Stereospecific Synthesis of 16α-Hydroxy-17-oxo Steroids by Controlled Alkaline Hydrolysis of Corresponding 16-Bromo 17-Ketones and Its Reaction Mechanism[1]

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Synthesis of 16α-hydroxy-17-oxo steroids **3**, **5b**, and **6b** and 3β,16α-dihydroxy-5-17-oxoandrosten-3-yl sulfate (**7**) from 16α-bromo-17-oxo steroids **1**, **5a**, and **6a** and the reaction mechanism of the controlled alkaline hydrolysis are described. Treatment of the bromo ketones with NaOH in aqueous DMF gave the 16α-hydroxy 17-ketones stereoselectively in 95% yield without formation of other ketols. The sodium salt of 3-sulfate **7** was also obtained in one step in 85% yield from the corresponding bromo ketone (**1a**). Isotope-labeling experiments and time-course studies showed that equilibration between the 16-bromo epimers **1** and **2** precedes the formation of **3**, in which the true intermediate is **2** and not **1**, and that the ketol **3** is formed by the direct S_N2 displacement of the 16β-bromine. The 16β-morpholino derivative **8** obtained by reaction of **1** with morpholine was shown to be an isomerized product of the 16α isomer which is produced also by S_N2 displacement of the 16β-bromine. The mechanism of ketol rearrangement of **3** to the 17β-hydroxy-16-oxo compound **4** was found to involve a hydration to the carbonyl function. The new hydration–dehydration mechanism is proposed for the ketol rearrangement.

Steroidal ring-D 16,17-ketols, especially 16α-hydroxy 17-ketones, have been known as major metabolites of C-18 and C-19 steroids and also as potentially useful intermediates in the synthesis of ring-D 16,17-glycols. It is desirable to provide a generally applicable and simple synthesis for these compounds. The previous observations on the relative stability of the 16,17-ketols toward alkali hydroxide[3] and the unsuccessful attempt[4] to isolate the thermodynamically unstable 16-hydroxy 17-ketone led to a general belief that it is impossible to isolate the corresponding ketol by hydrolysis of 16-bromo 17-ketone because of the instantaneous rearrangement.

Two types of nucleophilic reactions of the 16-bromo 17-ketone with strong bases are known, the direct displacement of bromine with amines[5] and thioacetate,[6] leading to the formation of 16β-substituted steroids, and the attack of methoxide ion[4,7] and hydrazine[8] at the 17-carbonyl function, leading to the 16α-hydroxy derivatives via three-membered-ring (epoxide) intermediates (Scheme I).

We report a controlled stereoselective alkaline hydrolysis, with pyridine or dimethylformamide (DMF) as a buffer, of 16α-bromo 17-ketones **1**, **5a**, and **6a** to the corresponding 16α-hydroxy 17-ketones **3**, **5b**, **6b**, and **7**. The isotope experiments showed the reaction mechanism to be S_N2 displacement of the 16β-bromine by hydroxide ion and refute the putative 16α,17α-epoxide mechanism.[8] It was also demonstrated that the bromo ketone **2** similarly reacts with morpholine as with hydroxide ion, thus refuting also the putative direct S_N2 displacement mechanism of the 16α-bromo isomer **1** as previously assumed.[5,6]

Results and Discussion

Synthesis of 16α-Hydroxy-17-oxo Steroids. When 3 molar equiv of $CuBr_2$ was used for the bromination of 3β-hydroxy-17-oxo steroids in dry MeOH, the 16α-bromo ketones **1a** and **1b** (Chart I) were obtained in a much improved yield (isolated yield 98%) with a relatively short

Scheme I

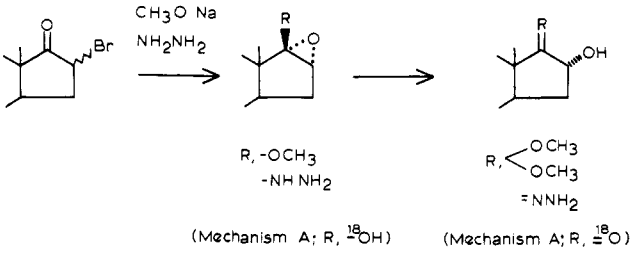

Chart I

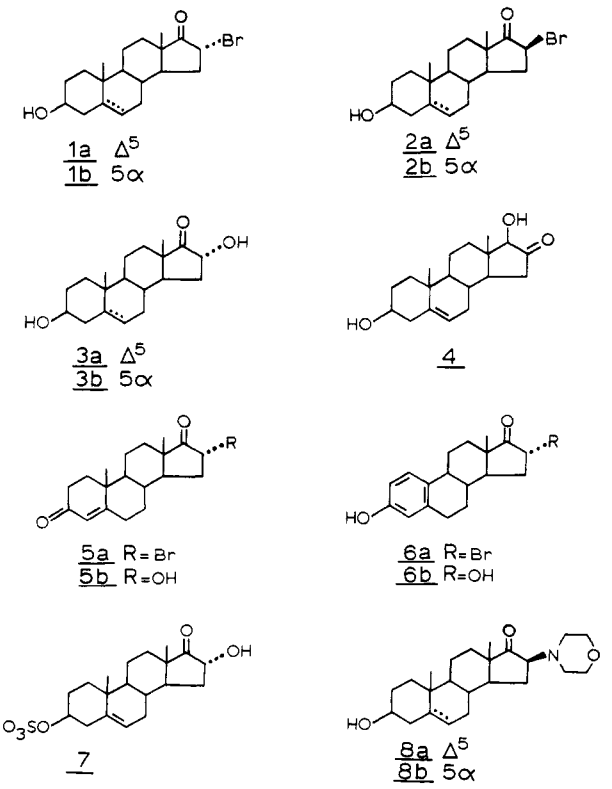

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Table I. Effects of Solvent on Epimerization of 16α-Bromo 17-Ketone 1a and Formation of 16,17-Ketols 3a and 4 with NaOH[a]

time, h	rel amt of products,[b] % 1a	2a	3a	4
	(a) 95% EtOH[c]			
0.17	30	36	31	0
1.5	18	20	42	20
6.5	5 (1a + 2a)		53	42
	(b) 75% Pyridine			
0.17	36	43	21	0
0.5	31	45	23	0
6.5	5 (1a + 2a)		95	0
	(b) 75% DMF			
0.17	1 (1a + 2a)		99	0
0.5	1 (1a + 2a)		99	0
1.0	0	0	100	0
1.5	0	0	100	0

[a] The 16α-bromo ketone 1a was treated with 1.2 equiv of NaOH at room temperature. [b] The relative amounts of products were obtained from the ^{1}H NMR spectra of the reaction mixtures without isolation. [c] The results obtained with 80% MeOH and 75% dioxane (not listed) are essentially the same as those for 95% EtOH.

reaction time (12 h of heating under reflux), compared to the reported one with 2 molar equiv of $CuBr_2$.[9] We initially explored the reaction of **1a** with alkali hydroxide in various polar solvents in order to clarify whether 16α-hydroxy 17-ketone **3a** could be isolated in a high yield without ketol rearrangement of **3a** which leads to formation of 17β-hydroxy 16-ketone **4**. The ^{1}H NMR spectra of the bromo ketones **1a** and **2a** and the ketols **3a** and **4** proved useful for the quantitative analysis of the reaction mixtures without isolation. The signals at δ 0.90 (s, 3 H) and 4.57 (m, 1 H) for **1a**, δ 1.09 (s, 3 H) and 4.14 (t, 1 H) for **2a**, δ 0.96 (s, 3 H) and 4.37 (t, 1 H) for **3a**, and δ 0.78 (s, 3 H) and 3.75 (m, 1 H) for **4** correspond to the H at the C-18 angular methyl and the H at C-16 or C-17, respectively. A brief treatment of **1a** with 1.2 equiv of NaOH in aqueous dioxane, EtOH, or MeOH at room temperature caused epimerization of **1a** to its 16β-bromo isomer **2a** together with the formation of the ketol **3a** (Table I). The 17β-hydroxy 16-ketone **4**, the rearranged product, however, was formed increasingly in proportion to the reaction time, maintaining the remaining bromo ketones in the same equilibrium (approximately a 1:1.2 ratio of **1a** to **2a**). In contrast, when aqueous pyridine or DMF was used as a solvent, the bromo ketone **1a** was converted almost completely to ketol **3** without the formation of ketol **4** (Table I). In view of the well-established relative stability of the 16,17-ketols toward alkali hydroxide, these surprising results, the controlled, quantitative, and stereospecific alkaline hydrolysis of bromo ketone **1a**, can be attributed to the buffer action of the protic solvents. The rearranged ketol **4** could not be detected in the reaction mixtures with DMF, even if a longer reaction time (2 h) was chosen, but approximately 5% of **4** was produced by exposure of **3a** to the NaOH–pyridine system for 12 h. Finally, ketol **3a** was obtained in above 95% yield on isolation from **1a** by use of 75% DMF and a 30-min reaction time or by use of 75% pyridine and an 8-h reaction time.

This synthesis has a great advantage in its simplicity and high yield over the previously reported and widely used method[3a] which involves epoxidation of ring-D enol acetates. This also promises a new utilization of the reaction

(9) (a) Glazier, E. R. *J. Org. Chem.* **1962**, *27*, 4397. (b)Numazawa, M.; Osawa, Y. *Steroids* **1978**, *32*, 519.

Table II. Epimerization of 16-Bromo 17-Ketones 1a and 2a and Formation of 16α-Hydroxy 17-Ketone 3a in the NaOH–Pyridine System[a]

	conditions NaOH, equiv	time, min	rel amt of products,[b] % 1a	2a	3a
			(a) Compound 1a		
A	0.06	10	45 (76)[c]	55 (99)	1
B	0.12	10	41	54	5
C	1.20	10	36	43	21
D	1.20	20	25 (99)	30 (99)	45 (99)
E	1.20	480	5 (1a + 2a)		95 (99)
			(b) Compound 2a		
F	0.06	10	20 (99)	80 (35)	1
G	0.12	10	38	57	5
H	1.20	10	35	43	22
I	1.20	20	25 (99)	30 (99)	45 (99)

[a] The bromo ketones 1a and 2a were treated in 75% pyridine at room temperature under the conditions described. [b] The relative amounts of products were obtained by ^{1}H NMR. [c] Deuterium content of the steroids at C-16 obtained by use of D_2O (99.5 atom % of theoretical D content) in the reaction mixture is shown in parentheses and was obtained by measuring the peak area of the C-16 proton in the ^{1}H NMR after purification by TLC of the product.

for practical syntheses of other steroidal α-ketols which are important to steroid chemistry. By use of the discovery of the controlled conditions of hydrolysis in aqueous DMF, the other 16α-hydroxy 17-ketones **3b** and **5b** were quantitatively obtained from the corresponding bromo ketones, **1b** and **5a**,[10] under the same conditions. For the quantitative synthesis of estrogen ketol **6b** from **6a**,[11] 2.0 equiv of NaOH and 1 h of reaction time were required because of the participation of the phenolic hydroxyl group of estrogen in an acid–base reaction with NaOH.

Furthermore, using pyridine as a solvent, we could synthesize sodium 3β,16α-dihydroxy-17-oxo-5-androsten-3-yl sulfate (**7**), the major human fetal 19-carbon steroid found in the umbilical cord blood and hitherto unavailable in crystalline salt form, in one step in 85% yield from bromo ketone **1a**. The bromo ketone **1a** was first sulfated with pyridine–$ClSO_3H$ complex in pyridine under ice cooling and then hydrolyzed with a chilled 0.1 N NaOH solution at 0 °C for 3 h. After Amberlite XAD-2 column chromatography of the reaction mixture, recrystallization of the crude product from MeOH–Et_2O gave pure sulfate **7**. The sulfate **7** was identified by solvolysis back to the aglycon **3a**, by ^{1}H NMR and IR spectra, and by elemental analysis. This synthesis using pyridine will be applicable to the synthesis of other steroidal sulfates having the α-ketol structure.

Mechanism of the Controlled Alkaline Hydrolysis. Aqueous pyridine was chosen as a solvent for the elucidation of the reaction mechanism because the rates of epimerization and hydrolysis in the solvent are easily controlled. The dynamic aspects of the equilibrium between bromo ketones **1a** and **2a** and of the production of the ketol **3a** are shown in Table II. Although epimerization of 16α-bromo-17-oxo steroids to the 16β isomer in an alkaline[12] and in an acid medium[13] was previously reported, a detailed examination has not been carried out. Treatment of **1a** with 0.06 equiv of NaOH gave an ap-

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(11) Fishman, J.; Biggerstaff, W. R. *J. Org. Chem.* **1958**, *23*, 1190.
(12) Fajkos, J.: Sorm, F. *Collect, Czech. Chem. Commun.* **1959**, *24*, 766.
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Table III. Isotope Analysis by Mass Spectrometry

	content, %				
		[M − 3β-AcOH]⁺		[M − 3β,16α-(AcOH)₂]⁺	
steroid analyzed	m/e 328 ($^{16}O^{16}O$)	m/e 330 ($^{16}O^{18}O$)	m/e 332 ($^{18}O^{18}O$)	m/e 268 (^{16}O)	m/e 270 (^{18}O)
I 3a-Ac_2 from 1a treatment	0.5	18	81.5	17	83
3a-Ac_2 from 3a treatment	20	80	0	22	78

	content, %	
steroid analyzed	m/e 348, 350 ($^{16}O^{79}Br^{81}Br$)	m/e 350, 352 ($^{18}O^{79}Br^{81}Br$)
1a-Ac and 2a-Ac recovered[a]	83	17

	content, %				
				[M − 3β,17α-(AcOH)₂]⁺	
steroid analyzed	m/e 328 ($^{16}O^{16}O$)	m/e 330 ($^{16}O^{18}O$)	m/e 332 ($^{18}O^{18}O$)	m/e 268 (^{16}O)	m/e 270 (^{18}O)
II 4-Ac_2 from 3a treatment	3	30	67	26	74

[a] The recovered bromo ketones 1a and 2a were analyzed as a mizture (16α-Br/16β-Br ratio of 1:1.25).

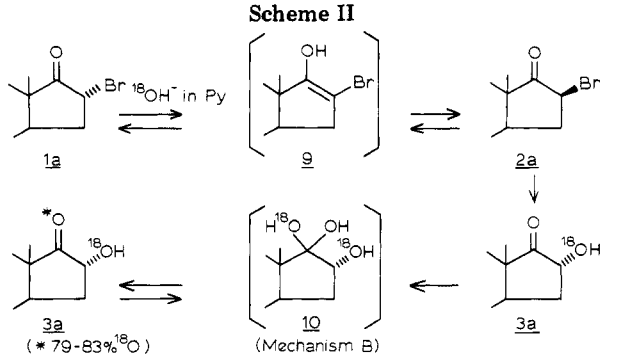
proximate 1:1.2 equilibrium between **1a** and **2a** without formation of **3a**. The bromo ketone **2a** was also epimerized back to **1a**, resulting in the same equilibrium. Its rate, however, was distinctly slower than that of the reverse. On the other hand, Fishman[14] reported a distinct preference for removal of the 16α-proton in the enolization of 17-oxo steroids which was explained in terms of steric hindrance by the C-18 methyl group to the development of an intramolecular transition complex for the removal of the 16α proton. The IR spectra in solution of **1a** and **2a** showed the same shift, +16 cm^{-1} (1748 cm^{-1}), of the carbonyl group's frequency caused by the introduction of 16α- or 16β-bromine. Considering the same shift, which indicates the same projected angle viewed along the C—C bond of BrC—C=O,[15] together with the stereoelectronic effects, first invoked by Corey[16] in the enolization of a conformationally rigid carbonyl function, it is suggested that the observed difference in the epimerization rate between **1a** and **2a** should be due to the conformational difference of ring D between them.[17]

When deuterium oxide was used in the reaction mixture (99.5 atom % of theoretical D content), the deuterium labeling at C-16 of both **1a** and **2a** was observed in the recovered compounds under conditions A and F (Table II). The observed differences in the degree of labeling, 76% for **1a** and 35% for **2a**, were as expected from the difference of the epimerization rate between **1a** and **2a** (Table II). The results show that the equilibration between **1a** and **2a** precedes the formation of **3a**. Observation over the course of time of the **1a**, **2a**, and **3a** concentrations during the reaction indicates that the conversion of **2a** to **3a** is the rate-limiting step.

The conversion of the 16β-bromo ketone **2a** to the 16α-hydroxy 17-ketone **3a** could go through the epoxide intermediate formed by hydroxide attack at the 17β-position (mechanism A, Scheme I), as previously postulated[8] for nucleophilic reactions, or proceed by the direct S_N2 displacement of the 16β-bromine with hydroxide ion (mechanism B, Scheme II). We then 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 under mechanism B. In addition, we aimed to confirm or refute the mechanism of ketol rearrangement[3,4] 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 atom %) was used with pyridine under condition E (Table II) for the controlled hydrolysis (**1a** to **3a**). The product, **3a**, and the recovered bromo ketones, **1a** and **2a**, were acetylated with Ac_2O–pyridine and then purified by TLC. The ^{18}O content of the isolated acetates was analyzed with solid samples by mass spectroscopy. The results are shown in Table III. Although the molecular ion was insignificant in all of the analyzed steroids, as is common in such steroidal acetates, strong fragment ions representing [M − acetic acid]⁺ [base peak for **3a**-Ac_2 and **4**-Ac_2 at m/e 328 and 50% of base peak (m/e 43) for **1a**-Ac and **2a**-Ac at m/e 348 and 350] and [M − diacetic acid]⁺ (18–22% of base peak for **3a**-Ac_2 and **4**-Ac_2 at m/e 268) were observed. Unexpectedly, the mass spectra of the diacetate derivative of **3a** obtained from the bromo ketone **1a** showed over 80% ^{18}O labeling at each of the 16α- and 17-positions.

Although the initial premise we made to directly determine the reaction mechanism was thus found to be invalid, careful analysis of the mass spectral data revealed not only evidence to determine the reaction mechanism but also new information about hydration of α-substituted

Scheme II



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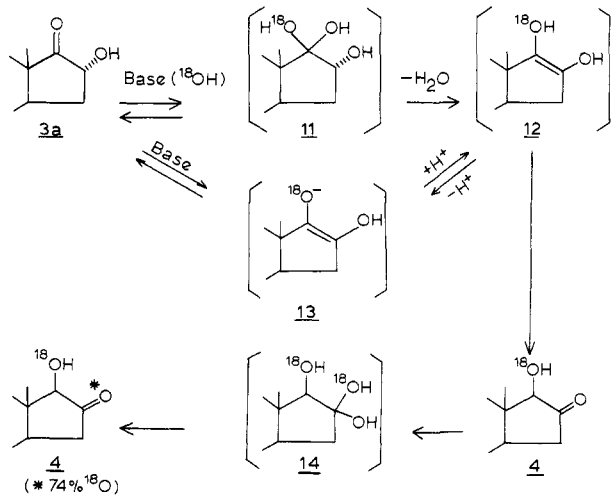
(16) Corey, E. J. *J. Am. Chem. Soc.* **1954**, *76*, 175. Corey, E. J.; Sneen, R. A. *Ibid.* **1956**, *78*, 6269. Kirk, D. N.; Hartshon, M. P. "Steroid Reaction Mechanisms"; Elservier: Amsterdam, 1968; p 154.

(17) The solid-state structure of **1a** is known and has an unstrained D-ring conformation and a 14α-envelope form (Duax, W. L.; Brennan, T. F.; Weeks, C. M.; Osawa, Y. *Cryst. Struct. Commun.* **1975**, *4*, 249). The crystal structure determination of **2a** showed that the conformation of the D ring was intermediate between a 14α,15β half-chair and a 15β envelope (Swenson, D. C.; Duax, W. L.; Osawa, Y.; Numazawa, M. *Cryst. Struct. Commun.* **1982**, *11*, 701).

Scheme III



ketones. The equal content of ^{18}O found in [M – acetic acid]$^+$ and [M – diacetic acid]$^+$ fragments gave the assignment of the [M – acetic acid]$^+$ quantitatively to be [M – 3β-acetic acid]$^+$. The percent of ^{18}O labeling at the 16α-hydroxyl group in **3a** diacetate was then calculated from the data in Table III by the following equations:

$$[16\text{-}^{18}O, 17\text{-}^{16}O] + [16\text{-}^{16}O, 17\text{-}^{16}O](0.5\%, m/e\ 328) = [17\text{-}^{16}O](17\%, m/e\ 268) \quad (1)$$

$$[16\text{-}^{18}O] = [16\text{-}^{18}O, 17\text{-}^{16}O] + [16\text{-}^{18}O, 17\text{-}^{18}O](81.5\%, m/e\ 332) \quad (2)$$

The analysis gave 83% ^{18}O labeling at the 17-carbonyl and 98% ^{18}O labeling at the 16α-hydroxyl group.[18] Ketol **3a** treated under the same condition used for **1a** 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 16α-hydroxy 17-ketone (Table III). In contrast, the recovered bromo ketone after the same 8-h treatment showed only 17% ^{18}O incorporation into the 17-carbonyl group, showing that the exchange rate for the α-bromo ketone is much slower than that for the α-hydroxy ketone. The 17β-hydroxy 16-ketone **4** obtained by ketol rearrangement of **3a** 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- and 16β-bromo ketones 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). Once the 16α-hydroxy 17-ketone is formed by the displacement the 17-ketone is hydrated easily even under controlled conditions (mechanism B, Scheme II). Under the drastic ketol rearrangement conditions (Scheme III), the hydrate **11**, which should initially place the ^{18}O label at C-17, may be dehydrated to [17-^{18}O]-enediol **12** or, alternatively, may be equilibrated back to [17-^{18}O]-17-ketone **3a**. The labeled **3a** could be enolyzed to **13** and give rise to [17-^{18}O]-enediol **12**. The ^{18}O labeling at the 17-position is definitive and extensive and therefore shows that the enediol mechanism previously formulated[3] is in error. The first step must be the 17-hydration and not the enolization.

(18) The details for the assignment of the [M – AcOH]$^+$ peak to be quantitatively [M – 3β-AcOH]$^+$ and for quantitative analysis of the ^{18}O labeling at the 16α-hydroxyl group were discussed in the preliminary paper.

Table IV. Isotope Effects on Base-Catalyzed Epimerization of 16α-Bromo Ketone **1b**[a]

NaOH, equiv	substr	rel amt of products,[b] % 1b	2b	3b
0.12	**1b**	41	50	9
	1b-*16β-d*	60	37	3
0.30	**1b**	31	41	28
	1b-*16β-d*	40	42	18
0.60	**1b**	18	22	60
	1b-*16β-d*	17	23	60

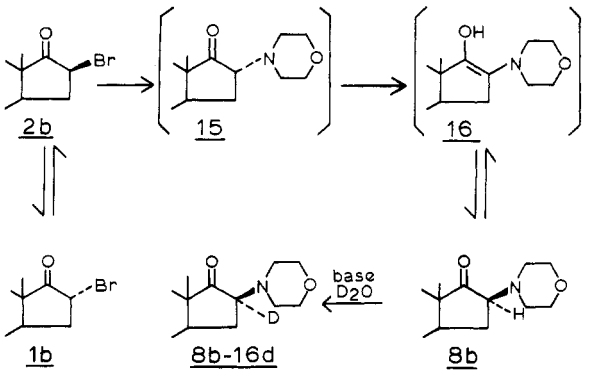
[a] The 16α-bromo 17-ketone **1b** was treated with NaOH in aqueous pyridine at room temperature for 10 min. [b] The relative amounts of products were determined by ^1H NMR.

Although the possibility of the enolization of 17-labeled **3a** to give intermediate **13** cannot be eliminated on the basis of currently available evidence, there is no reason to believe that the rate of enolization of the nonlabeled **3a** should be slower than that of the labeled **3a**. If the ketol rearrangment involves the enol **13** as an obligatory intermediate, the ^{18}O content at the 17-hydroxyl group of **13**, then, is expected to be lower than that of **11**. The observed labelings of 91% at the 17β-hydroxyl and of 74% at the carbonyl, which is the extent of the equilibrium after the ketol rearrangement, strongly favors the hydration–dehydration mechanism (Scheme III, **3a** → **11** → **12**). In this mechanism, the 17-hydration is the first step, with the degree of ^{18}O labeling equal to the initial ^{18}O content of the medium, and gives the intermediate triol **11**, and this is followed by the 1,2-dehydration to preferentially remove nonlabeled water from 16- and 17-positions, presumably the 17β-hydroxide addition[19] and the 17α-hydroxyl and 16β-proton elimination in the trans quasi-diaxial conformation, to give the 16-ene-16,17-diol **12**. Ketonization of **12** then gives highly 17β-labeled, thermodynamically stable ketol **4**. The ^{18}O labeling at the 16-position of **4** under the drastic conditions should be equal to the ^{18}O content of the diluted medium after the ketol rearrangement which was analyzed to be 74%. If the initial ^{18}O labeling at the 17-carbonyl is very fast and the enolization to form **13** is slow and obligatory, the ^{18}O labeling at the 17- and 16-positions should have been equal. The large discrepancy of the two labelings disputes the enolization mechanism, and we postulate the hydration–1,2-dehydration mechanism for the ketol rearrangement. The formation of the 16β-hydroxy compound should be a positive indication of the enolization mechanism involving the intermediate **13**. The fact that it was not detected at all during the rearrangement further supports the hydration–dehydration mechanism.

Isotope Effect on Epimerization of the 16α-Bromo 17-Ketone 1a. When 3β-hydroxy-5α-androstan-17-one-*16,16-d*$_2$ (d_0, 12%; d_1, 6%; d_2, 82%) was subjected to the bromination of $CuBr_2$, the 16α-bromo 17-ketone **1b**-*16-d* (62 atom %) was quantitatively obtained. The deuterium labeling at the 16β-position of **1b** was less by approximately 20% than that of the starting material. This should be due to the enolization–ketonization reaction of the starting material before the bromination and not to the enolization

(19) The first β-side attack by the nucleophile at the 17-carbonyl function is favored in this mechanism. Nucleophilic 17β additions of MeO^- and NH_2NH_2 to the 16β-bromo 17-ketone **2** have been reported to form the 17β-substituted 16α,17α-epoxide.[4,7,8] The β side of the 17-carbonyl in the compound **2** is sterically more hindered by the presence of bromine than in the 16α-hydroxylated compound **3**. Recent total structure analysis showed that the addition product of CN^- to **2** is the 17β-cyano 16α,17α-epoxide (Swenson, D. C.; Duax, W. L.; Numazawa, M.; Osawa, Y. *Cryst. Struct. Commun.* **1982**, *11*, 617.

Scheme IV



of **1b**-*d* produced because **2b** is not detected under the reaction conditions. Treatment of **1b**-*d* with 0.12 and 0.30 equiv of NaOH in aqueous pyridine gave rise to an isotope effect on the C–H(D) breaking process in the enolization of the 17-carbonyl function (Table IV), even though the deuterium labeling of **1b**-*d* was only 62%. The observed production ratios of the 16α-hydroxy derivative **3b** would primarily be due to the isotope effect in the enolization process, because the epimerization of the bromo ketone **1b** to **2b** precedes the hydrolysis under the controlled conditions. The results also demonstrate that the C–H(D) breaking process should be the rate-limiting step in the equilibration between the 16-bromo ketones, similar to substitution reactions of enolizable ketones.[16]

Reaction of the Bromo Ketones 1 and 2 with Morpholine. The discrepancy between the 16β assignment for substitution by other nucleophiles[5,6] and the 16α configuration for hydroxide substitution presented here should be noted. The configuration of the 16β-morpholino 17-ketone **8** was first assigned by molecular rotational evidence.[5] Recently its total structure was unambiguously determined by X-ray crystallography.[20] Conformational analysis of the solid-state structure shows that the 16β-morpholino derivative **8a** has a thermodynamically stable conformation. It has an unstrained D-ring conformation, the 14α-envelope form, and an unstrained morpholino substituent at C-16 in a chair conformation. Nonbonded interaction between the C-18 angular methyl and the 16β-morpholino group could not be detected in **8a**.

When the bromo ketones **1a** and **2a** were separately subjected to the reaction with morpholine at room temperature for 30 min, the recovered bromo ketones (yield 83%) were shown by NMR analysis to give the same equilibrium between **1a** and **2a** as observed under the controlled conditions. On the other hand, the product obtained upon heating either the 16α isomer **1a** or the 16β isomer **2a** with the base was seemingly only the 16β-morpholino isomer as previously reported.[5] Treatment of the 16β-morpholino derivative **8b** with morpholine–D_2O (2:1 v/v) under the drastic conditions gave in an almost quantitative yield monodeuterated **8b** (Scheme IV). The signal at δ 2.98 (t, 1 H) of the 16-proton of **8b**-*d* by NMR and M + 1 (*m/e* 384) and M + 2 (*m/e* 385) fragments of **8b**-*d* by mass spectrometry[21] demonstrated that the deuterium was incorporated regiospecifically at the 16α-position of **8b** with an 82 atom % abundance.

These results suggest that the thermodynamic control of the substitution reaction strongly prefers the formation of the 16β-isomer through enolization and that the displacement by morpholine of the 16-bromo 17-ketone occurs initially to form 16α-morpholino 17-ketone **15**, by the S_N2 substitution on the 16β-bromo isomer **2b** as elucidated unambiguously for the controlled hydroxide reaction, and then the 16α-morpholino isomer **15** epimerizes to the thermodynamically stable 16β-isomer **8b** in the presence of heated basic morpholine (Scheme IV).

Experimental Section

General Methods. Melting points were measured on a Fisher-Jones melting point apparatus and were uncorrected. IR spectra were recorded on a Perkin-Elmer 267 spectrophotometer in KBr pellets. ^{1}H NMR spectra were obtained with a Varian EM-360 spectrometer and a JEOL JNM-PMX 60 spectrometer at 60 MHz with tetramethylsilane as an internal standard. Mass spectra were measured on a Hitachi RMU-7 spectrometer.

16α-Bromo-17-oxo Steroids 1. A solution of 27.7 mmol of 3β-hydroxy-5α-androstan-17-one or 3β-hydroxy-5-androsten-17-one and 83.5 mmol of $CuBr_2$ in 300 mL of dry MeOH was heated under reflux for 12 h. After the same workup as previously reported,[9] the bromo ketone **1b** and **1a** were obtained in 95% and 98% yields, respectively. **1a**: mp 177–178 °C (MeOH) (lit.[9] mp 175–176 °C); ^{1}H NMR ($CDCl_3$) δ 0.93 (3 H, s, 18-CH_3), 1.00 (3 H, s, 19-CH_3), 3.45 (1 H, br m, 3α-H), 4.40 (1 H, m, 16β-H), 5.40 (1 H, m, 6-H). **1b**: mp 164–165 °C (MeOH) (lit.[22] mp 164–165 °C); ^{1}H NMR ($CDCl_3$) δ 0.83 (3 H, s, 19-CH_3), 0.90 (3 H, s, 18-CH_3), 3.46 (1 H, br m, 3α-H), 4.55 (1 H, m, 16β-H).

16β-Bromo-3β-hydroxy-5-androsten-17-one (2a). Repeated crystallization from MeOH of the reaction mixtures of **1a** and **2a** obtained from the epimerization of **1a** with 0.12 equiv of NaOH in pyridine gave a pure 16β isomer, **2a**, mp 171–173 °C (lit.[23] mp 176–177 °C).

16α-Bromo-3β-hydroxy-5α-androstan-17-one-*16-d* (1b-*16-d*). 3β-Hydroxy-5α-androstan-17-one-*16,16-d*$_2$ was synthesized according to Tökés et al.[24] and consisted of the following mixtures as determined by mass spectroscopy using M^+ ion peaks: 12% d_0, 6% d_1, 82%, d_2. The deuterated compound was converted to the 16α-bromo ketone **1b** as described above: mp 163–164 °C; 38% d_0 and 62% d_1 by MS.

Epimerization of the 16-Bromo 17-Ketones and Formation of 16α-Hydroxy 17-Ketones in NaOH–Aqueous Polar Solvent. To a solution of 16α- or 16β-bromo 17-ketone (0.41 mmol) in an aqueous polar solvent (8 mL) was added 0.48 mL of NaOH solution, and the mixture was allowed to stand at room temperature for an appropriate period. The mixture was poured into 1% HCl solution and then extracted with AcOEt. The organic layer was washed with 5% $NaHCO_3$ and water and dried with Na_2SO_4. After evaporation of the solvent, the residue (110–140 mg) was submitted to NMR analysis.

3β,16a-Dihydroxy-5-androsten-17-one (3a). The hydrolyzed residue of **1a** obtained with 75% DMF, 1.2 equiv of NaOH, and a 30-min reaction time was recrystallized from MeOH to give **3a** (118 mg, 95%) as colorless needles: mp 188–190 °C (lit.[8] mp 187–189 °C); ^{1}H NMR ($CDCl_3$) δ 0.98 (3 H, s, 18-CH_3), 1.00 (3 H, s, 19-CH_3), 3.45 (1 H, br m, 3α-H), 4.40 (1 H, m, 16β-H), 5.41 (1 H, m, 6-H).

3β,16α-Dihydroxy-5α-androstan-17-one (3b). The hydrolyzed residue of **1b** obtained under the same conditions as above was recrystallized from MeOH to give **3b** (110 mg, 89%) as colorless needles: mp 183–185 °C (lit.[8] mp 181–184 °C) ^{1}H NMR ($CDCl_3$) δ 0.83 (3 H, s, 19-CH_3), 0.90 (3 H, s, 18-CH_3), 3.66 (1 H, br m, 3α-H), 4.55 (1 H, m, 16β-H).

16α-Hydroxy-4-androstene-3,17-dione (5b). The 16α-bromo compound **5a**[10] was hydrolyzed in the same manner as above. The hydrolyzed product was recrystallized from acetone to give **5b** (108 mg, 89%) as colorless plates: mp 188–190 °C (lit.[9b] mp 188–191 °C); ^{1}H NMR ($CDCl_3$) δ 1.00 (3 H, s, 18-CH_3), 1.21 (3 H, s, 19-CH_3), 4.36 (1 H, m, 16β-H), 5.72 (1 H, s, 4-H).

3,16α-Dihydroxy-1,3,5(10)-estratrien-17-one (6b). The crude **6b** obtained from **6a**[11] by use of 75% DMF, 2 equiv of NaOH,

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(21) The mass spectrum of the standard **8b** showed M^+ and M^+ + 1 peaks (1:1) at *m/e* 383 and 384.
(22) Fajkos, J.; Sorm, F. *Chem. Listy* **1958**, *52*, 505.
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and a 1-h reaction time was recrystallized from MeOH to give a pure **6b** (112 mg, 90%) as colorless plates: mp 203–206 °C (lit.[25] mp 205–207 °C); ^{1}H NMR ($CDCl_3$) δ 0.97 (3 H, s, 18-CH_3), 4.40 (1 H, m, 16β-H), 6.51–7.20 (3 H, m, aromatic protons).

Sodium 3β,16α-Dihydroxy-17-oxo-5-androsten-3-yl Sulfate (7). The 16α-bromo 17-ketone **1a** (2 g, 4.88 mmol) in 10 mL of dry pyridine was added to 1.5 equiv of pyridine–$ClSO_3H$ complex in 20 mL of pyridine with stirring under ice cooling. After 20 min the reaction mixture was poured into 1 L of chilled 0.1 N NaOH solution 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 column was washed with water (1 L), the sulfate was eluted with MeOH (1 L). The eluate was condensed to 20 mL and allowed to stand at 4 C for 24 h. The precipitates (1.95 g) were collected by filtration and recrystallized from MeOH–Et_2O to give **7** (1.63 g, 85%) as colorless needles: mp > 280 °C; IR (KBr) ν_{max} 3440, 1738, 1235 cm^{-1}; ^{1}H NMR [pyridine-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 $C_{19}H_{27}O_6SNa\cdot H_2O$: C, 53.76; H, 6.89; S, 7.55. Found: C, 53.54; H, 6.83; S, 7.42.

Hydrolysis of 16α-Bromo Ketone 1a with the NaOH–$H_2{}^{18}O$–Pyridine System. Bromo ketone **1a** or ketol **3a** (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 F, Table II). The reaction mixture was poured into 5% HCl solution and extracted with AcOEt (2 × 20 mL). The organic layer was washed with 5% $NaHCO_3$ and water and dried with Na_2SO_4. After evaporation of the solvent, the residue (30–32 mg) was acetylated with pyridine (1 mL)–Ac_2O (0.5 mL). The crude acetates obtained by evaporation of the solvents under the reduced pressure were purified by TLC (*n*-hexane–AcOEt, 4:1) to give the pure **3a** diacetate [mp 167–168 °C (lit.[8] mp 167–168 °C); ^{1}H NMR ($CDCl_3$) δ 1.00 (3 H, s, 18-CH_3), 1.06 (3 H, s, 19-CH_3), 2.00 (3 H, s, 3β-$OCOCH_3$), 2.12 (3 H, s, 17β-$OCOCH_3$), 4.60 (1 H, br m, 3α-H), 5.43 (2 H, m, 6-H and 16β-H)] and the acetates of the recovered bromo ketones **1a** and **2a** (16α-Br/16β-Br ratio of 1:1.25).

Ketol Rearrangement of 3a. The ketol **3a** (25 mg, 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 reaction mixture was poured into 5% HCl solution and then extracted with AcOEt (2 × 10 mL). The organic layer was washed with 5% $NaHCO_3$ and water and dried with Na_2SO_4. After evaporation of the solvent, the rearranged product was recrystallized from MeOH to give **4** (13 mg, 52%) as colorless needles: mp 204–206 °C (lit.[26] mp 202–205 °C); ^{1}H NMR ($CDCl_3$) δ 0.78 (3 H, s, 18-CH_3), 1.05 (3 H, s, 19-CH_3), 3.45 (1 H, br m, 3α-H), 3.73 (1 H, s, 17α-H), 5.43 (1 H, m, 6-H). **4** was acetylated with pyridine–Ac_2O as usual to give the **4** diacetate: mp 125 °C; ^{1}H NMR ($CDCl_3$) δ 0.83 (3 H, s, 18-CH_3), 1.06 (3 H, s, 19-CH_3), 2.00 (3 H, s, 3β-$OCOCH_3$), 2.13 (3 H, s, 17β-$OCOCH_3$), 4.66 (1 H, br m, 3α-H), 5.00 (1 H, s, 17α-H), 5.40 (1 H, m, 6-H).

16β-Morpholino-3β-hydroxy-5-androsten-17-one (8a). A solution of **1a** or **2a** (300 mg, 0.86 mmol) in morpholine (3 mL) was heated under reflux for 2 h. After removal of the majority of the amine by distillation under reduced pressure, the products were precipitated with water, and the gummy solid was washed with water. The crude 16β-morpholino 17-ketone was recrystallized from MeOH to give a pure **8a** as colorless plates: mp 201–202 °C (lit.[5a] mp 200 °C); ^{1}H NMR ($CDCl_3$) δ 0.90 (3 H, s, 18-CH_3), 1.03 (3 H, s, 19-CH_3), 2.67 (4 H, t, J = 5 Hz, 3′-H), 2.98 (1 H, q, J = 4, 2 Hz, 16α-H), 3.55 (1 H, br m, 3α-H), 3.90 (4 H, t, J = 5 Hz, 2′-H), 5.40 (1 H, m, 6-H).

16β-Morpholino-3β-hydroxy-5α-androstan-17-one (8b). A solution of **1b** (300 mg, 0.86 mmol) in morpholine was heated under reflux for 1 h. After the same workup as above, the crude 16β-morpholino compound was recrystallized from aqueous MeOH to give a pure **8b** as colorless needles: mp 195–198 °C (lit.[5a] mp 192–197 °C); ^{1}H NMR ($CDCl_3$) δ 0.83 (3 H, s, 19-CH_3), 0.86 (3 H, s, 18-CH_3), 2.67 (4 H, t, J = 5 Hz, 3′-H), 2.98 (1 H, q, J = 4, 2 Hz, 16α-H), 3.50 (1 H, m, 3α-H), 3.72 (4 H, t, J = 5 Hz, 2′-H).

Epimerization of 16β-Morpholino Derivative 8b. The 16β-morpholino derivative **8b** (90 mg, 0.24 mmol) was heated under reflux in morpholine (1 mL)–D_2O (0.5 mL) for 1 h. The solvent was evaporated under reduced pressure to give deuterated **8b**. After crystallization from MeOH–water, **8b**-*d* was isolated: 65% yield; mp 193–194 °C (lit.[5a] mp 192–197 °C).

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Registry No. **1a**, 1093-91-0; **1a** acetate, 24335-49-7; **1b**, 28507-02-0; **1b-16-d**, 82865-80-3; **2a**, 74644-60-3; **2a** acetate, 24335-50-0; **3a**, 1232-73-1; **3a** diacetate, 10587-75-4; **3b**, 10459-27-5; **4**, 1159-66-6; **4** diacetate, 1249-72-5; **5a**, 61145-69-5; **5b**, 63-02-5; **6a**, 71765-95-2; **6b**, 566-76-7; **7**, 35420-26-9; **8a**, 5986-91-4; **8b**, 3000-34-8.

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An Excursion into the Synthesis of Potential Angiotensin Converting Enzyme Inhibitors

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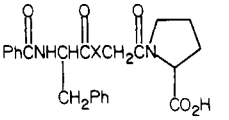
An attempt to prepare dehydro analogues of **1** gave the expected tripeptide **2a**, but rearrangement of a thiazolone derivative of Δ^Z-Phe made only a thiazolinecarboxylic acid (**8**) available. The latter was also converted into a tripeptide (**11**) and both compounds, **2a** and **11**, showed moderate angiotensin converting enzyme inhibition.

Angiotensin converting enzyme (ACE) is responsible for the hydrolysis[1] of a decapeptide, angiotensin I, to give the potent pressor octapeptide, angiotension II. Inhibition of this process allows control of hypertension in some human subjects. Recently reported ACE inhibitors include the simple tripeptide,[2a] *N*-Bz-Phe-Gly-Pro-OH (**1a**), which



1a, X = NH
b, X = CH_2

shows an IC_{50}[3] for ACE of 9.4 × 10^{-6} M, and its "keto methylene" modification[2a] (**1b**), which has an IC_{50} of 7 ×

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