hand, treatment of the 17β-alcohol **3a** with the deuterated acid or base efficiently introduced the isotope at C-17 of the 17β-alcohol **3a** (83 or more than 98 atom%), as shown in Table II.

From the results obtained above, it is concluded that the intramolecular 1,2-hydride shift mechanism is principally operative in the rearrangement of 16β-hydroxy-17-ones to 17β-hydroxy-16-ones with base and acid, and that the enolization process accounts for only a minor fraction even if it is operative (Fig. 4). It is well known that with acid only 16β-hydroxy-

TABLE I. Analysis of Deuterium Content of the Ketols **2a** and **3a** Obtained by Treatment of the Deuterated Ketol **2**-*16-d* with NaOH and H_2SO_4

Conditions Base or acid[c]	Time	Relative amounts of products[a] (%)		^{2}H-Content[b] (atom%)	
		2a	**3a**	**2a**	**3a**
Substrate: **2a**-*16-d* (90 atom%)					
3 M H_2SO_4	10 d	58	42	91	63
3 M H_2SO_4	20 d	40	60	90	36
0.025 M NaOH	1 h	74	26	89	65
0.025 M NaOH	7 h	30	70	—	57
1 M NaOH	1 h	0	100	—	46
1 M NaOH	3 h	0	100	—	16
Substrate: **2b**-*16-d* (90 atom%)[d]					
3 M H_2SO_4	25 d	37	63	—	31
1 M NaOH	1 h	0	100	—	50

a) The relative amounts of products were determined by measuring the peak height of the C-18 angular methyl resonance in the ^{1}H-NMR spectrum of the reaction mixtures without isolation. *b*) The deuterium content was determined by MS analysis (*m/z* 306 and 307, M^+) and the location of the isotope was found by ^{1}H-NMR analysis. *c*) For this treatment, 3 M H_2SO_4 (6 ml) was added to a solution of the substrate (0.35 mmol) in 14 ml of MeOH, or 0.025 or 1 M NaOH (4 ml) was added to that in 40 ml of 60% aqueous MeOH. *d*) The relative amounts of products and ^{2}H-contents were determined after derivatization of the products to the acetates **2b** and **3b**. MS: M^+ *m/z* 390 and 391.

TABLE II. Enolization of the Ketol **3a** with NaOD and D_2SO_4

Substrate	Conditions Base or acid[a)]	Time	^{2}H-Content at C-17 of **3a** atom% (^{2}H-distribution)
3a	3 M D_2SO_4[b)]	15 d	83[c)] (13% d_0, 46% d_1, 41% d_2)
3a	1 M NaOD[b)]	1 h	98[c)] (5% d_2, 95% d_3)
3a-*17-d*	3 M H_2SO_4[d)]	7 d	46[e)] (54% d_0, 46% d_1)
3a-*17-d*	1 M NaOD[d)]	1 h	40[e)] (60% d_0, 40% d_1)

a) For this treatment, 3 M D_2SO_4 or 1 M NaOD was added to the reaction mixture using deuterated solvents as described in Table I. *b*) The ketol **3a** was treated with the deuterated acid or base under the rearrangement conditions using D_2O and MeOD as solvents. *c*) ^{2}H-Content was determined by ^{1}H-NMR analysis. *d*) The ketol **3a**-*16-d* (57 atom%) was exposed to the rearrangement conditions. *e*) ^{2}H-Content was determined by MS analysis.

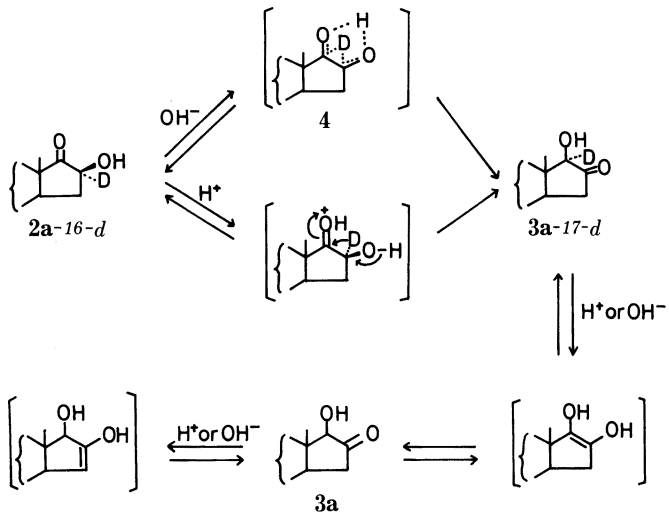


Fig. 4

17-ones are isomerized to 17β-hydroxy-16-ones, while the 16α-isomers are unchanged.[2c,3a)] To account for such a difference in the isomerization, the hydride shift mechanism was proposed for acid-catalyzed rearrangement.[3a)] The present results showed that the mechanism is operative in both acid- and base-catalyzed reactions. The reaction with an acid may principally proceed through a protonated form, as shown in Fig. 1, whereas with a base, it may proceed through transition state represented diagrammatically by **4** in Fig. 4 rather than the protonated form.

To our knowledge, no detailed study of enolization of the 16-oxo function of 17β-hydroxy-16-ones has previously been reported. Treatment of the 16-oxo derivative **3a** with the D_2O/NaOD/MeOD or D_2O/D_2SO_4/MeOD system led to the introduction of deuterium at C-17 at a rate greater than that at C-15 in each experiment (Table II), in accordance with the previous results[7)] on 17-unsubstituted 16-oxo steroids. Johnson *et al.*[3a)] assumed the 1,2-hydride shift process to be reversible. We could detect only enolization of the 16-oxo function of the ketol **3a**; hence there may be no apparent reversible reaction under the conditions employed.

When a deuterated 16β-acetoxy-17-one **2b**-*16-d* was treated with H_2SO_4 and NaOH as above, the rearranged product **3a** retained deuterium at C-17 to almost the same extent as the

16β-alcohol **2a** (Table I). It has been reported that α-acetoxyketone may rearrange during chromatography on alumina to give more stable isomers[3b,8] through an *ortho*-ester of the ene-diol. The present results show that this process involves first hydrolysis of the acetoxy group leading to the 16β-alcohol **2a**, followed by ketol rearrangement principally through the 1,2-hydride shift process as described above.

Recently we[9] proposed a new hydration–dehydration mechanism for the rearrangement of 16α-hydroxy-17-ones to 17β-hydroxy-16-ones with base. The case for the enolization mechanism, if not invalidated, is also weakened in that reaction. The present results along with the our previous findings[9] suggest that rearrangement of steroidal D-ring ketols has interesting theoretical implications. Further studies on the ketol rearrangement are in progress in this laboratory.

Experimental

Melting points were measured on a Yanagimoto melting point apparatus. ^{1}H-NMR spectra were obtained on a JEOL PMX 60 spectrometer with tetramethylsilane as an internal standard. Mass spectra were measured on a Hitachi RMU-7 spectrometer.

[16,16-$^2H_2$]3β-Hydroxy-5α-androstan-17-one (1-*16,16-d*$_2$)——A mixture of **1** (1.0 g, 3.45 mmol) was heated under reflux for 4 h with 20 ml of CH_3OD, 1.0 g (43.5 mmol) of Na metal and 3 ml of D_2O essentially according to the method reported by Tokes *et al.*[4] After this time, the reaction mixture was poured into 100 ml of chilled 6 M HCl solution. The precipitate was collected by filtration, washed with water and recrystallized from acetone to give the desired material **1**-*16,16-d*$_2$ (96%) as colorless needles, mp 173—174 °C (lit.[10] 172—174 °C, reported for non-labeled **1**), which consisted of the following mixture as determined by MS using the M^+ ion peak: 3% d_0, 6% d_1, 91% d_2.

[16α-^{2}H]3β,16β-Diacetoxy-5α-androstan-17-one (2b-*16-d*)——The deuterated compound **1**-*16,16-d*$_2$ (900 mg, 3.10 mmol) was converted to **2b**-*16-d* (36%) according to the previously reported method,[3a] mp 154—156 °C (lit.[3a] 153—155.5 °C, reported for non-labeled **2b**). 10% d_0 and 90% d_1 by MS. ^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, s, 19-Me), 0.93 (3H, s, 18-Me), 2.00 (3H, s, 3β-OAc), 2.10 (3H, s, 16β-OAc), 4.67 (1H, br m, 3α-H).

[16β-^{2}H]3β,16β-Dihydroxy-5α-androstan-17-one (2a-*16-d*)——The diacetate **2b**-*16-d* (600 mg, 1.61 mmol) was hydrolyzed with H_2SO_4 to yield **2a**-*16-d* (45%) according to the method previously reported by Kincl,[5] mp 181—183 °C (acetone) (lit.[4] 195—200 °C ($CHCl_3$–ether), reported for non-labeled **2a**). 10% d_0 and 90% d_1 by MS. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, s, 19-Me), 0.93 (3H, s, 18-Me), 3.50 (1H, br m, 3α-H).

Rearrangement Experiments——(A) Acid Catalysis: Compound **2a** or **2**-*16-d* (0.35 mmol) was dissolved in 14 ml of MeOH, then 6 ml of 3 M H_2SO_4 was added. The solution was allowed to stand at room temperature for an appropriate time and then neutralized with 5% $NaHCO_3$ solution. After removal of most of MeOH under reduced pressure at below 30 °C, the product was extracted with AcOEt (100 ml × 3). The organic layer was washed with water and dried (Na_2SO_4). After evaporation of the solvent, the residue (75—90 mg) was submitted to ^{1}H-NMR analysis and then purified by fractional crystallization from aqueous MeOH to give pure **2a** and **3a**. **2a**: mp 180—182 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.93 (1H, m, 16α-H). **3a**: mp 200—203 °C (lit.[4] 202—205 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.73 (3H, s, 18-Me), 0.83 (3H, s, 19-Me), 3.53 (1H, br m, 3α-H), 3.73 (1H, s, 17α-H).

(B) Base Catalysis: Solutions of **2a** and **2**-*16-d* (0.35 mmol) in 40 ml of 60% aqueous MeOH were each admixed with 4 ml of NaOH solution and then allowed to stand at room temperature for an appropriate time. Acidification with 5% HCl solution was followed by extraction with AcOEt and the usual work-up. The crude residue (83—92 mg) obtained was submitted to ^{1}H-NMR analysis and fractional crystallization as above. When **2b**-*16-d* was used as a substrate, the crude residue was acetylated with pyridine (1 ml)–Ac_2O (0.5 ml). The crude acetate obtained by evaporation of the solvent under reduced pressure was purified by fractional crystallization from acetone–water to give pure **2b** and **3b**. **3b**: mp 181—183 °C (lit.[2b] 179—181 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.82 (3H, s, 18-Me), 0.87 (3H, s, 19-Me), 2.03 (3H, s, 3β-OAc), 2.17 (3H, s, 16β-OAc), 4.83 (1H, br m, 3α-H), 5.00 (1H, s, 17α-H).

Treatment of the 17β-Hydroxy-16-ones 3a and 3a-*16-d* with Acid or Base——The 17β-alcohol **3a** (20 mg, 0.71 mmol) was treated with 3 M D_2SO_4 (15 d) or 1 M NaOD (1 h) under the above rearrangement conditions using deuterated solvents (MeOD and D_2O). After work-up as above, the deuterated compound **3a** was quantitatively recovered in each experiment. D_2SO_4 treatment: **3a** (mp 201—203 °C) consisted of 13% d_0, 46% d_1 and 41% d_2 by MS; the ^{1}H-NMR spectrum showed that the 17α-H/17α-D ratio was about 17/83. NaOD treatment: **3a** (mp 200—202 °C) consisted of 5% d_2 and 95% d_3 by MS; the ^{1}H-NMR spectrum demonstrated that 17α-H of **3a** was almost completely exchanged for deuterium (more than 98%).

The 17β-alcohol **3a**-*16-d* (43% d_0 and 57% d_1), which was obtained by the rearrangement experiment using **2a**-*16-d* and H_2SO_4, was similarly treated with 3 M H_2SO_4 (7 d) and 1 M NaOH (1 h). The recovered **3a** was submitted to MS analysis. The 3 M H_2SO_4 treatment: **3a** (mp 199—203 °C) consisted of 54% d_0 and 46% d_1. The 1 M NaOH

treatment: **3a** (mp 200—203 °C) consisted of 60% d_0 and 40% d_1.

Acknowledgment We thank Professor Toshio Nambara and Dr. Kadzutake Shimada of Tohoku University for MS analysis.

References and Notes

1) A preliminary note was published in *J. Chem. Soc., Chem. Commun.*, **1982**, 530.
2) *a*) M. N. Huhman and M. H. Lott, *J. Am. Chem. Soc.*, **71**, 719 (1949); *b*) N. S. Leeds, D. K. Fukushima, and T. F. Gallagher, *ibid.*, **76**, 2943 (1954); *c*) J. Fishman, *ibid.*, **82**, 6143 (1960); *d*) S. Bernstein and E. W. Cantrall, *J. Org. Chem.*, **26**, 3560 (1961).
3) *a*) W. S. Johnson, B. Gastambide, and R. Pappo, *J. Am. Chem. Soc.*, **79**, 1957 (1957); *b*) D. N. Kirk and M. P. Hartshorn, "Steroid Reaction Mechanisms," Elsevier Publishing Co., Amsterdam, 1968, p. 388.
4) L. Tokes, R. L. LaLonde, and C. Djerassi, *J. Org. Chem.*, **32**, 1012 (1967).
5) F. A. Kincl, *J. Steroid Biochem.*, **7**, 419 (1976).
6) The k_H/k_D value shown in the text is not exact, because the deuterium content at C-16 of the ketol **2a**-*16-d* is not 100 atom% but 90 atom%. However, the value is assumed to be almost the same as that obtained using 100 atom%-labeled substrate.
7) J. Fishman, *J. Org. Chem.*, **27**, 1745 (1962).
8) L. F. Fieser and R. Stevenson, *J. Am. Chem. Soc.*, **76**, 1728 (1954); K. L. Williamson and W. S. Johnson, *J. Org. Chem.*, **26**, 4563 (1961); H. B. Henbest, D. N. Jones, and G. P. Slater, *J. Chem. Soc.*, **1961**, 4472.
9) M. Numazawa, M. Nagaoka, and Y. Osawa, *J. Org. Chem.*, **47**, 4024 (1982).
10) E. Elisberg, H. Vanderhaeghe, and T. F. Gallagher, *J. Am. Chem. Soc.*, **74**, 2814 (1962).