Catalysis and Mechanism of the Isomerization of a Δ^5-3-Keto Steroid

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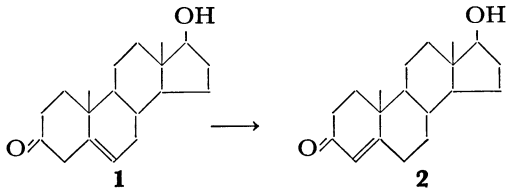
(Received February 25, 1977)

The acid- and base-catalyzed isomerizations of Δ^5- to Δ^4-testosterone have been kinetically studied in aqueous solution. Solvent isotope effects of $k_{H_3O^+}/k_{D_3O^+}=0.69$ and $k_{OH^-}/k_{OD^-}=3.1$ were obtained. It was thus concluded that the acid-catalyzed isomerization proceeds through rate-determining enolization but the base-catalyzed reaction through rate-determining protonation of an enolate ion. It was found that primary amines efficiently catalyze the isomerization *via* an iminium ion intermediate. Possible bifunctional catalysis was suggested for the high catalytic activity of polyfunctional primary amines.

The isomerization of β,γ-unsaturated ketones to their conjugated isomers has been shown to be catalyzed by both acids[1-6] and bases.[4,6] The acid-catalyzed isomerization involves the formation of a dienol intermediate followed by protonation at the γ carbon to give an α,β isomer.[3,5] A similar pathway through a dienolate anion intermediate has been presented for the base-catalyzed reaction.[4,6] The rate-determining step of these reactions seems to depend on the ketone structure.[5,6] Recently, a primary amine has been found to efficiently catalyze the isomerization through a dienamine intermediate.[7,8] Closely related enzymatic reactions have received considerable attention.[9,10] The Δ^5-3-keto steroid isomerase from *P. testosteroni* has most extensively been studied, although the mechanism for this reaction has yet to be fully elucidated.[9]

In this paper, acid- and general base-catalyzed isomerization of Δ^5-(**1**) to Δ^4-testosterone (**2**) has been investigated to determine the rate-determining step.



1 **2** (1)

Various primary amines, including polyfunctional amines, have been examined for their catalytic activities.

Experimental

Materials. Δ^5-Testosterone (**1**) was prepared by the isomerization of testosterone (**2**) catalyzed by potassium *t*-butoxide in *t*-butyl alcohol.[11] Crude products were recrystallized from acetone to give white plates melting at 160 °C. Calcd for $C_{19}H_{28}O_2$: C, 79.17; H, 9.72%. Found: C, 79.40; H, 9.72%.

Inorganic salts of reagent grade were used without further purification. Organic buffers were distilled or recrystallized before use. Freshly boiled, glass distilled water was used for all the rate determinations.

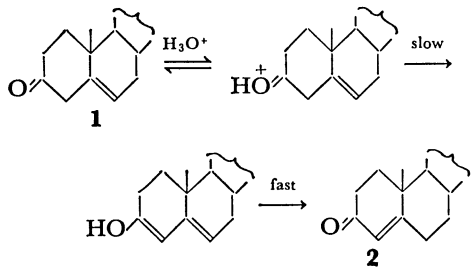
Kinetics. Rates were measured at 30±0.1 °C in aqueous buffer solutions containing 1% methanol, ionic strength being maintained at 0.50 with the addition of KCl. Three ml of buffer solution was equilibrated at 30 °C in a stoppered quartz cuvette inserted in a water-jacketted cell holder. Into the buffer solution was injected 30 μl of stock solution of **1** in methanol (4×10^{-3} M) with use of a microsyringe. After thorough mixing, the reaction was monitored spectrophotometrically using a Shimadzu spectrophotometer UV-200. First-order plots were linear over 90% conversion as monitored at 245 nm (λ_{max} of **2**) in the absence of primary amines.

Values of pH of buffer solutions and reaction mixtures were determined with a Hitachi-Horiba pH meter CTE F-5 calibrated in +0.01 pH unit.

To determine solvent isotope effects, D_2O and DCl and NaOD solutions supplied by E. Merck (isotopic purity >99.5 %) were used. Protic methanol was used for the preparation of the stock solution of **1**. Thus, deuterium purity of the reaction mixture was >98%.

Results and Discussion

Acid-catalyzed Isomerization. The rates of isomerization of **1** were measured in HCl (H_2O) and DCl (D_2O) solutions. An inverse isotope effect of $k_{H_3O^+}/k_{D_3O^+}=0.69\pm0.02$ was observed (Table 1). The result is consistent with the rate-determining enolization and in agreement with that observed for androst-5-ene-3,17-dione.[3]



1 **2** (2)

With 3-cyclohexen-1-one a normal isotope effect was found, indicating the rate-determining protonation of

Table 1. Acid-catalyzed isomerization of **1**

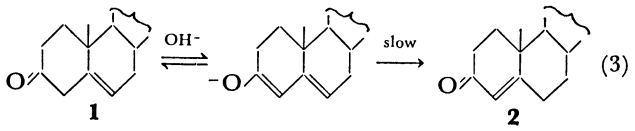
Acid	Acid concn, M	$10^3\ k_{obsd}$, s^{-1}	$10^2\ k_2$, $M^{-1}\,s^{-1}$
HCl	0.099	1.12	1.13
		1.07	1.08
		1.14	1.15
	0.0495	0.553	1.12
		0.532	1.07
		0.556	1.12
		Av	1.11±0.013
DCl	0.104	1.71	1.63
		1.64	1.58
	0.052	0.837	1.61
		0.802	1.54
		Av	1.60±0.024

Table 2. Base-catalyzed isomerization of **1**

Base	Base concn, M	$10^3\ k_{obsd}$, s^{-1}	k_2, $M^{-1}\,s^{-1}$
NaOH	0.00495	5.37	1.08
		5.77	1.17
		5.70	1.15
	0.0099	10.5	1.06
		11.7	1.19
		11.6	1.17
	0.0198	21.5	1.09
		20.7	1.05
		23.6	1.19
		Av	1.13±0.02
NaOD	0.00496	1.61	0.325
		1.65	0.332
	0.00993	3.59	0.363
	0.00960	3.69	0.384
		3.65	0.380
	0.0199	7.29	0.366
		7.53	0.378
		Av	0.361±0.009

a dienol intermediate.[5a] The reason for the mechanistic difference of the latter reaction was nicely interpreted by the conformational aspects.[6]

Base-catalyzed Isomerization. The rate of the isomerization of **1** were compared in NaOH (H_2O) and NaOD (D_2O) solutions (Table 2). The isotope effect is normal $k_{OH^-}/k_{OD^-}=3.13\pm0.14$, indicating an equilibrium formation of a dienolate ion intermediate, followed by the rate-determining protonation of the intermediate by water.



This observation is consistent with the preferential α-protonation (deconjugation) of a dienolate anion in *t*-butyl alcohol.[11,12] That is, even in aqueous solution the base-catalyzed isomerization proceeds by the rate-determining protonation or the dienolate anion undergoes the preferential α-C protonation. A similar observation has recently been made with 3-cyclohexen-1-one.[6]

General Base Catalysis. The isomerization was also carried out in various buffer solutions. The observed rates were strongly dependent on buffer concentrations. Buffer-dependent rate constants were partitioned into the acid- and base-catalytic constants in a usual way as seen in Fig. 1. The acid-catalytic term was negligible at pH>7 except for primary amine buffers. The general base catalytic constants obtained are given in Table 3 and the Brønsted plots are shown in Fig. 2 ($\beta=0.48$). The apparent general base catalysis observed here must be a consequence of combined specific hydroxide and general acid catalyses since the rate-determining step is protonation (Eq. 3). In the same way, the apparent general acid catalysis

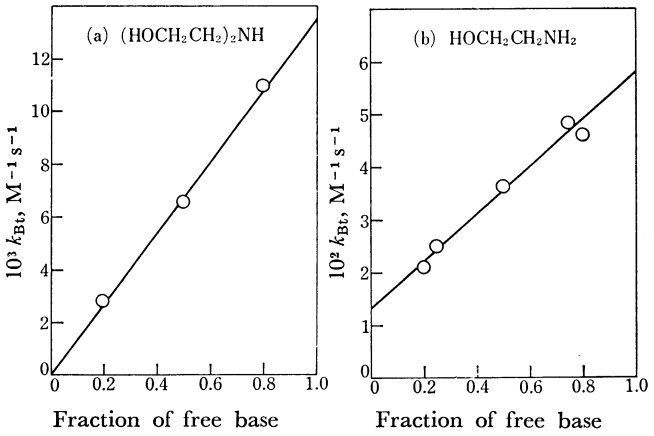


Fig. 1. Buffer-dependent rate constants, k_{Bt}, for the isomerization of **1** in (a) 2,2′-iminodiethanol and (b) 2-aminoethanol buffer solutions.

Table 3. General base catalysis in the isomerization of **1**

No.	Base	pK_a[a]	$10^2\ k_B$, $M^{-1}\,s^{-1}$
1	$(C_2H_5)_2NH$	11.1	19.7
2	$(C_2H_5)_3N$	10.9	7.8
3	Caps[b]	10.4	5.3
4	CO_3^{2-}	9.8	7.0
5	$HOCH_2CH_2NH_2$	9.6	5.8
6	$(HOCH_2CH_2)_2NH$	9.1	1.34
7	Morpholine	8.7	5.0
8	$(HOCH_2CH_2)_3N$	7.9	0.328
9	Imidazole	7.0	0.155

a) pH of a buffer solution of [acid]/[base]=1. b) Cyclohexylaminopropanesulfonate.

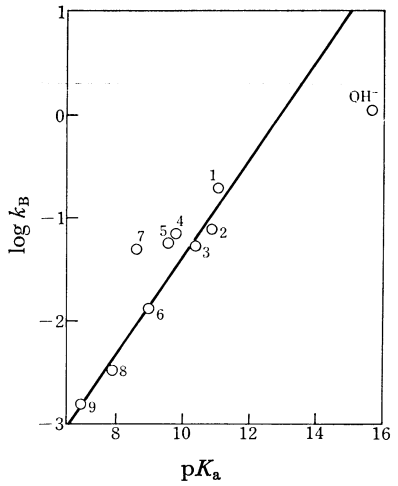
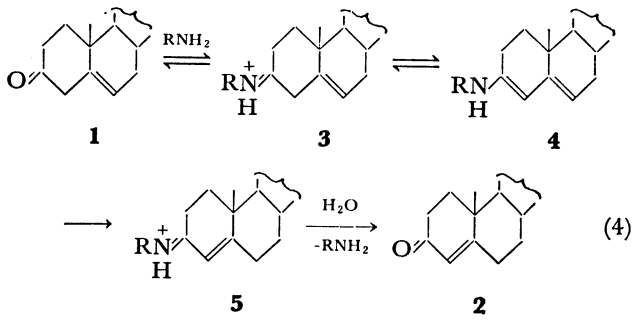


Fig. 2. Brønsted plot for general base catalysis of the isomerization of **1**. For numbering see Table 3.

is actually specific oxonium-general base catalysis. Thus, the reasoning previously made by Jones and Wigfield[4] that the phenolic group of the enzyme acts as a general acid because of insufficient base catalytic activity of phenolate anion is weak.

Primary Amine Catalysis. The reactions in primary amine buffers showed some induction period with higher concentrations, and rates showed non-linear buffer de-

pendence. With 2-aminoethanol as a buffer, the rate constants obtained with low buffer concentrations (< 0.06 M) showed an acid-catalytic term as is seen in Fig. 1(b). With primary amine buffers of lower pK_a, the anomalous behavior was more apparent and the transient intermediate formation was observed by the scannings of UV spectra of the reaction mixture. An intermediate having the absorption maximum at ≈280 nm appeared rapidly, followed by slower decay for all the primary amines of $pK_a<9$ studied here. Similar observations were noted previously with trifluoethylamine[7] and glycylglycine[8] and the intermediate of λ_{max} ≈280 nm was identified as an α,β-unsaturated iminium ion of type **5**.



(4)

Kinetics of the primary amine-catalyzed isomerization of 3-methyl-3-cyclohexen-1-one has recently been investigated with trifluoroethylamine as a buffer, and a mechanism similar to Eq. 4 has been presented by Pollack and Kayser.[7] Some kinetic results preliminarily obtained with our system are consistent with the mechanism 4.[13] Because of the kinetic complexity and the publication of Pollack's data, thorough kinetic investigation of the present system was abandoned.

We compared relative catalytic effects of various primary amines. For the sake of kinetic simplicity,

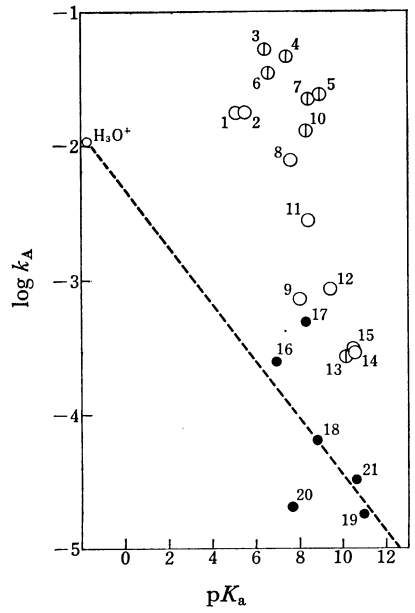


Fig. 3. Amine catalysis of the isomerization of **1**. For numbering see Table 4. ○, Primary amines. ⊕, Polyfunctional primary amines. ●, Sceondary and tertiary amines.

Table 4. Amine catalysis in the isomerization of **1** in phosphate buffer[a]

No.	Amine	pK_a[b]	$10^4\ k_A$, $M^{-1}\ s^{-1}$
1	$NCCH_2NH_2$	5.3	176
2	$CF_3CH_2NH_2$	5.6	174
3	$(CH_3)_2\overset{+}{N}HCH_2CH_2NH_2$	6.7	520
4	$H_3\overset{+}{N}CH_2CH_2NH_2$	7.5	450
5	$[H(NHCH_2CH_2)_2NH_2]H^+$	9.0[c]	240
6	$[H(NHCH_2CH_2)_3NH_2]H_2^{2+}$	6.8[d]	334
7	$[H(NHCH_2CH_2)_4NH_2]H_2^{2+}$	8.5[e]	225
8	$NCCH_2CH_2NH_2$	7.7	78.9
9	$(HOCH_2)_3CNH_2$	8.1	7.4
10	$^-OCOCH_2NHCOCH_2NH_2$	8.4	130
11	$ClCH_2CH_2NH_2$	8.5	28
12	$HOCH_2CH_2NH_2$	9.5	8.6
13	$^-OCOCH_2CH_2NH_2$	10.2	2.7
14	$CH_3CH_2NH_2$	10.6	2.9
15	CH_3NH_2	10.6	3.1
16	Imidazole	7.1	2.5
17	Morpholine	8.4	5.0
18	$(HOCH_2CH_2)_2NH$	8.9	0.64
19	$(CH_3CH_2)_2NH$	11.0	0.18
20	$(HOCH_2CH_2)_3N$	7.8	0.21
21	$(CH_3CH_2)_3N$	10.7	0.33

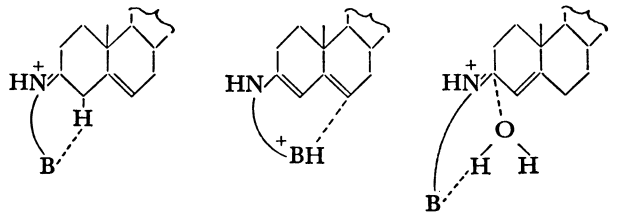
a) [Phosphate]=0.1 M, pH=6.6±0.1. Without added amines, $k_{obsd}=2.04\times10^{-5}\ s^{-1}$. b) Values taken from "CRC Handbook of Biochemistry," H. A. Sober, Ed, Chem. Rubber Co., Cleveland (1968), unless otherwise noted. c) R. L. Pecsok, R. A. Garber, and L. D. Shields (*Inorg. Chem.*, **4**, 447 (1965)) report $pK_a=4.22, 8.95, 9.79$ at 26 °C. d) D. B. Rorabacher, W. J. MacKeller, F. R. Shu, and S. M. Bonavita (*Anal. Chem.*, **43**, 561 (1971)) report $pK_a=3.39, 6.75, 9.31, 10.09$ at 25 °C. e) D. B. Rorabacher, W. J. MacKeller, F. R. Shu, and S. M. Bonavita (*Anal. Chem.*, **43**, 561 (1971)) report $pK_a=2.40, 4.70, 8.50, 9.65, 10.36$ at 25 °C.

very low concentrations of amines (≦0.02 M) were employed to determine the catalytic constants at pH near neutrality. To maintain pH constant (pH=6.6± 0.1), phosphate buffer of 0.1 M was used. Under these conditions, the formation of **2** (245 nm) was of the first-order in rate. From the rate increase by the addition of an amine, the apparent catalytic constant k_A was calculated and given in Table 4.

Logarithms of k_A are plotted against pK_a of the conjugate acid in Fig. 3. A reference line was drawn through the points for usual general acids because the acidic term of primary amine catalysis is important in mechanism 4.[7,13] Points for primary amines show large upward deviations from the reference line by the magnitude of *ca.* 2 in the log unit. The reaction in a 1-M buffer of *N,N*-dimethylethylenediamine of neutral pH would be more than 10^5 times as rapid as the uncatalyzed reaction at the same pH. A primary amine effectively attacks the β,γ-unsaturated ketone as a nucleophile to give an iminium ion followed by rapid isomerization to the α,β-unsaturated iminium ion, which is finally hydrolyzed to give the α,β-unsaturated ketone

(Eq. 4). During the conversion of the iminium intermediate, a second molecule of an amine or an external buffer acts as a general base or acid.

When a bifunctional primary amine having a second base group is used as a catalyst, intramolecular base (acid) catalysis may operate in manners like **6**—**8** in each step of Eq. 4.



6 **7** **8**

Some polyfunctional primary amines were examined and are included in the plots of Fig. 3. Small such effects seem to be actually operative, though not definite. Intramolecular catalysis similar to **8** was previously found in the dehydration of a carbinolamine (the reverse of the present reaction).[14] Although the catalysis like **6** may also be possible, the step that follows (γ protonation) would hardly be catalyzed intramolecularly because of the remoteness of an acid group (**7**). Thus, whether or not the bimolecular catalysis by primary amines is actually effective depends on the rate-determining step of the overall reaction. Further details on this problem is now under investigation.

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