Table II. Effect of Solvent and Counteranion

compd	λ_{max}, nm MeOH	λ_{max}, nm $CHCl_3$ (1% EtOH)
3 (transoid)	470 (Cl^-)	496 (Cl^-)
	471 (ClO_4^-)	510 (ClO_4^-)
5 (cisoid)	480 (Cl^-)	500 (Cl^-)
	478 (ClO_4^-)	526 (ClO_4^-)

transoid to some intermediary state between transoid and cisoid to reduce the serious twist involved in transoid by the sacrifice of the electrostatic effect.

Also an environmental effect seems to be operating to some extent, which we conclude by considering that in **1** the two N^+ interact in a somewhat hydrophobic environment which may enhance the interaction energy. The effect of solvent was investigated together with the effect of a counteranion to ascertain this mechanism (Table II). In such an apolar solvent as $CHCl_3$, the red shift became very significant as expected, especially for the cisoid chromophore, and this red shift was more significant when the cation was more "naked" (see Table II). This enormous enhancement of the electrostatic interaction in a less polar medium is also seen in the specific phase transfer of a hydrophilic anion by a lipophilic ammonium ion.[8]

Thus, a conclusion may be drawn that a moderate red shift is observed for the protonated Schiff base with a proximal ammonium ion, and the shift is further enhanced either (i) when the system has a loose counteranion and especially is embedded into the less polar environment or (ii) when the system is restricted in its motion by the binding and both the electrostatic destabilization and the unfavorable twist of the conjugated chromophore are operating. Interestingly, the latter mechanism is important even in aqueous solution and without any loose counteranion.

The present situation combined with the electrostatic stabilization of the excited state by a proximal anion[4] seem to be closely correlated with bacteriorhodopsin[9] and rhodopsin. An attempted a priori general estimation of the red shift is now under way.

(8) Waddel, W. H.; Schaffer, A. M.; Becker, R. S. *J. Am. Chem. Soc.* **1977**, *99*, 8456.
(9) Tabushi, I.; Imuta, J.; Seko, N.; Kobuke, Y. *J. Am. Chem. Soc.* **1978**, *100*, 6287.
(10) In rhodopsin, the proximal Lys is believed to be present. The sequence of the retinal-binding peptide from the bacteriorhodopsin was shown to be Val-Ser-Asp-Pro-Asp-Lys-Lys: Bridgen, J.; Walker, I. B. *Biochemistry* **1976**, *15*, 792.

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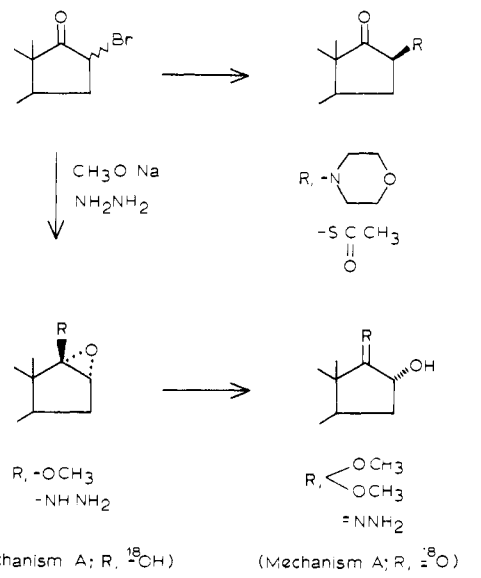
Controlled Alkaline Hydrolysis of 16-Bromo-17-keto Steroids without Ketol Rearrangement and Its Reaction Mechanism

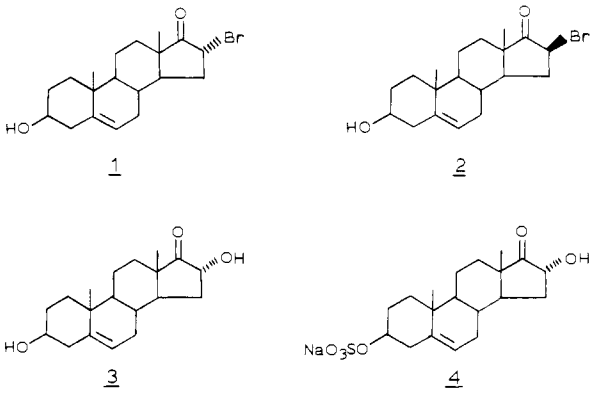
Sir:

From the observations[1,2] on the relative stability of steroidal 16,17-ketols toward alkali hydroxide and the unsuccessful attempts to isolate thermodynamically unstable 16-hydroxy 17-ketone, it has been considered impossible to isolate the ketol by hydrolysis of 16-bromo 17-ketone. Two types of reactions of 16-bromo 17-ketone with other nucleophiles have been known, the direct displacement of bromine with amines[3] and thioacetate,[4] leading

Scheme I



to formation of the 16β-substituted steroids, and the attack of methoxide ion[2,5] and hydrazine[6] at the 17-carbonyl function, leading to 16α-hydroxy derivatives via 16α,17α-epoxide intermediates (Scheme I). We wish to report the controlled stereospecific alkaline hydrolysis, with pyridine as a buffer,[7] of 16α-bromo 17-ketone **1** to 16α-hydroxy 17-ketones **3** and **4** and elucidation of the reaction mechanism by use of deuterium- and ^{18}O-labeling experiments. The isotope experiments showed the mechanism to be nucleophilic displacement of bromine of the 16β-bromo isomer **2** by hydroxide ion and refutes the putative 16α,17α-epoxide mechanism.



The dynamic aspects of equilibrium between the bromo ketones **1** and **2**[8] and of production of the 16α-hydroxy 17-ketone **3** are shown in Table I.[9] Treatment of **1** with 0.012 equiv of NaOH in aqueous pyridine at room temperature did not cause any change,

(1) (a) Leeds, N. S.; Fukushima, D. K.; Gallagher, T. F. *J. Am. Chem. Soc.* **1954**, *76*, 2943. (b) Fishman, J. *Ibid.* **1960**, *82*, 6143. (c) Kirk, D. N.; Hartshorn, M. P. "Steroid Reaction Mechanisms", Elsevier: Amsterdam, 1968; pp 388.
(2) Hassner, A.; Catsoulacos, P. *J. Org. Chem.* **1966**, *31*, 3149.
(3) (a) Hewett, C. L.; Savage, D. S. *J. Chem. Soc. C* **1966**, 484. (b) Hassner, A.; Catsoulacos, P. *J. Org. Chem.* **1967**, *32*, 549.
(4) Takeda, K.; Toneno, T. *Chem. Pharm. Bull.* **1964**, *12*, 905.
(5) Mueller, G. P.; Johns, W. F. *J. Org. Chem.* **1961**, *26*, 2403.
(6) Catsoulacos, P.; Hassner, A. *J. Org. Chem.* **1967**, *32*, 3723.
(7) When MeOH, EtOH, and dioxane were used as a solvent, the formation of the rearranged product, 3β,17β-dihydroxy-5-androsten-16-one, together with the ketol **3** were observed in 5–20% yield with 1.2 equiv of NaOH and 2-h reaction time.
(8) The 16α-bromo 17-ketone **1** was prepared according to the paper: Numazawa, M.; Osawa, Y. *Steroids* **1978**, *32*, 519. Upon a fractional crystallization of the mother liquor of **1**, the 16β-bromo isomer **2** was obtained; mp 171–173 °C.
(9) The bromo ketones **1** and **2** and the ketol **3** were quantified by the peak areas corresponding to both the C-16 proton and the C-18 angular methyl of ^1H NMR spectra of the reaction mixtures without isolation. ^1H NMR ($CDCl_3$): **1** δ 0.90 (s, 3 H), 4.57 (t, 1 H); **2** δ 1.09 (s, 3 H), 4.14 (t, 1 H); **3** δ 0.96 (s, 3 H), 4.37 (t, 1 H).

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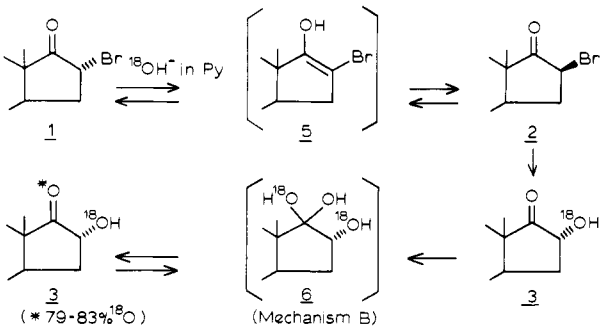
Table I. Epimerization of the 16-Bromo 17-Ketones 1 and **2** and Formation of the 16α-Hydroxy 17-Ketone 3 in Sodium Hydroxide–Aqueous Pyridine System[a]

	conditions		relative amount of products, %[b]		
	NaOH, equiv	time, min	1	**2**	3
			Compound 1		
A	0.012	10	100	0	0
B	0.06	10	45 (76)[c]	55 (99)	<1
C	0.12	10	41	54	5
D	0.60	10	40	50	10
E	1.20	10	36	43	21
F	1.20	20	25 (99)	30 (99)	45 (99)
G	1.20	480	5 (1 and **2**)		95 (99)
			Compound **2**		
H	0.06	10	20 (99)	80 (35)	<1
I	0.12	10	38	57	5
J	1.20	10	35	43	22
K	1.20	20	25 (99)	30 (99)	45 (99)

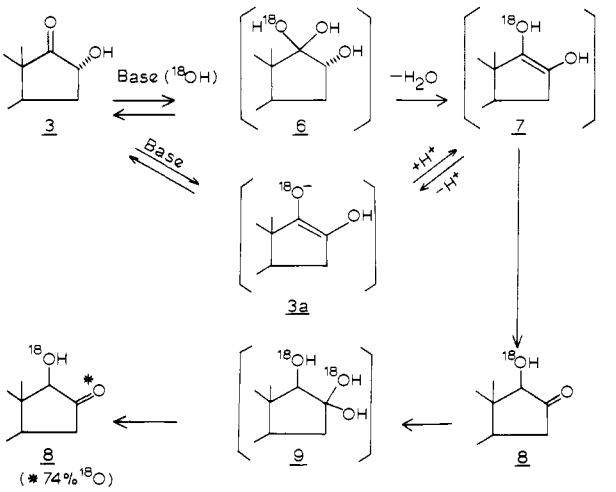
[a] To a solution of 16α- or 16β-bromo 17-ketone **1** or **2** (150 mg, 0.41 mmol) in 75% aqueous pyridine (8 mL) was added 0.48 mL of sodium hydroxide solution and the mixture allowed to stand at room temperature. The mixture was poured into 1% HCl solution and then extracted with AcOEt. The organic layer was washed with 5% $NaHCO_3$ and H_2O and dried (Na_2SO_4). After evaporation of the solvent the residue obtained (110–140 mg) was submitted to NMR analysis. [b] Relative amount of product was obtained by ^1H NMR. [c] Deuterium content of the steroids at C-16 is shown in parentheses and was obtained by measuring the peak areas of the C-16 proton in ^1H NMR after purification by TLC of the product obtained by use of D_2O (99.8 atom %).

Scheme II



but a fivefold increment of NaOH in pyridine was enough to give an approximate 1:1.2 equilibrium between **1** and **2**, in favor of **2** without the formation of **3**.[10] An increasing production of **3**[11] in proportion to the amount of the base and to the reaction time was observed, maintaining the remaining bromo ketone in the same equilibrium between **1** and **2**. The ketol **3** was stereoselectively obtained in 95% yield from **1** without formation of other ketols by using 8-h reaction time and 1.2 equiv of alkali. The bromo ketone **2** was also epimerized back to **1**, resulting in the same equilibrium (approximately 1:1.2 of **1** to **2**). Its rate, however, was distinctly slower than that of the reverse, when 0.06 equiv of NaOH was used (compare condition H with B). Deuterium contents of **1** and **2** recovered from conditions B and H in D_2O also clearly show the difference of the epimerization rate between **1** and **2** (Table I). On the other hand, deuterium was almost completely incorporated, not only into C-16 of **3** but also into C-16 of **1** and **2** under conditions F, G, and K, respectively, by using 1.20 equiv of NaOH. The results show that the equilibration between **1** and **2** precedes the formation of **3**. Kinetic observation

Scheme III



of **1**, and **2**, and **3** concentrations during the reaction indicates that the conversion of **2** to **3** is the rate-limiting step.

Since this reaction could proceed through the 16α,17α-epoxide[2,5,6] intermediate formed by hydroxide attack at the 17β position (mechanism A, Scheme I), as previously postulated for nucleophilic reactions, or by the direct S_N2 displacement of the 16β-bromine with hydroxide ion (mechanism B, Scheme II), we attempted to determine the mechanism by use of [^{18}O]water with the premise that the primary product should be 17-^{18}O labeled under mechanism A and 16α-^{18}O labeled if it is by mechanism B. In addition, we aimed to confirm or refute the mechanism of ketol rearrangment[1,2] which had been formulated to involve the direct enolization of the 17-carbonyl group, but the alternate mechanism of hydration to the carbonyl followed by dehydration to form the enediol had been overlooked.

^{18}O-enriched water (99.5 atom %) was used with pyridine under condition G (Table I) for the controlled hydrolysis (**1** to **3**) and with methanol for the ketol rearrangement (**3** to **8**). The product was isolated as acetate and analyzed as solid sample by mass spectrometry (MS). The results are shown in Table II. Unexpectedly, the mass spectra of the diacetate derivative of **3** obtained from bromo ketone **1** showed over 80% oxygen labeling at each of the 16α and 17 positions, thus contradicting the plausible first hypothesis to elucidate the mechanism directly. However, quantitative analysis of the data gave 83% ^{18}O labeling at the 17-carbonyl and 98% ^{18}O labeling at the 16α-hydroxyl group.[12]

Ketol **3** treated under the same condition with bromo ketone **1** for the controlled hydrolysis showed no labeling at the 16α-hydroxyl group and 78–80% ^{18}O labeling at the 17-carbonyl group, showing the extent of oxygen equilibrium of the 17-ketone (Table II). In contrast, the recovered bromo ketone after 8 h of treatment showed only 17% ^{18}O incorporation into the 17-carbonyl group.

(10) Epimerization of 16α-bromo 17-ketone steroids to the 16β isomer in alkaline medium (Fajkos, J.; Sorm, F. *Collect. Czech. Chem. Commun.* **1959**, *24*, 766) and in acid medium (Fajkos, J. *J. Chem. Soc.* **1959**, 3966) has also been reported. The dynamic aspects of the epimerization, however, have not been clarified.

(11) The ketol **3** obtained was identical with the standard sample in every respect.

(12) Although the molecular ion was insignificant in all of the steroids analyzed, as is common in such steroidal acetates, strong fragment ions representing [M – acetic acid]$^+$ [base peak for **3**-Ac_2 and **8**-Ac_2 at *m/e* 328 and 50% of base peak (*m/e* 43) for **1**-Ac and **2**-Ac at *m/e* 348 and 350] and [M – diacetic acid]$^+$ (18–22% of base peak for **3**-Ac_2 and **8**-Ac_2 at *m/e* 268) were observed. The ^{18}O content in [M – diAcOH]$^+$ derived from **3**-Ac_2 (*m/e* 270 vs. 268) was found to be 83%, which shows the extent of the labeling at the 17-carbonyl group. The assignment of the [M – AcOH]$^+$ peak to be quantitatively [M – 3β-AcOH]$^+$ was made on the basis of the following reasons: If the [M – AcOH]$^+$ fragment is derived exclusively by 16α-acetoxyl elimination from 83% 17-^{18}O-labeled **3**-Ac_2, the [M – AcOH]$^+$ fragment should have 0% for M + 4 (*m/e* 332) and 83% for M + 2 (*m/e* 330). On the other hand, exclusive 3β-acetoxyl elimination should give maximally 83% for M + 4 (when the 16α-^{18}O labeling is 100%) and minimally 17% for M + 2. The observed values (Table II) of 81.5% for M + 4 (*m/e* 332) and 18% for M + 2 (*m/e* 330) show that this fragment quantitatively represents [M – 3β-AcOH]$^+$. Similar observations were made for the assignment of the [M – 3β-AcOH]$^+$ peak in the fragmentation of the 3β,17β-diacetate (**8**-Ac_2). The percent ^{18}O labeling at the 16α-hydroxyl group in **3**-Ac_2 (98%) was calculated from the data in Table II by the following equations: (1) [16-^{18}O,17-^{16}O] + [16-^{16}O,17-^{16}O] (0.5%, *m/e* 328) = [17-^{16}O] (17%, *m/e* 268) and (2) [16-^{18}O] = [16-^{18}O,17-^{16}O] + [16-^{18}O,17-^{18}O] (81.5%, *m/e* 332).

Table II. Isotope Analysis by Mass Spectrometry

		content, %				
			m/e, [M − 3β-AcOH]+		m/e, [M − 3β, 16α-diAcOH]+	
	steroid analyzed	328 ($^{16}O^{16}O$)	330 ($^{16}O^{18}O$)	332 ($^{18}O^{18}O$)	268 (^{16}O)	270 (^{18}O)
I[a]	3-Ac_2 from 1 treatment	0.5	18	81.5	17	83
	3-Ac_2 from 3 treatment	20	80	0	22	78
		348, 350 ($^{16}O^{79}Br$, ^{81}Br)		350, 352 ($^{18}O^{79}Br$, ^{81}Br)		
	1-Ac and 2-Ac recovered	83		17		
					m/e, [M − 3β, 17β-diAcOH]+	
		328 ($^{16}O^{16}O$)	330 ($^{16}O^{18}O$)	332 ($^{18}O^{18}O$)	268 (^{16}O)	270 (^{18}O)
II[b]	8-Ac_2 from 3 treatment	3	30	67	26	74

[a] Bromo ketone 1 or ketol 3 (0.11 mmol) in 0.75 mL of pyridine was treated with NaOH–$H_2{}^{18}O$ [5 mg (0.125 mmol) of NaOH in 0.25 mL (13.89 mmol) of 99.5 atom % [^{18}O]water; theoretical ^{18}O content of the solution 98.6 atom %] for 8 h at room temperature (condition G). The products were washed with water, acetylated, separated by TLC, and subjected to MS as solid. The recovered bromo ketones **1** and **2** were analyzed as a mixture (16α-Br/16β-Br = 1:1.25). [b] Ketol 3 (0.082 mmol) in 3.5 mL of MeOH was treated with NaOH–$H_2{}^{18}O$ (7 mg of NaOH in 0.19 mL of 99.5 atom % [^{18}O]water) for 3 days at room temperature. The product was isolated and analyzed in the same manner described for 3-acetate.

3β,17β-Dihydroxy-5-androsten-16-one (**8**) obtained by ketol rearrangement of **3** in CH_3OH–NaOH–$H_2{}^{18}O$ showed 74% ^{18}O labeling at the 16-carbonyl and 91% incorporation into the 17β-hydroxyl group.

The results demonstrate that the formation of 16α-hydroxy 17-ketone by alkaline hydrolysis of 16α- or 16β-bromo ketone is by the direct S_N2 displacement of the 16β-bromine (mechanism B, Scheme II) and not by the putative epoxide mechanism (mechanism A, Scheme I). The quantitative incorporation (98% observed compared to 98.6% theoretical) at the 16α position which was not affected by the equilibrium at the 17-carbonyl (17% in the bromo ketone and 79–83% in the ketol) eliminates the epoxide mechanism which requires the transfer of the carbonyl oxygen to the 16α position. The results also show that epimerization of 16-bromo 17-ketone through the enol does not involve an incorporation of hydroxide ion but that ketol rearrangement through the enediol involves participation of hydroxide ion and results in the ^{18}O exchange of both functions. The reaction mechanisms are formulated in Schemes II and III. 16α-Bromo 17-ketone (**1**) and 16β-bromo 17-ketone (**2**) undergo a rapid equilibrium slightly in favor of 16β.[13] The initial product of alkaline hydrolysis should primarily be [16α-^{18}O]**3**, but with further contact with [^{18}O]-hydroxide ion the 17-carbonyl group exchanges its oxygen through an intermediate **6** and gives rise to doubly labeled **3**. The 16-carbonyl of the ketol **8** also undergoes ^{18}O exchange to give doubly labeled **8**. Under a drastic ketol rearrangement condition (Scheme III), the initial ^{18}O labeling at C-17 would occur by formation of the hydrate **6**. The hydrate may be dehydrated to form [17-^{18}O]enediol **7** and rearrange to give the 16-ketone **8** or, alternatively, may be equilibrated back to form 17-^{18}O-labeled 17-ketone **3**. The labeled **3** may be enolized under the condition to **3a** and give rise to the [17-^{18}O]enediol **7** by protonation. After the rearrangement to 17-^{18}O-labeled 16-ketone **8**, similar ^{18}O-exchange reactions are assumed to occur through an intermediate **9** to give the final product labeled at both the 16 and 17 positions.

By this discovery of the controlled condition of hydrolysis, one of our long-time goals to synthesize sodium 3β,16α-dihydroxy-17-oxo-5-androsten-3-yl sulfate (**4**), the major human fetal 19-carbon steroid found in the umbilical cord blood and hitherto unavailable in crystalline salt form,[14] was achieved in one step in 85% yield from readily available bromo ketone **1** by sulfation with pyridine–chlorosulfonic acid complex followed by addition of NaOH solution.[15]

Acknowledgments. This research was supported in part by USPHS Research Grants HD04945 from the National Institute of Child Health and Human Development and RR05716 from the Division of Research Resources. Assistance by Carol Yarborough is greatly appreciated.

(13) Discrepancy between the 16β assignment for substitution by other nucleophiles[3,4] and the 16α configuration for hydroxide substitution presented here should be noted. Irrespective of which of the bromo ketones (**1** or **2**) is used, the product is seemingly in only one configuration in all cases. When **1** and **2** were separately subjected to the reaction with morpholine under the same condition reported previously,[3] the same equilibrium between **1** and **2** as reported in this paper was observed by NMR analysis. Determination of the total structure of the 16-morpholino derivative by X-ray crystallography is under way to further investigate the stereochemistry of nucleophilic substitution.

(14) Triethylamine and ammonia salts of the sulfate **4** were previously synthesized in a relatively low yield by involving five steps from 16α,17α-epoxy-3β-hydroxy-5-pregn-20-one (Wynne, K. N.; Renwick, A. G. C. *Biochem. J.* **1976**, *156*, 419) and three steps from the 16α-bromo ketone **1** (Numazawa, M.; Osawa, Y. *Steroids* **1978**, *32*, 519).

(15) The 16α-bromo 17-ketone **1** (2 g, 4.88 mmol) in 10 mL of dry pyridine was added to 1.5 equiv of pyridine–chlorosulfonic acid complex in 20 mL of pyridine with stirring under ice cooling. After 20 min, the reaction mixture was poured into a chilled 0.1 N NaOH solution (1 L) and allowed to stand at 0 °C for 3 h. The solution was passed through a column of Amberlite XAD-2 (4 × 100 cm). After the solution was washed with H_2O, the absorbed steroid was eluted with MeOH. The eluate was condensed to 20 mL and allowed to stand at 4 °C for 24 h. The solid (1.95 g) precipitated was collected by filtration and recrystallized from MeOH–Et_2O to give **4** (1.63 g, 4.21 mmol) as colorless needles: mp >280 °C; IR (KBr) ν_{max} 3440 (OH), 1738 (C=O), 1235 (SO_4); ^1H NMR [Py-d_5–CD_3OD (1:3)] δ 0.92 (3 H, s, 18-CH_3), 0.97 (3 H, s, 19-CH_3), 4.19–4.70 (2 H, m, 3α-H and 16β-H). Anal. Calcd for $C_{19}H_{27}O_6SNa·H_2O$: C, 53.76; H, 6.89; S, 7.55. Found: C, 53.54; H, 6.83; S, 7.42.

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Contribution of Orbital Alignment to Organic and Enzymatic Reactivity

Sir:

Storm and Koshland[1] have proposed that a 10° misalignment of reactant groups relative to an ideal orientation can cause a massive decrease in rate. If this is true, then enzymes could achieve much of their catalytic ability by optimizing orientational relationships at the active sites ("orbital steering").[1] The evidence supporting the significance of orbital alignment in catalysis rests mainly on the widely differing lactonization rates found among structurally similar hydroxy acids.[2]

(1) D. R. Storm and D. E. Koshland, Jr., *J. Am. Chem. Soc.*, **94**, 5815 (1972).

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