NOVEL EPIMERIZATION OF STEROIDAL ALLYLIC ALCOHOLS

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ABSTRACT

Epimerization of 4-pregnene-3β,20α-diol into 4-pregnene-3α,20α-diol was achieved under the mild condition of an acidic medium at room temperature. This reaction was favorable for synthesis of 4-pregnene-3α,20α-diol in better yield, after chemical reduction of 20α-hydroxy-4-pregnen-3-one with metal hydrides, which resulted in predominant production of 4-pregnene-3β,20α-diol. The by-product which was formed more by raising the temperature was identified as 3,5-pregnadien-20α-ol. This method was also applicable for epimerization of other Δ^4-3β-hydroxysteroids.

INTRODUCTION

In our previous investigations on the steroid metabolism in the mammary glands of mice (2) and rats (3), formation of steroidal allylic alcohols, 4-pregnene-3α,20α-diol (2a) and 4-androstene-3α,17β-diol (2d), was established. Besides these reports, Δ^4-3α-hydroxysteroid formation has been observed in a variety of tissues, such as adrenal gland, ovary (4), fetal liver (5,6), and uterus (7,8). Although considerable interest has been centered on the physiological significance of steroidal allylic alcohols, synthesis of the Δ^4-3α-hydroxysteroids in sufficient yield has been found difficult. In fact, this kind of steroids has been obtained as the minor product (5 - 10 %) of the metal hydride reduction of Δ^4-3-oxosteroids. For example,

the reduction of 20α-hydroxy-4-pregnen-3-one by lithium aluminum hydride ($LiAlH_4$) yielded the corresponding diols, 4-pregnene-3β,20α-diol (1a) and its 3α-isomer (2a), in a ratio of 94 : 6 (2). Burstein and Ringold (9) reported another synthetic approach in which the stereo-controlled synthesis of Δ^4-3α-hydroxysteroid was involved, but required five steps for the synthesis.

In this paper, the simple and efficient synthesis of Δ^4-3α-hydroxysteroid by the single step epimerization of Δ^4-3β-hydroxysteroid is reported.

RESULTS AND DISCUSSION

Allylic alcohols in an acidic medium are readily dehydroxylated to yield relatively stable allylic cations, whose recombination with a hydroxyl group is expected to afford a mixture of the equilibrated allylic alcohol isomers. Thus, when Δ^4-3β-hydroxysteroids which are usually the major product of hydride reduction of Δ^4-3-oxosteroids are epimerized partially in an acidic medium, a mixture of Δ^4-3β- and 3α-hydroxysteroids can be obtained as a thermodynamically equilibrated mixture.

Initial studies were carried out with [^{3}H]4-pregnene-3β, 20α-diol (1a), obtained predominantly by $LiAlH_4$ reduction of [^{3}H]20α-hydroxy-4-pregnen-3-one. The steroid (1a) was treated with 1 % sulfuric acid in aqueous acetone at 4°C, 21°C, and 60°C. Samples taken at various time intervals were analyzed on thin layer chromatography and the products were quantitated from their radioactivities. As a result

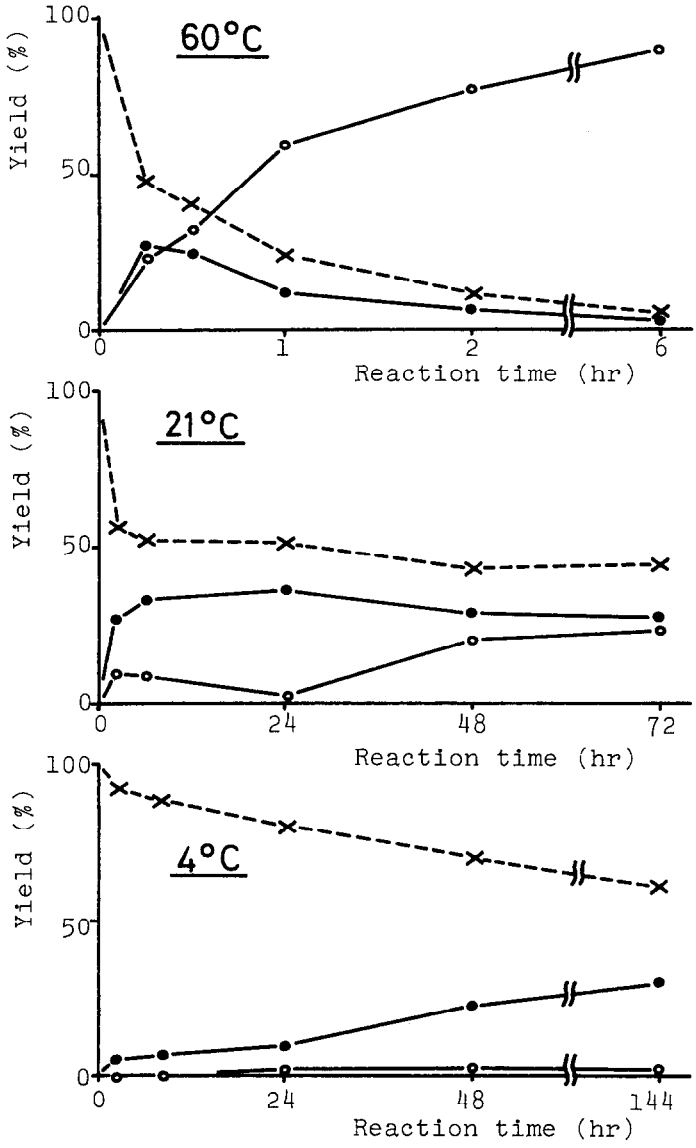


Fig. 1. Effect of Reaction Temperature for Epimerization of 4-Pregnene-3β,20α-diol (1a).

×---× 4-Pregnene-3β,20α-diol (1a)
•——• 4-Pregnene-3α,20α-diol (2a)
o——o 3,5-Pregnadien-20α-ol (3a)

of the epimerization reaction, only three products were obtained and almost all radioactivities could be recovered at each time intervals. The typical reaction patterns at the three different temperatures are shown in Fig. 1. At 60°C, the epimerized diol, 4-pregnene-3α,20α-diol (2a), increased at remarkably fast rate with decreasing of the starting diol (1a), but both were completely transformed to the thermodynamically more stable diene, 3,5-pregnadien-20α-ol (3a). This procedure may be generally applicable for preparation of steroidal 3,5-dienes from Δ^4-3-hydroxy-steroids. The reaction at 4°C was not suitable for the preparative method, because of the slow reaction and poor yield. The appropriate rate for the epimerization was observed at 21°C, resulting the maximum formation of Δ^4-3α-hydroxysteroid (2a) at 24 hrs.

The effect of acid concentration on the epimerization was also studied. As shown in Table 1, increment of the acid concentration resulted in the undesired products (3a). It was also demonstrated that perchloric acid could be

Table 1. Effect of Acid Concentration for Epimerization of 4-Pregnene-3β,20α-diol (1a).

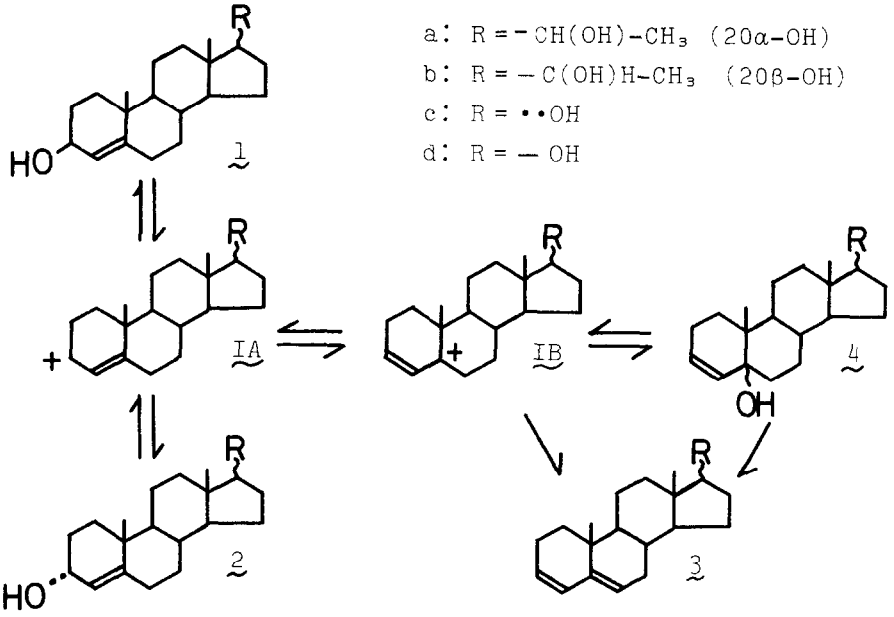
		Starting Material	Yield (%) at 24 hrs	
Acid	Concentration	1a	2a	3a
H_2SO_4	10 %	24	13	63
	5 %	21	12	68
	1 %	57	33	10
$HClO_4$	10 %	36	15	50
	5 %	26	14	60
	1 %	59	36	6

substituted for sulfuric acid.

From these results, it can be concluded that 4-pregnene-3β,20α-diol (1a) is effectively epimerized to the 3α-isomer (2a) in 83.5 % aqueous acetone containing 1 % sulfuric or perchloric acid at room temperature for 24 hrs with constant stirring.

This condition was applied to other Δ^4-3β-hydroxysteroids such as 4-pregnene-3β,20β-diol (1b), 4-androstene-3β,17α-diol (1c), or 4-androstene-3β,17β-diol (1d). Resulting Δ^4-3α-hydroxysteroids were identified by digitonin precipitability (10), oxidation by manganese dioxide (MnO_2) (11), color reaction with trichloroacetic acid in CH_2Cl_2 (12), and finally by ^{1}H-NMR and mass spectrometric analyses. Oxidation of Δ^4-3α-hydroxysteroids with MnO_2 resulted in the corresponding Δ^4-3-oxosteroids. Treatment of allylic alcohols with trichloroacetic acid gave the characteristic pink color, while Δ^4-3-oxosteroids did not react.

The structural characterization of all products was confirmed by spectroscopical methods. Comparison of the ^{1}H-NMR spectra of the C_4 proton of Δ^4-3-oxosteroids to the corresponding Δ^4-3β- and Δ^4-3α-hydroxysteroids led the same result as reported by Burstein and Ringold (9). The peak of the C_4 proton of Δ^4-3α-hydroxysteroids was observed as doublet ($J = 5$ Hz) and shifted 0.26 - 0.27 ppm upper field from those of the corresponding Δ^4-3-oxosteroids, while Δ^4-3β-hydroxysteroids exhibited the C_4 proton as a broad



Scheme 1. Proposed Mechanism of the Epimerization.

singlet peak with 0.43 - 0.45 ppm upper field shift.

Proposed mechanism of the epimerization is illustrated in Scheme 1. The allylic cation (IA), which is derived initially from the acid-catalyzed dehydroxylation of 1, is an equilibrium with the tertiary allylic cation (IB). The non-stereospecific attack of a hydroxy anion to IA forms 1 and 2. Similarly, the ion (IB) might give the corresponding allylic tertiary alcohol (4). Because of the remarkable instability of 4, it goes back to the ion (IB) under the present condition or is directly transformed to yield the diene (3). It is also possible that the diene (3) can be generated directly from the ion (IB).

The present findings offer the simple procedures of not only the synthesis of Δ⁴-3α-hydroxysteroids from Δ⁴-3β-

hydroxysteroids, but also that of $\Delta^{3,5}$-steroids by one step elimination, due to the employed conditions.

EXPERIMENTAL

Physical constant Melting points were determined with a micro melting point apparatus (Yanagimoto Co., Tokyo) and are not corrected. Nuclear magnetic resonance spectra were recorded on Varian FT-80A (80 MHz) and XL-100 (100 MHz) spectrometers in deutriochloroform using tetramethylsilane as an internal reference. Mass spectra were recorded by electron impact method on a mass spectrometer (Model D-300, Japan Electronic Optics Laboratory, Tokyo). Ultraviolet and visible spectra were recorded on spectrophotometers (Cary 17D and Union SM401).

Preliminary experiment [^{3}H]4-Pregnene-3β,20α-diol (1a, 1.27 mg, m.p.182.5 - 184), which was synthesized from [1,2-^{3}H]20α-hydroxy-4-pregnen-3-one (specific activity, 1.73×10^5 cpm/mg) by $LiAlH_4$ (E. Merck, Darmstadt, Germany) reduction in anhydrous ethyl ether (2), was dissolved in 1.0 ml of acetone and 0.2 ml of 1 % H_2SO_4 was added. While the mixture kept at 4°C, 21°C, or 60°C with stirring, 10 µl of each sample was taken at various time intervals, and was analyzed on thin layer chromatography developing with benzene-acetone (4 : 1) system. The spots which were detected by exposing the plate to iodine vapor or by spraying phosphomolybdate solution in 95 % ethanol followed by a short heating were scraped off from the plate and their radioactivities were measured by a liquid scintillation counter (Nuclear Chicago, System 725, Des Plaines, IL).

Another series of experiment was also performed with [^{3}H]4-pregnene-3β,20α-diol (1a) 0.64 mg dissolved in 0.5 ml of acetone. After adding 0.1 ml of various concentration of H_2SO_4 and $HClO_4$, the mixture was maintained at 21°C for 24 hrs with stirring. The sample was analyzed in the same manner as state above.

Identification of the products For identification of the reaction products, the mixture obtained from the above experiments were pooled and acetone was evaporated under a reduced pressure. The steroids were extracted with ethyl acetate and separated by thin layer chromatography. There were three spots on the chromatogram, one U.V. positive spot and two U.V. negative spots. Each spot was extracted from silica gel with ethanol-ether (3 : 7) mixture.

The extract from the U.V. positive spot (Rf 0.54) was concentrated and crystallized from ethanol to afford 3a: m.p. 113.5 - 115; U.V. spectrum (methanol) λ_{max} 228 ($\log \varepsilon$ 4.23), 235 (4.26), 243 (4.07); ^{1}H-NMR spectrum ($CDCl_3$, ppm) 5.89 (m, 1H, 4-H), 5.64 (m, 1H, 3-H or 6-H), 5.40 (m, 1H, 6-H or 3-H), 3.73 (m, 1H, 20β-H); mass spectrum (EI) m/e

300 (M^+), 285 (M^+-CH_3), 282 (M^+-H_2O).

The extracts from two U.V. negative spots were oxidized with MnO_2 to give 20α-hydroxy-4-pregnen-3-one, and reacted to trichloroacetic acid in CH_2Cl_2 for a color test. Crystallization of the extract from the spot which showed the identical chromatographic behavior (Rf 0.30) with 4-pregnene-3β,20α-diol (1a) and precipitated with digitonin yielded 1a: m.p. 182.5 - 184; ^{1}H-NMR spectrum ($CDCl_3$, ppm) 5.25 (bs, 1H, 4-H), 4.12 (m, 1H, 3α-H), 3.68 (m, 1H, 20β-H).

After concentrating the extract from the other U.V. negative spot (Rf 0.22), which was not precipitated with digitonin, crystallization from ethanol gave 2a: m.p. 206 - 209.5; ^{1}H-NMR spectrum ($CDCl_3$, ppm) 5.42 (bd, J = 5 Hz, 1H, 4-H), 4.04 (m, 1H, 3β-H), 3.67 (m, 1H, 20β-H).

^{1}H-NMR Spectrum of 20α-hydroxy-4-pregnen-3-one in $CDCl_3$ showed peaks at 5.68 ppm (s, 1H, 4-H) and 3.69 ppm (m, 1H, 20β-H).

General epimerization procedure Δ^4-3β-Hydroxysteroids (1) 20mg was dissolved in 20 ml of acetone and 4 ml of 1 % H_2SO_4 was added. Final concentration of the steroid was 2.62 to 2.87 mM and the final concentration of H_2SO_4 and water were 0.167 % and 16.5 %, respectively. After stirring the above mixture at 21°C for 24 hrs, an equimolar amount of NaOH (3.0 ml of 0.5 N NaOH) was added and acetone was evaporated under a reduced pressure. The steroids were extracted with ethyl acetate and the extract was evaporated to dryness. Resulting solid was dissolved in 2 ml of dehydrated ethanol and digitonin solution (36.9 mg digitonin in 80 % aqueous ethanol) was added and allowed to stand at 4°C overnight. After resulting precipitate was filtered off, the filtrate was evaporated at 30 - 35°C and the residue was resuspended in ether. This suspension was filtered on a glass wool to remove the colloidal digitonin and the filtrate was applied to thin layer chromatography, using 20 x 20 cm pre-coated TLC plate (silica gel 60, F-254, layer thickness 0.25 mm, E. Merck).

Detection of the bands on the chromatogram was performed by spraying a phosphomolybdate solution to a part of the plate and the band of Δ^4-3α-hydroxysteroids was scraped off from the plate, which was not treated with the phosphomolybdate, and steroids were eluted from the silica gel with ethanol-ether (3 : 7) mixture. Final crystallization was performed from ethanol.

4-Pregnene-3α,20β-diol (2b) This compound (2b, 3.34 mg, 17 % yield) was obtained from 19.46 mg of 4-pregnene-3β,20β-diol (1b) by the general epimerization procedure stated above. The crystalline 4-pregnene-3α,20β-diol (2b) gave the following physical constants: m.p. 172 - 174; Rf 0.26; ^{1}H-NMR spectrum ($CDCl_3$, ppm), 5.48 (bd, J = 5 Hz, 1H, 4-H), 4.08 (m, 1H, 3β-H), 3.74 (m, 1H, 20α-H); mass spectrum (EI) m/e 318 (M^+), 300 (M^+-H_2O), 285 ($M^+-H_2O-CH_3$).

The peak of the C_4 proton of 4-pregnene-3β,20β-diol (1b) on ^{1}H-NMR spectrum was 5.30 ppm (bs), and that of 20β-hydroxy-4-pregnen-3-one was 5.75 ppm (s).

4-Androstene-3α,17α-diol (2c) 4-Androstene-3β,17α-diol (1c, 18.98 mg) was epimerized according to the general procedure and 5.46 mg (29 %) of crystalline 4-androstene-3α,17α-diol (2c) was obtained: m.p. 180 - 182; Rf 0.13; ^{1}H-NMR spectrum ($CDCl_3$, ppm) 5.49 (bd, J = 5 Hz, 1H, 4-H), 4.08 (m, 1H, 3β-H), 3.75 (d, J = 6 Hz, 1H, 17β-H); mass spectrum (EI) m/e 290 (M^+), 272 (M^+-H_2O).

The ^{1}H-NMR peaks of the C_4 proton of 4-androstene-3β,17α-diol (1c) and the corresponding 3-oxosteroid were 5.31 ppm (s) and 5.76 ppm (s) respectively.

4-Androstene-3α,17β-diol (2d) From 18.35 mg of 4-androstene-3β,17β-diol (1d), 4.98 mg (27 %) of 4-androstene-3α,17β-diol (2d) was obtained: m.p. 233.5 - 237; Rf 0.22; ^{1}H-NMR spectrum ($CDCl_3$, ppm) 5.48 (bd, J = 5 Hz, 1H, 4-H), 4.08 (m, 1H, 3β-H), 3.64 (m, 1H, 17α-H); mass spectrum (EI) m/e 290 (M^+), 272 (M^+-H_2O).

The C_4 proton of 4-androstene-3β,17β-diol (1d) showed peak at 5.29 ppm (s) on ^{1}H-NMR spectrum, and that of 17β-hydroxy-4-androsten-3-one showed a singlet peak at 5.74 ppm.

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