On the Acid-Catalyzed D-Homoannulation of Pregnanetriol 20-Sulfate and Its C-20 Isomeric Sulfate

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Received February 14, 1994; accepted April 28, 1994

To demonstrate the rearrangement reactions of pregnanetriol 20-sulfates in hot acid hydrolysis, 5β-pregnane-3α,17α,20α-triol 20-sulfate (8a) and its C-20 isomer 5β-pregnane-3α,17α,20β-triol 20-sulfate (12a) were heated in 3 M hydrochloric acid. As the sole D-homosteroidal product of each sulfate, 3α-hydroxy-17aβ-methyl-D-homo-5β-androstan-17-one (13a) and 3α-hydroxy-17α-methyl-D-homo-5β-androstan-17a-one (17a) were obtained from 8a and 12a, respectively, accompanied with several kinds of degradation products considered to be monohydroxysteroidal dienes. It became clear that the reaction of 8a proceeds *via* two steps: ring enlargement of 8a occurred at once to give 3α-hydroxy-17aα-methyl-D-homo-5β-androstan-17-one (14) as the intermediate, followed by isomerization to 13a as the final product.

The mechanism of D-homoannulation was elucidated by hydrolysis of [20-^{13}C]pregnanetriol 20-sulfate (8b, 12b). The D-homosteroid obtained from 8b contained a quantitative amount of the isotope only at C-17a, indicating that the ring-enlargement reaction of the 20α-sulfate proceeds with stereospecific migration of the C_{13}–C_{17} bond. Compound 12b gave the ^{13}C-labelled D-homosteroid enriched solely at C-17, which means that the D-homoannulation of 20β-sulfate occurs by stereospecific migration of the C_{16}–C_{17} bond.

The diene products from 8a and 12a were formed from the reaction intermediates 17α,20β-oxido-5β-pregnan-3α-ol (19) and 17α,20α-oxido-5β-pregnan-3α-ol (20), respectively.

The mechanism of these rearrangement reactions is discussed.

Keywords D-homoannulation; pregnanetriol 20-sulfate; ^{13}C-NMR; rearrangement reaction; acid-catalyzed hydrolysis

Hydrolysis of pregnanediol disulfate (**1**) in 3 M hydrochloric acid in a refluxing water bath gave 17α-ethyl-17β-methyl-18-nor-5β-androst-13-en-3α-ol (**3**) as the major product, accompanied with many kinds of degradation products, including 17α-methyl-D-homo-5β-androstane-3α,17aβ-diol (**4**) as the second major degradation product.[1] Under similar conditions, 5β-pregnane-3α,20β-diol disulfate (**2**), the C-20 isomeric sulfate of **1**, also gave a mixture of degradation products common with those of **1**. In contrast to **1**, however, the main product of **2** was the D-homosteroid (**4**).[2] Although it was shown that the ring-enlargement reactions of both sulfates occurred with the migration of C_{16}–C_{17} bond to C-20 at the side chain, the mechanisms were different for **1** and **2**. D-Homoannulation of **1** proceeded through the C-20 carbocation formed by elimination of sulfuric acid during the reaction (stepwise mechanism), while that of **2** proceeded by a concerted mechanism like the uranediol rearrangement reaction.[3]

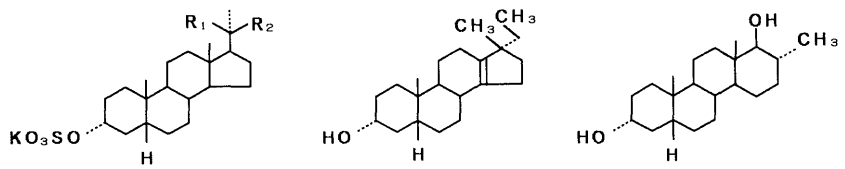
We then became interested in what kinds of reactions would occur in two C-20 isomeric pregnanetriol 20-sulfates (**8a**, **12a**) having an additional hydroxyl group at C-17α.

This paper describes the structural elucidation of the D-homosteroidal products obtained by heating pregnanetriol 20-sulfate (**8a**) and its C-20 isomeric sulfate (**12a**) in 3 M hydrochloric acid and also the mechanism of the D-homoannulation.

Results and Discussion

Preparation of Sulfates Pregnanetriol 20-sulfate (**8a**) was prepared from pregnanetriol (**5**) as a starting material. By the method of Lewbart and Schneider,[4] **5** was converted in two steps to 5β-pregnane-3α,17α,20α-triol 3-acetate (**6a**), which was treated with a sulfur trioxide–pyridine complex to give the 20-monosulfate (**7a**). Saponification of **7a**, followed by treatment with an ion-exchange resin, gave the desired sulfate as the potassium salt (**8a**). The overall yield of **8a** from **5** was 49%.

The isomeric sulfate (**12a**) was obtained in a similar way. 3α,17α-Dihydroxy-5β-pregnan-20-one 3-acetate (**24a**) was derived by the method of Lewbart[5] to 5β-pregnane-3α,17α,20β-triol 3-acetate (**10a**), which was converted to the 20-monosulfate (**11a**). The desired sulfate (**12a**) was finally obtained by saponification of **11a**. The overall yield of **12a** from **10a** was 37%.

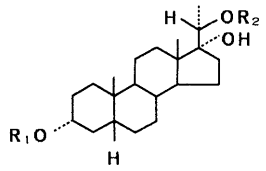


1 : R_1=H, R_2=OSO_3K
2 : R_1=OSO_3K, R_2=H

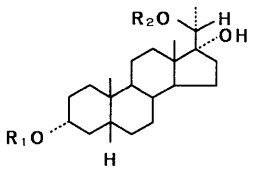
3

4

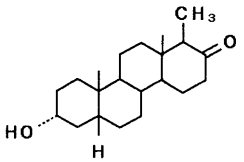
Chart 1



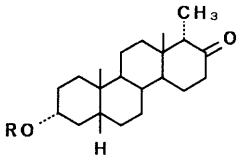
5 : R_1=H, R_2=H
6a : R_1=Ac, R_2=H
7a : R_1=Ac, R_2=SO_3K
(7b : [20-^{13}C])
8a : R_1=H, R_2=SO_3K
(8b : [20-^{13}C])
16 : R_1=H, R_2=CH_3-C$_6$H$_4$-SO_2



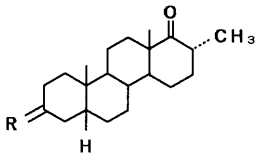
9 : R_1=H, R_2=H
10a : R_1=Ac, R_2=H
11a : R_1=Ac, R_2=SO_3K
(11b : [20-^{13}C])
12a : R_1=H, R_2=SO_3K
(12b : [20-^{13}C])



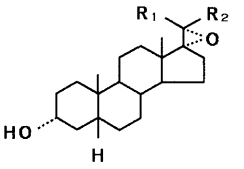
13a



14 : R=H
15 : R=Ac



17a : R=
18 : R=O



19 : R_1=H, R_2=CH_3
20 : R_1=CH_3, R_2=H

Chart 2

The structures of both sulfates (**8a**, **12a**) were confirmed by instrumental and elemental analyses.

Hydrolysis Product of 8a An aqueous solution of **8a** at 95 °C was combined with the same volume of 6 M hydrochloric acid at 95 °C, and the resultant solution was heated at the same temperature for 15 min. The gas chromatogram of the hydrolyzate obtained is shown in Fig. 1a, where the peaks are divided into two groups, A and B. By GC-MS of the product, group A was shown to be a mixture of at least four isomeric products having a molecular ion *m/z* of 372 ($C_{21}H_{32}O$), corresponding to monohydroxysteroidal dienes. By MS of the trimethylsilylmethoxime derivative of the hydrolyzate, group B was estimated to be a D-homosteroid, isolation of which was undertaken.

Column chromatography of the hydrolyzate using silica gel gave group A and group B, of which the latter, obtained as a crystalline material (yield from **8a**: 15%), was identified as 3α-hydroxy-17aβ-methyl-D-homo-5β-androstan-17-one (**13a**),[6] by instrumental analyses including IR, ^{1}H- and ^{13}C-NMR. The β-configuration of the methyl group at C-17a was supported by comparison of the circular dichroism (CD) with that of the 17a-isomer (**14**).

By analogy with the similar reaction observed in the solvolytic D-homoannulation of 5α-pregnane-3β,17α,20α-triol 3-acetate 20-tosylate by Williams *et al.*,[7] **13a** may be produced by a concerted mechanism involving two steps, as shown in Chart 3: Elimination of sulfuric acid from C-20, migration of the C_{13}–C_{17} bond to C-20, and deprotonation from the hydroxy group at C-17α may occur simultaneously to produce the ring-enlargement product (**14**). Because this product has an axial methyl group at C-17a, it should convert to a thermodynamically more stable isomer (**13a**), where the methyl group at C-17a is in a β-configuration. To test this assumption, we have synthesized the supposed intermediate (**14**) to observe its isomerization.

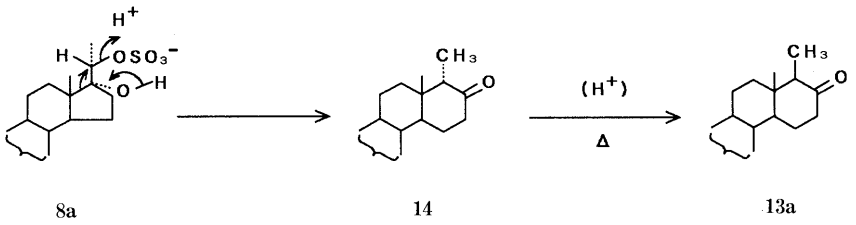
By referring to the method of Williams *et al.*,[7] pregnanetriol 3-monoacetate (**6a**) was derived to its tosylate (**16**), which was solvolyzed in aqueous acetone containing potassium acetate. The solvolyzate obtained was chromatographed on alumina to give 3α-acetoxy-17aα-methyl-D-homo-5β-androstan-17-one (**15**), saponification of which gave **14**.

Because of its insolubility in aqueous solution, **14** was dissolved in 50% (v/v) aqueous alcoholic 3 M hydrochloric acid. Refluxing the solution gave **13a** quantitatively. Because the conditions of this isomerization were not the same as those of the hydrolysis of **8a**, this dose not prove that D-homoannulation of **8a** to **13a** proceeds *via* intermediate **14**, but it seems likely.

On the other hand, monohydroxysteroidal diene products (group A) were considered to be formed *via* the 17α,20β-oxide (**19**). This was confirmed in the following way. The epoxide (**19**) prepared by the method of Lewbart[5] was heated in 3 M hydrochloric acid in 10% (v/v) aqueous alcohol to give a mixture of products corresponding to monohydroxysteroidal diene, the retention times of which on gas chromatograms were identical with those of group A (Fig. 1). Thus, the diene products (group A) may be formed *via* the epoxide intermediate.

Hydrolysis Product of 12a The sulfate **12a** was hydrolyzed in a similar way to that described for **8a**. The product was also divided into two groups as shown in Fig. 1b: group C, composed of at least five products having retention times from 4.5 to 7.0 min, and group D. Similarly to the case of **8a**, group C was considered on the basis of GC-MS to be a mixture of monohydroxysteroidal dienes and group D a monohydroxyketogenic steroid, probably an isomer of **13a**.

Crystalline material corresponding to group D was



8a 14 13a

Chart 3

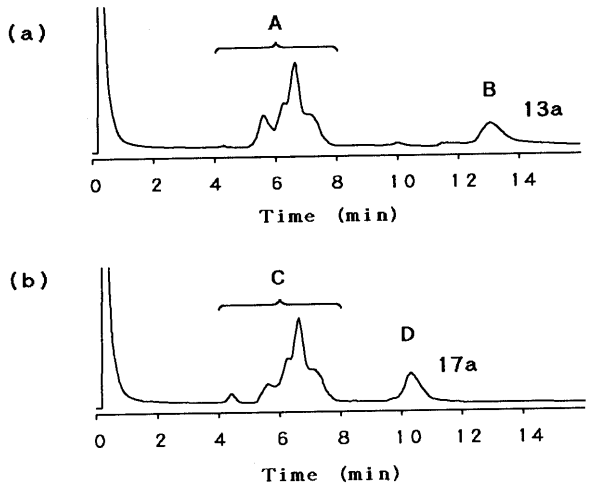


Fig. 1. Gas Chromatogram of (a) the Degradation Product of 5β-Pregnane-3α,17α,20α-triol 20-Sulfate and That of (b) Its C-20 Isomeric Sulfate

obtained by column chromatography of the hydrolyzate (yield from **12a**: 13%), and the structure was assigned to be 3α-hydroxy-17α-methyl-D-homo-5β-androstan-17a-one (**17a**). The position of the carbonyl group at C-17a was confirmed by the finding that Jones' oxidation of **17a** and **4** gave the same diketone (**18**) as the sole product. The configuration of the C-17 methyl group of **17a** was considered to be α because it showed a negative Cotton effect in the CD spectrum. D-Homoannulation of **12a** may occur *via* such a concerted mechanism as reported in the reaction of 5α-pregnane-3β,17α,20β-triol 20-sulfate by Williams *et al.*[7]

The same steroidal diene mixture corresponding to group C was obtained when the 17α,20α-oxide (**20**)[5] was heated under acidic conditions, which means that the diene products of sulfate **12a** were formed *via* the epoxide. In accordance with the results of Williams *et al.*,[7] 5α-pregnane 17α,20α-epoxide gave no D-homosteroid but only non-polar steroids.

Studies of [20-^{13}C]Sulfates (8b, 12b) Based on the present results and also those of Williams *et al.*,[7] we proposed for the D-homoannulation of **8a** a mechanism involving the migration of the C_{13}–C_{17} bond to C-20, followed by isomerization of the intermediate product as shown in Chart 3. For the D-homoannulation of **12a**, the reaction was considered to occur by migration of the C_{16}–C_{17} bond to the same carbon as speculated by Williams *et al.*[7] To confirm these mechanisms, we decided to prepare two [20-^{13}C]pregnanetriol sulfates (**8b**, **12b**) and to analyze the location of carbon-13 in their hydrolysis products. First, the synthetic procedures for the ^{13}C-labelled sulfates will be described.

The Wittig reaction of 3α-hydroxy-5β-androstan-17-one (**21**) with [1-^{13}C]iodoethane gave a satisfactory yield of (*Z*)-[20-^{13}C]-5β-androst-17(20)-en-3α-ol (**22b**),[8] which was acetylated in the usual way to afford the acetate (**23b**). From the ^{13}C-NMR, it was clear that only C-20 was labelled quantitatively with carbon-13. Oxidation of **23b** with osmium tetroxide gave a glycol (**6b**), from which one of the desired sulfates was obtained through **7b** by a procedure similar to that described for the preparation of **8a** from **6a**. No change in labelled position or isotope content was observed in the transformation from **23b** to **8b**.

Another desired sulfate (**12b**) was synthesized using **6b** as a starting material. Oxidation of **6b** under mild conditions[9] gave its C-20 ketone (**24b**) in satisfactory yield. Stereospecific reduction of **24b** with sodium borohydride produced mainly the 20β-ol (**10b**). The synthetic routes from **10b** to **12b** were as described in the preparation of **12a** from **10a**. In this case, too, neither isotope content nor labelling position was changed during the transformation from **6b** to **12b**.

Next, hydrolysis of both ^{13}C-labelled sulfates (**8b**, **12b**) was carried out in the same way as described in the experiments with **8a** and **12a** to give their hydrolyzate (D-homosteroid). In Fig. 2, the ^{13}C-NMR spectrum (B) of the D-homosteroid obtained from the [20-^{13}C]sulfate (**8b**) is compared with that of an authentic steroid (A). In spectrum A, peaks at 213.2 and 71.7 ppm correspond to the carbons at C-17 and C-3, respectively. The peak at 56.2 ppm was assigned to the methine carbon at C-17a. In spectrum B, on the other hand, only the peak at 56.2 ppm shows a remarkably large peak height, which means that the D-homosteroid obtained from **8b** is enriched with ^{13}C only at C-17a of the molecule. In contrast to the product of **8b**, the D-homosteroid obtained from **12b** was shown to be labelled only at C-17 of the molecule.

Table I shows a comparison of the isotope abundance at C-17 and C-17a with that at C-3 for the D-homosteroid from **13a** and **13b**, where the natural abundance of the isotope at C-3 is taken as unity. For example, the ^{13}C-abundances at C-17 and C-17a of **13a** were both 1, whereas those of **13b** were 1 and 10, respectively. Thus, the ratio of isotope content at C-17 and C-17a between ^{13}C-enriched and standard steroids can be calculated as 1 and 10, respectively. The ratio (10) at C-17a of the product is equal to that at C-20 of the original steroids (**23b**, **6b**, **7b**, **8b**). On the other hand, the ratio at C-17 is 1, indicating no enrichment of ^{13}C at this position. In sharp contrast, the ^{13}C-enriched carbon of the D-homosteroid obtained from the [20-^{13}C]sulfate (**12b**)

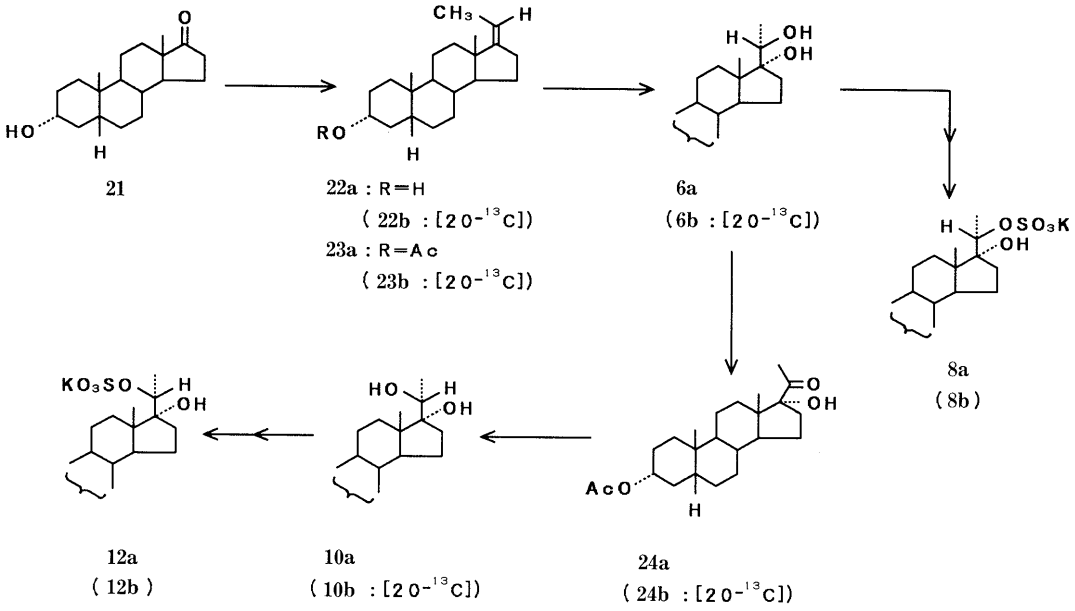


Chart 4

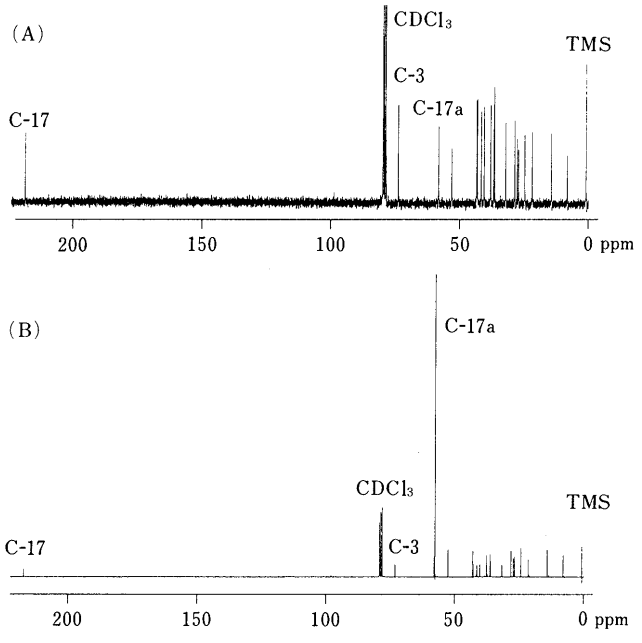


Fig. 2. ^{13}C-NMR Spectrum of (A) 3α-Hydroxy-17aβ-methyl-D-homo-5β-androstan-17-one (**13a**) and That of (B) the Degradation Product of [20-^{13}C]-Pregnane-3α,17α,20α-triol 20-Sulfate (**8b**)

Spectra were determined in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard.

Table I. Comparison of ^{13}C-Contents at C-3, C-17, and C-17a between D-Homosteroid (**13a**) and the Product Obtained from [20-^{13}C]-Pregnane-3α,17α,20α-triol 20-Sulfate (**8b**)[a]

Position	**13a**	Product	Ratio (Product/**13a**)
^{13}C at C-3	1	1	—
^{13}C at C-17	1	1	1
^{13}C at C-17a	1	10	10

a) ^{13}C-NMR spectra were measured in $CDCl_3$ with TMS as an internal standard. The isotope content at C-3 of each steroid was taken as unity.

Table II. Comparison of ^{13}C-Contents at C-3, C-17, and C-17a between D-Homosteroid (**17a**) and the Product Obtained from [20-^{13}C]-Pregnane-3α,17α,20β-triol 20-Sulfate (**12b**)[a]

Position	**17a**	Product	Ratio (Product/**17a**)
^{13}C at C-3	1	1	—
^{13}C at C-17	1	9	9
^{13}C at C-17a	1	1	1

a) ^{13}C-NMR spectra were measured in $CDCl_3$ with TMS as an internal standard. The isotope content at C-3 of each steroid was taken as unity.

was shown to be C-17 but not C-17a (Table II).

It may be concluded from the above results that D-homoannulation of pregnanetriol 20-sulfate (**8a**) proceeds with migration of the C_{13}–C_{17} bond to C-20, whereas that of the C-20 isomeric sulfate (**12a**) proceeds with migration of the C_{16}–C_{17} bond.

On the Mechanism of D-Homoannulations Because D-homoannulation of pregnanes starts with the elimination of a leaving group from C-20,[10] it is unlikely that elimination of the C-17α hydroxyl group can be an initial step in the D-homoannulation of the two isomeric sulfates (**8a**, **12a**). Thus, it may be sufficient to consider the elimination of only the sulfoxy group.

Chart 5 shows three possible Newman projections of **8a** (A, B, C) along with the C_{17}–C_{20} bond on the assumption that they are in staggered conformations. According to the Büchi model, the order of stability of three conformers in the ground state is C > A > B, and that in the transition state is C′ ≧ A′ ≫ B′. In conformer A, some steric hindrance exists between the C-21 methyl group and the C-12β hydrogen. This hindrance disappears in the transition state (A′), where migration of the C_{13}–C_{17} bond to C-20 and elimination of the sulfoxy group from C-20 occur simultaneously. The product (**14**) thus formed may be isomerized to the more stable isomer (**13a**) as described above. This process is supported by the isolation of **13a** and also by experiments using the [20-^{13}C]sulfate (**8b**).

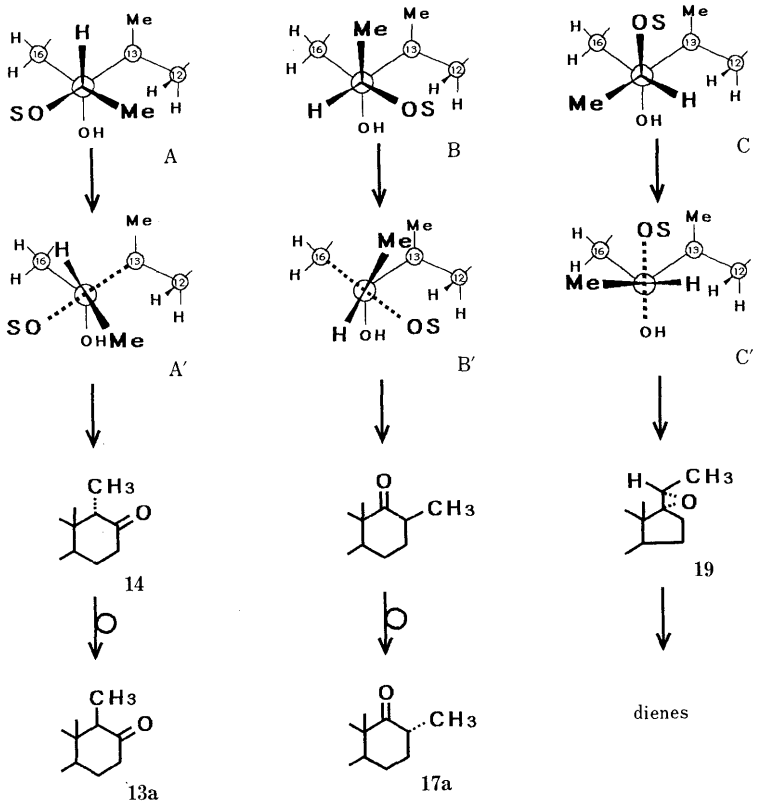


Chart 5. Partial Structures of 5β-Pregnane-3α,17α,20α-triol 20-Sulfate (**8a**) and Their Staggered Conformations, Showing the Reaction Pathways Producing Rearranged Products

Conformations are shown along the C_{17}–C_{20} bond visualized from the C-21 side.

There is marked steric hindrance in conformer B among the two methyl groups at C-18 and C-21, and the C-16β hydrogen, and the hindrance between C-18 and the C-21 methyl group becomes rather greater in the transition state (B′), where the migration of the C_{16}–C_{17} bond to C-20 may be difficult. In fact, neither the product **17a** nor its precursor (C-17β methyl isomer) was obtained from **8a**. Thus, this pathway may not be involved in the reaction of **8a**. Even the small hindrance present in conformation C becomes smaller in the transition state (C′), from which the 17α,20β-oxide (**19**) should be produced. This oxide is considered as an intermediate not to D-homosteroid but to steroidal dienes (group A in Fig. 1a); this is supported by the present experiments, as described.

Similarly, D-homoannulation of **12a** is illustrated in Chart 6, which shows three possible conformers for the ground state (D, E, F) and transition state (D′, E′, F′). The stability in the ground and the transition states by the Büchi model is D≫F>E and D′>E′≧F′, respectively. Because no remarkable steric hindrance exists in conformer D′, where the ester group and the C_{16}–C_{17} bond are arranged in antiparallel relation, ring-enlargement may occur easily through the removal of the sulfoxy group with the simultaneous migration of the C_{16}–C_{17} bond to C-20. This is supported by the isolation of **17a** as expected, and also by the experiments using the [20-^{13}C]sulfate (**12b**). Because the steric hindrance caused by interaction between the C-18 and C-21 methyl groups in conformer E is reduced in the transition state (E′), migration of the C_{13}–C_{17} bond to C-20 accompanied by simultaneous removal of the sulfoxy group may occur to produce **13a**. As described above, however, no such product was obtained from **12a**, which means that this pathway is not involved in the reaction of **12a**. Conformer F may be fairly unstable because of the interaction between the C-21 methyl group and the C-12β hydrogen. In the transition state, however, the hindrance becomes greater because of the approach of the C-21 methyl group to the C-18 methyl group. If the reaction proceeded beyond this transition state, the oxide **20** shown in Chart 6 should be formed. Thus, D-homoannulation of **12a** occurs only by the pathway *via* conformer D′.

A question remains concerning the lack of contribution of the pathway E→E′→**13a** in the reaction of **12a**, because there seems to be no remarkable difference in steric hindrance between E′ and F′. The predominancy of the pathway through F′ may be explained by considering the characteristics of the 17α,20α-oxide (**20**) intermediate. Probably, no sooner had the oxide **20** been formed than it would have been removed from the reaction system because of its conversion to steroidal dienes.

In conclusion, the ^{13}C-NMR spectra of the two D-homosteroids (**13b**, **17b**) obtained from the [20-^{13}C] sulfates (**8b**, **12b**, respectively) clearly demonstrate that the ring-enlargement reaction of **8a** occurred with migration of the C_{13}–C_{17} bond, whereas that of **12a** occurred with migration of the C_{16}–C_{17} bond.

Experimental

Apparatus All melting points were determined on a Yanagimoto

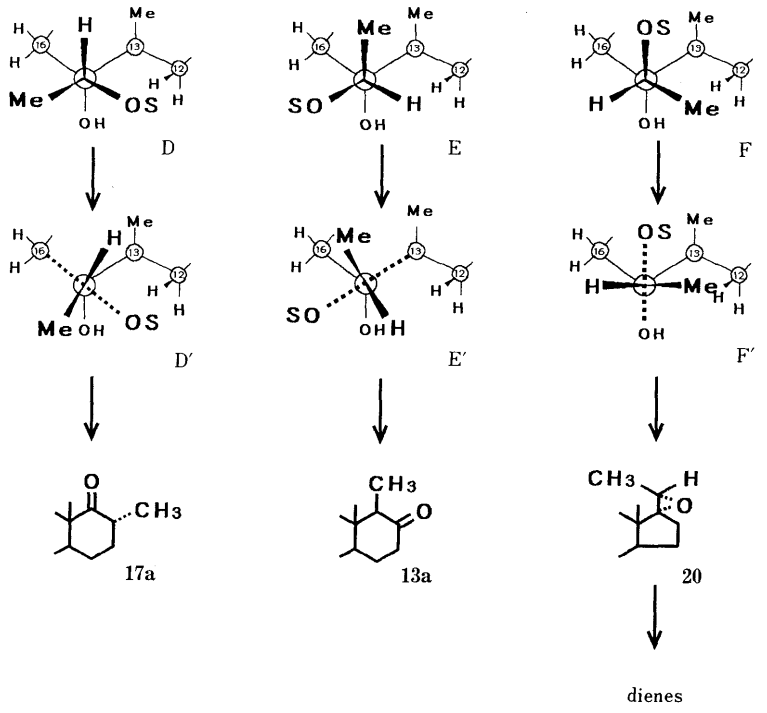


Chart 6. Partial Structures of 5β-Pregnane-3α,17α,20β-triol 20-Sulfate (**12a**) and Their Staggered Conformations, Showing the Reaction Pathways Producing Rearranged Products

Conformations are shown along the C_{17}–C_{20} bond visualized from the C-21 side.

MP-500D micro melting point apparatus and are uncorrected. IR spectra were measured on a JASCO FT/IR-7000 spectrophotometer. ^{1}H-NMR spectra were recorded at 270 MHz and ^{13}C-NMR spectra at 67.8 MHz, on a JEOL JNM-GX270 spectrometer. Chemical shifts are expressed in ppm relative to TMS as an internal standard. The following abbreviations are used: s=singlet, d=doublet, q=quartet, dqu=doublet of quintets, tt=triplet of triplets, m=multiplet. EI-MS (ionization voltage, 20 eV) and negative ion secondary ion mass spectrometry (SI-MS) were measured with a Hitachi M-2000 mass spectrometer using a direct inlet system; glycerol was used as a matrix in the negative ion SI-MS measurements. CD spectra were recorded in ethanol at 22 °C on a JASCO J-20C recording spectropolarimeter. Gas liquid chromatography (GLC) was carried out on a Shimadzu GC-4CM gas chromatograph with a glass column (2 m×3 mm i.d.) packed with 1.5% OV-1 on Shimalite W (80—100 mesh) and a flame ionization detector (FID), using N_2 (40 ml/min) as a carrier gas. The column temperature employed was 220 °C. GC-MS was carried out with a Hitachi M-2000 mass spectrometer with a glass column (2 m×1.8 mm i.d.) using the same packings as described above and He (30 ml/min) as a carrier gas. Other conditions employed were as follows: column temperature, 240 °C; ionization voltage, 20 eV.

Materials 5β-Pregnane-3α,17α,20α-triol (**5**), 5β-pregnane-3α,17α,-20β-triol (**9**) and 3α-hydroxy-5β-androstan-17-one (**21**) were obtained from Sigma (St. Louis, MO., U.S.A.). 5β-Pregnane-3α,17α,20α-triol 3-acetate (**6a**, mp 138—140 °C, lit.[4] 140 °C) was prepared by the method of Lewbart and Schneider[4] from **5**. 5β-Pregnane-3α,17α,20β-triol 3-acetate (**10a**, mp 93—95 °C, lit.[5] 92.5—93.5 °C) was prepared by the method of Lewbart[5] from 3α,17α-dihydroxy-5β-pregnane-20-one 3-acetate (**24a**, mp 199—200 °C, lit.[11] 198.5—199.5 °C), which was prepared by acetylation of 3α,17α-dihydroxy-5β-pregnane-20-one (Sigma). 17α,20β-Oxido-5β-pregnan-3α-ol (**19**, mp 132—134 °C, lit.[5] 137—139 °C) and 17α,20α-oxido-5β-pregnan-3α-ol (**20**, mp 167—168 °C, lit.[5] 162—164 °C) were prepared by the method of Lewbart[5] from **6a** and **10a**, respectively. 17α-Methyl-D-homo-5β-androstane-3α,17aβ-diol (**4**) was prepared by the method reported previously.[12] TLC was performed on Kieselgel 60 F_{254} (Merck). For column chromatography, silica gel (Kieselgel 60, 70—230 mesh, Merck) and aluminum oxide (Alumina: activated, neutral, activity I, ICN Biomedicals GmbH) were used. [1-^{13}C]Iodoethane (99.4 atom %) was purchased from Isotec Inc. (Miamisburg, Ohio, U.S.A.). Other reagents and their sources are as follows: *N*-Trimethylsilylimidazole (TMSI) from GL Sciences Inc. (Tokyo), *O*-methylhydroxylammonium chloride from Wako Pure Chemical Industries, Ltd. (Osaka), and Amberlite XAD-2 from Organo (Tokyo). All other reagents and solvents were of reagent grade and were used without further purification.

1. Preparation of Sulfates. Potassium 5β-Pregnane-3α,17α,20α-triol 3-Acetate 20-Sulfate (7a) Chlorosulfonic acid (0.54 ml, 8 mmol) was added with stirring to dry pyridine (15 ml, 0.19 mol) under cooling. After being warmed to 60 °C, the solution was gradually added over 1 h to a stirred solution of **6a**[4] (1.00 g, 2.64 mmol) in dry pyridine at 60 °C. The reaction mixture was cooled to room temperature and adjusted to pH 7 with 0.3 N KOH. The solution was passed through a column packed with XAD-2 resin (300 ml). The column was washed with water, and the methanolic eluate was concentrated *in vacuo* to give a crystalline residue (1.19 g), which was recrystallized from a mixture of methanol and ethanol to give fine needles (738 mg, 56%), mp 170—172 °C. *Anal.* Calcd for $C_{23}H_{37}KO_7S \cdot 1/2H_2O$: C, 54.62; H, 7.57; S, 6.34. Found: C, 54.92; H, 7.69; S, 6.76. IR (KBr) cm^{-1}: 3500 (OH), 1740 (C=O), 1243 (SO_2). ^{1}H-NMR (DMSO-d_6): 4.59 (1H, m, 3β-H), 4.20 (1H, q, J=6.3 Hz, 20β-H), 1.96 (3H, s, CH_3COO), 1.14 (3H, d, J=6.3 Hz, 21-H), 0.90 (3H, s, 19-H), 0.64 (3H, s, 18-H). MS (SI-MS) *m/z*: 457 $[M-K]^-$.

Potassium 5β-Pregnane-3α,17α,20α-triol 20-Sulfate (8a) The acetate (**7a**, 650 mg, 1.31 mmol) was dissolved in 250 ml of 0.1 M KOH (95% (v/v) aqueous methanol solution), and the solution was allowed to stand for 3 h at room temperature. The reaction mixture was diluted with water (800 ml), and passed through a column packed with XAD-2 resin (240 ml). The fraction eluted with methanol was concentrated *in vacuo* to give a crystalline residue (560 mg), which was recrystallized from a mixture of methanol and ethanol to give fine needles (530 mg, 87%), mp 157—159 °C. *Anal.* Calcd for $C_{21}H_{35}KO_6S \cdot 1/2H_2O$: C, 54.40; H, 7.83; S, 6.91. Found: C, 54.14; H, 7.93; S, 7.31. IR (KBr) cm^{-1}: 3458 (OH), 1241 (SO_2). ^{1}H-NMR (DMSO-d_6): 4.39 (1H, d, J=4.9 Hz, 3α-OH), 4.18 (1H, q, J=6.3 Hz, 20β-H), 1.18 (3H, d, J=6.3 Hz, 21-H), 0.87 (3H, s, 19-H), 0.63 (3H, s, 18-H). MS (SI-MS) *m/z*: 415 $[M-K]^-$.

Potassium 5β-Pregnane-3α,17α,20β-triol 3-Acetate 20-Sulfate (11a) Using the same procedure as described for the preparation of **7a**, a crude product (1.53 g) was obtained when **10a**[5] (1.20 g, 3.17 mmol) was used. Recrystallization of the product from methanol gave fine needles (700 mg, 44%), mp 150—151 °C. *Anal.* Calcd for $C_{23}H_{37}KO_7S \cdot 1/2H_2O$: C, 54.62; H, 7.57; S, 6.34. Found: C, 54.43; H, 7.65; S, 6.67. IR (KBr) cm^{-1}: 3446 (OH), 1738 (C=O), 1245 (SO_2). ^{1}H-NMR (DMSO-d_6): 4.60 (1H, m, 3β-H), 4.33 (1H, q, J=6.3 Hz, 20α-H), 1.97 (3H, s, CH_3COO), 1.17

(3H, d, J=6.3 Hz, 21-H), 0.91 (3H, s, 19-H), 0.75 (3H, s, 18-H). MS (SI-MS) m/z: 457 $[M-K]^-$.

Potassium 5β-Pregnane-3α,17α,20β-triol 20-Sulfate (12a) Using the same procedure as described for the preparation of **8a**, a crude product (530 mg) was obtained from **11a** (600 mg, 1.19 mmol). Recrystallization of the product from a mixture of methanol and ethanol gave fine prisms (460 mg, 85%), mp 166—167 °C. *Anal.* Calcd for $C_{21}H_{35}KO_6S$: C, 55.47; H, 7.76; S, 7.05. Found: C, 55.58; H, 8.04; S, 6.76. IR (KBr) cm^{-1}: 3452 (OH), 1226 (SO_2). ^{1}H-NMR (DMSO-d_6): 4.38 (1H, d, J=4.6 Hz, 3α-OH), 4.30 (1H, q, J=6.3 Hz, 20α-H), 1.18 (3H, d, J=6.3 Hz, 21-H), 0.88 (3H, s, 19-H), 0.75 (3H, s, 18-H). MS (SI-MS) m/z: 415 $[M-K]^-$.

2-1. Hydrolysis of 8a and Product Analysis. Hydrolysis A heated aqueous solution (450 ml) of the sulfate **8a** (450 mg, 0.97 mmol) in a water bath (95 °C) was combined with the same volume of 6 M HCl heated at the same temperature. After 15 min, the solution was cooled on ice, followed by neutralization with 6 N NaOH solution (450 ml). The solution was extracted (600 ml × 3) with a mixture of chloroform and ethyl acetate (3 : 1, v/v). The combined organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give the residue (292 mg). This was chromatographed on a column (2.6 cm, i.d.) packed with 50 g of silica gel and eluted with a mixture of hexane and chloroform (1 : 1, v/v). Each fraction (20 ml) was collected automatically and monitored by GLC and TLC (acetone and benzene, 1 : 3, v/v). Eluates were divided into fr. I (Rf 0.56, 161 mg, corresponding to group A in Fig. 1a) and fr. II (Rf 0.44, 51 mg, corresponding to group B in Fig. 1a).

GC-MS of the Degradation Product A solution of *O*-methylhydroxylammonium chloride (20 mg) in pyridine (1 ml) was added to a test tube containing a part of the degradation product of **8a**. The solution was allowed to stand overnight at room temperature and then dried under an N_2 stream. The residue obtained was dissolved in ethyl acetate (5 ml), and the resultant solution was washed with 5% $NaHCO_3$ (3 ml × 2) and 10% NaCl (3 ml × 2). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under an N_2 stream. To the residue obtained, pyridine (20 μl) and TMSI (100 μl) were added. The mixture was warmed at 50 °C for 10 min, then dried under an N_2 stream. The residue obtained was taken up in hexane (200 μl), and the supernatant was submitted to GC-MS. MS of fr. I (corresponding to group A) m/z: 372 (M^+), 282 (M^+-90) in every peak. MS of fr. II (corresponding to group B) m/z: 419 (M^+), 389 $[M^+-30\ (CH_2O)]$, 388 $[M^+-31\ (CH_3O)]$, 298 $[M^+-31-90$ (TMSOH)], 114 $[C_2H_5-C(=N-OCH_3)-C^+H-CH_3]$.

3α-Hydroxy-17aβ-methyl-D-homo-5β-androstan-17-one (13a) Recrystallization of fr. II (51 mg) from a mixture of hexane and acetone gave fine needles (47 mg, 15%), mp 197—198 °C (lit.[6] 200 °C). *Anal.* Calcd for $C_{21}H_{34}O_2$: C, 79.19; H, 10.76. Found: C, 79.01; H, 10.67. IR (KBr) cm^{-1}: 3430 (OH), 1709 (C=O). ^{1}H-NMR ($CDCl_3$): 3.66 (1H, tt, $J_1=J'_1$=10.9 Hz, $J_2=J'_2$=4.7 Hz, 3β-H), 2.24 (1H, q, J=6.7 Hz, 17aα-H), 0.92 (3H, d, J=6.7 Hz, 17aβ-CH_3), 0.90 (3H, s, 19-H), 0.64 (3H, s, 18-H). ^{13}C-NMR ($CDCl_3$): 213.2 (C-17), 71.7 (C-3), 56.2 (C-17a), 23.2 (C-19), 13.2 (C-18), 7.1 (17aβ-CH_3). MS m/z: 318 (M^+), 276 $[M^+-42\ (CH_2CO)]$, 246 $[M^+-72\ (C_4H_8O)]$, 228 ($M^+-72-18$). CD (c=0.30, ethanol) $[\theta]^{22}$ (nm): −4800 (273) (negative maximum).

Heating of the Oxide 19 in 3 M HCl A solution of **19**[5] (0.9 mg) in 3 M 10% (v/v) ethanolic HCl (2.6 ml) was heated at 95 °C for 15 min. The solution was treated in a way similar to that described for the hydrolysis of **8a** to give the degradation product, which was submitted to GC and GC-MS. MS m/z: 300 (M^+), 285 (M^+-15), 267 ($M^+-15-18$) in every peak.

2-2. Preparation of D-Homosteroid (14) and Its Isomerization. 5β-Pregnane-3α,17α,20α-triol 3-Acetate 20-Tosylate (16) A pyridine solution (30 ml) containing **6a** (142 mg, 0.38 mmol) and *p*-toluenesulfonyl chloride (366 mg, 1.93 mmol) was allowed to stand for 24 h at room temperature. After addition of chloroform (200 ml), the solution was washed with a saturated solution of $NaHCO_3$ (100 ml × 5) and water (100 ml × 4). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give an oily residue (220 mg). ^{1}H-NMR ($CDCl_3$): 7.79 (2H, d, J=8.4 Hz, tosyl), 7.33 (2H, d, J=8.4 Hz, tosyl), 4.83 (1H, q, J=6.3 Hz, 20β-H), 4.69 (1H, tt, $J_1=J'_1$=11.3 Hz, $J_2=J'_2$=4.9 Hz, 3β-H), 2.45 (3H, s, tosyl), 2.01 (3H, s, CH_3COO), 1.25 (3H, d, J=6.3 Hz, 21-H), 0.91 (3H, s, 19-H), 0.70 (3H, s, 18-H).

3α-Acetoxy-17aα-methyl-D-homo-5β-androstan-17-one (15) A 20% (v/v) aqueous acetone solution (38 ml) containing **16** (200 mg) and potassium acetate (250 mg) was warmed at 50 °C for 18 h. After being diluted with water (100 ml), the mixture was extracted with chloroform (50 ml × 3). The combined organic layer was washed with a saturated solution of $NaHCO_3$ (100 ml × 5) and water (100 ml × 3), dried over anhydrous Na_2SO_4, and finally concentrated *in vacuo*. The oily residue (106 mg) obtained was chromatographed on a column (2 cm, i.d.) packed with alumina (80 g), which was eluted with a mixture of hexane and chloroform (4 : 1, v/v). Fractions (20 ml) were collected automatically and monitored by TLC (cyclohexane and ethyl acetate, 7 : 3, v/v). Eluates were divided into fr. I (Rf 0.52, 48 mg) and fr. II (Rf 0.38, 38 mg). Recrystallization of fr. II from aqueous acetone gave plates (36 mg, 29% from **6a**), mp 134—135 °C. *Anal.* Calcd for $C_{23}H_{36}O_3$: C, 76.62; H, 10.07. Found: C, 76.78; H, 10.32. IR (KBr) cm^{-1}: 1738, 1719 (C=O). ^{1}H-NMR ($CDCl_3$): 4.75 (1H, tt, $J_1=J'_1$=11.3 Hz, $J_2=J'_2$=4.8 Hz, 3β-H), 2.04 (3H, s, CH_3COO), 2.01 (1H, q, J=7.5 Hz, 17aβ-H), 1.14 (3H, d, J=7.5 Hz, 17aα-CH_3), 0.92 (3H, s, 19-H), 0.81 (3H, s, 18-H). MS m/z: 360 (M^+), 318 $[M^+-42\ (CH_2O)]$, 300 $[M^+-60\ (CH_3COOH)]$, 288 $[M^+-72\ (C_4H_8O)]$, 228 ($M^+-72-60$).

3α-Hydroxy-17aα-methyl-D-homo-5β-androstan-17-one (14) A methanolic solution (20 ml) of **15** (20 mg, 0.06 mmol) was treated with 0.1 M KOH (1.1 ml), and the solution was allowed to stand for 24 h at room temperature. After being diluted with water (50 ml), the mixture was extracted with chloroform (50 ml × 3). The combined organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give a crystalline residue (16 mg). Recrystallization of the product from a mixture of hexane and acetone gave needles (12 mg, 67%), mp 165—166 °C. *Anal.* Calcd for $C_{21}H_{34}O_2$: C, 79.19; H, 10.76. Found: C, 79.12; H, 10.82. IR (KBr) cm^{-1}: 3426 (OH), 1713 (C=O). ^{1}H-NMR ($CDCl_3$): 3.67 (1H, tt, $J_1=J'_1$=10.9 Hz, $J_2=J'_2$=4.8 Hz, 3β-H), 2.01 (1H, q, J=7.3 Hz, 17aβ-H), 1.11 (3H, d, J=7.3 Hz, 17aα-CH_3), 0.91 (3H, s, 19-H), 0.81 (3H, s, 18-H). MS m/z: 318 (M^+), 300 $[M^+-18\ (H_2O)]$, 276 (M^+-42), 246 (M^+-72), 228 ($M^+-72-18$). CD (c=0.119, ethanol) $[\theta]^{22}$ (nm): −1590 (283) (negative maximum).

Isomerization of 14 A solution of **14** (8 mg) in 3 M 50% (v/v) ethanolic HCl (20 ml) was refluxed for 15 min. The reaction was terminated by cooling the solution on ice, and the resultant solution was neutralized with 3 N NaOH. The solution was extracted (20 ml × 3) with a mixture of chloroform and ethyl acetate (3 : 1, v/v). The combined organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give a crystalline residue (6 mg). Recrystallization of the product from a mixture of hexane and acetone gave needles (3 mg), mp 194—197 °C. No depression of the melting point was observed on admixture of this compound with **13**. ^{1}H-NMR ($CDCl_3$): 3.66 (1H, tt, $J_1=J'_1$=10.9 Hz, $J_2=J'_2$=4.8 Hz, 3β-H), 2.23 (1H, q, J=6.7 Hz, 17aα-H), 0.91 (3H, d, J=6.7 Hz, 17aβ-CH_3), 0.90 (3H, s, 19-H), 0.64 (3H, s, 18-H).

3-1. Hydrolysis of 12a and Product Analysis. Hydrolysis Hydrolysis of **12a** (450 mg, 0.99 mmol) was carried out in the same manner as described above to give the degradation product (280 mg), which was submitted to column chromatography. The products were divided into fr. I (Rf 0.56, 196 mg) and fr. II (Rf 0.48, 52 mg).

GC-MS of the Degradation Product Using the same procedure as described for the degradation product of **8a**, the product was converted to the methyloxime and trimethylsilyl derivatives, followed by GC-MS examination. MS of fr. I m/z: 372 (M^+), 282 (M^+-90) in every peak. MS of fr. II m/z: 419 (M^+), 390 $[M^+-29\ (CHO)]$, 388 $[M^+-31\ (CH_3O)]$, 300 ($M^+-29-90$), 285 ($M^+-29-90-15$), 101 $[C_3H_7-CH=N-OCH_3]^+$.

3α-Hydroxy-17α-methyl-D-homo-5β-androstan-17a-one (17a) Recrystallization of fr. II (52 mg) from a mixture of chloroform and hexane gave needles (40 mg, 13%), mp 139—140 °C. *Anal.* Calcd for $C_{21}H_{34}O_2$: C, 79.19 ; H, 10.76. Found: C, 79.17; H, 10.96. IR (KBr) cm^{-1}: 3428 (OH), 1705 (C=O). ^{1}H-NMR ($CDCl_3$): 3.62 (1H, tt, $J_1=J'_1$=10.9 Hz, $J_2=J'_2$=4.8 Hz, 3β-H), 2.72 (1H, dqu, $J_{17\beta-16\alpha}$=12.8 Hz, $J_{17\beta-17\alpha}=J_{17\beta-16\beta}$=6.5 Hz, 17β-H). 1.07 (3H, s, 18-H), 0.97 (3H, d, J=6.5 Hz, 17α-CH_3), 0.91 (3H, s, 19-H). ^{13}C-NMR ($CDCl_3$): 217.5 (C-17a), 71.7 (C-3), 39.5 (C-17), 23.4 (C-19), 17.1 (C-18), 15.0 (17α-CH_3). MS m/z: 318 (M^+), 300 $[M^+-18\ (H_2O)]$, 285 $[M^+-18-15\ (CH_3)]$, 272 $[M^+-18-28\ (CO)]$. CD (c=0.31, ethanol) $[\theta]^{22}$ (nm): −830 (273) (negative maximum).

Heating of Oxide 20 in 3 M HCl A solution of **20**[5] (0.9 mg) was treated in the same way as described for **19** to give the degradation product, which was submitted to GC and GC-MS. MS m/z: 300 (M^+), 285 (M^+-15), 267 ($M^+-15-18$) in every peak.

3-2. Structural Characterization of 17a. 17α-Methyl-D-homo-5β-

androstane-3,17-dione (18) A suspension of 17α-methyl-D-homo-5β-androstane-3,17-diol (**4**, 80 mg, 0.25 mmol) in acetone (30 ml) was treated with Jones' reagent[13] (CrO_3: 0.64 mmol) at 0 °C, and the mixture was stirred for 15 min at 0 °C. The reaction was terminated by adding excess methanol. After addition of ethyl acetate (200 ml), the solution was washed with 5% $NaHCO_3$ and water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give a crystalline residue (79 mg). Recrystallization of the product from methanol gave fine needles (54 mg, 68%), mp 185—188 °C. *Anal.* Calcd for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19. Found: C, 79.56; H, 10.37. IR (KBr) cm^{-1}: 1717, 1702 (C=O). ^{1}H-NMR ($CDCl_3$): 2.75 (1H, dqu, $J_{17\beta-16\alpha}$=12.9 Hz, $J_{17\beta-17\alpha}=J_{17\beta-16\beta}$=6.4 Hz, 17β-H), 1.11 (3H, s, 18-H), 1.01 (3H, s, 19-H), 0.99 (3H, d, J=6.3 Hz, 17α-CH_3). MS *m/z*: 316 (M^+), 301 (M^+-15), 283 ($M^+-15-18$), 246.

Oxidation of 17a Using the same oxidation procedure as described for the preparation of **18**, a crude product (5 mg) was obtained from **17a** (5 mg). Recrystallization of the product from methanol gave fine needles (2 mg), mp 184—187 °C. No depression of the melting point was observed on admixture of this compound with **18**. ^{1}H-NMR ($CDCl_3$): 2.75 (1H, dqu, $J_{17\beta-16\alpha}$=12.8 Hz, $J_{17\beta-17\alpha}=J_{17\beta-16\beta}$=6.4 Hz, 17β-H), 1.11 (3H, s, 18-H), 1.01 (3H, s, 19-H), 0.99 (3H, d, J=6.6 Hz, 17α-CH_3).

4-1. Synthesis of the [20-^{13}C]Sulfate (8b) and Its D-Homoannulation. (*Z*)-[20-^{13}C]-5β-Pregn-17(20)-en-3α-ol (22b) The Wittig reaction of 3α-hydroxy-5β-androstan-17-one (**21**, 2.10 g, 5.72 mmol) using [1-^{13}C]iodoethane was carried out using the method reported previously[8] and gave a crude product (2.03 g). Recrystallization of the product from methanol gave needles (1.75 g, 80%), mp 188—189 °C (**22a**[1]: mp 185—187 °C). ^{13}C-NMR ($CDCl_3$): 150.4 (C-17), 113.3 (C-20), 71.9 (C-3), 23.4 (C-19), 16.9 (C-21), 13.1 (C-18). The ratio of ^{13}C-abundance (**22b**/**22a**) at C-20 was 11.

(*Z*)-[20-^{13}C]-5β-Pregn-17(20)-en-3α-yl Acetate (23b) The olefinic product (**22**, 1.73 g, 5.72 mmol) was acetylated in the usual manner to afford the crude acetate (2.00 g), recrystallization of which from methanol gave needles (1.77 g, 90%), mp 119—120 °C (**23a**[1]: mp 115—119 °C). ^{13}C-NMR ($CDCl_3$): 170.6 ($CH_3\underline{C}OO$), 150.3 (C-17), 113.3 (C-20), 74.4 (C-3), 23.3 (C-19), 21.5 ($\underline{C}H_3COO$), 16.9 (C-21), 13.1 (C-18). The ratio of ^{13}C-abundance (**23b**/**23a**) at C-20 was 11.

[20-^{13}C]-5β-Pregnane-3α,17α,20α-triol 3-Acetate (6b) A solution containing **23b** (1.61 g, 4.67 mmol) and osmium tetroxide (1.67 g, 6.57 mmol) in a mixture of dry benzene (160 ml) and dry pyridine (16 ml) was allowed to stand in the dark for 30 min at room temperature. The organic solvent was concentrated *in vacuo*. The residue was dissolved in a mixture of methanol (240 ml), water (320 ml) and benzene (160 ml) containing $KHCO_3$ (34 g) and Na_2CO_3 (34 g), followed by stirring for 48 h at room temperature. The solution was extracted with ethyl acetate (400 ml × 3), and the combined organic layer was washed with 5% Na_2CO_3, 0.5 M HCl, and water, dried over anhydrous Na_2SO_4 and finally concentrated *in vacuo* to give a crystalline residue (1.81 g). Recrystallization of the product from a mixture of hexane and acetone gave fine needles (1.63 g, 92%), mp 138—139 °C (**6a**: mp 138—140 °C). ^{13}C-NMR ($CDCl_3$): 170.7 ($CH_3\underline{C}OO$), 85.7 (C-17), 74.3 (C-3), 72.4 (C-20), 23.3 (C-19), 21.5 ($\underline{C}H_3COO$), 18.5 (C-21), 14.2 (C-18). The ratio of ^{13}C-abundance (**6b**/**6a**) at C-20 was 11.

Potassium [20-^{13}C]-5β-Pregnane-3α,17α,20α-triol 3-Acetate 20-Sulfate (7b) Using the same procedure as described for the preparation of **7a**, a crude product (940 mg) was obtained from **6b** (800 mg, 2.11 mmol). Recrystallization of the product from a mixture of methanol and ethanol gave fine needles (610 mg, 58%), mp 169—171 °C (**7a**: mp 170—172 °C). ^{13}C-NMR (DMSO-d_6): 169.7 ($CH_3\underline{C}OO$), 83.9 (C-17), 77.1 (C-20), 73.4 (C-3), 22.9 (C-19), 21.0 ($\underline{C}H_3COO$), 16.1 (C-21), 14.4 (C-18). The ratio of ^{13}C-abundance (**7b**/**7a**) at C-20 was 11.

Potassium [20-^{13}C]-5β-Pregnane-3α,17α,20α-triol 20-Sulfate (8b) Using the same procedure as described for the preparation of **8a**, a crude product (512 mg) was obtained from **7b** (560 g, 1.1 mmol). Recrystallization from a mixture of methanol and ethanol gave fine needles (428 mg, 83%), mp 155—157 °C (**8a**: mp 157—159 °C). ^{13}C-NMR (DMSO-d_6): 83.9 (C-17), 77.1 (C-20), 69.8 (C-3), 23.0 (C-19), 16.1 (C-21), 14.5 (C-18). The ratio of ^{13}C-abundance (**8b**/**8a**) at C-20 was 11.

Hydrolysis of 8b and Isolation of D-Homosteroid Using the same procedure as described for the treatment of **8a**, **8b** (390 mg, 0.86 mmol) was hydrolyzed to give a product (264 mg), which was submitted to alumina column chromatography. The product was divided into fr. I (*Rf* 0.56, 197 mg) and fr. II (*Rf* 0.44, 46 mg). Recrystallization of fr. II from a mixture of hexane and acetone gave **13b** as fine needles (41 mg, 15%), mp 197—199 °C (**13a**: mp 197—198 °C). ^{13}C-NMR ($CDCl_3$): 213.5 (C-17), 71.6 (C-3), 56.2 (C-17a), 23.3 (C-19), 13.2 (C-18), 7.1 (17aβ-CH_3). The ratio of ^{13}C-abundance (**13b**/**13a**) at C-17 was 1, and that at C-17a was 10.

4-2. Synthesis of [20-^{13}C]-Sulfate (12b) and Its D-Homoannulation. [20-^{13}C]-3α,17α-Dihydroxy-5β-pregnan-20-one 3-Acetate (24b) A solution of DMSO (0.43 ml, 6 mmol) in anhydrous CH_2Cl_2 (2 ml) was added to a stirred solution of oxalyl chloride (0.26 ml, 3 mmol) in anhydrous CH_2Cl_2 (8 ml) at −60 °C within 2 min. The mixture was stirred for 15 min at the same temperature, then to this mixture was added a solution of **6b** (570 mg, 1.5 mmol) in anhydrous CH_2Cl_2 (5 ml) at the same temperature within 5 min. Stirring was continued for an additional 30 min at −60 °C, followed by addition of triethylamine (1.8 ml, 13 mmol) and by additional stirring for 5 min. The solution was allowed to warm to room temperature. After being diluted with water (50 ml), the mixture was extracted with CH_2Cl_2 (40 ml × 3), and the combined organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give a residue (630 mg). The residue was chromatographed on a column (2.5 cm, i.d.) packed with silica gel (60 g). Fractions eluted with a mixture of hexane and chloroform (4:6, v/v) were concentrated *in vacuo* to give a crystalline residue (518 mg), which was recrystallized from methanol to give plates (465 mg, 82%), mp 198—200 °C (**24a**: mp 199—200 °C). ^{13}C-NMR ($CDCl_3$): 211.8 (C-20), 170.7 ($CH_3\underline{C}OO$), 90.1 (C-17), 74.2 (C-3), 27.9 (C-21), 23.2 (C-19), 21.4 ($\underline{C}H_3COO$), 15.6 (C-18). The ratio of ^{13}C-abundance (**24b**/**24a**) at C-20 was 10.

[20-^{13}C]-5β-Pregnane-3α,17α,20β-triol 3-Acetate (10b) A methanolic solution (100 ml) of sodium borohydride (83 mg, 2.2 mmol) and **24b** (433 mg, 1.15 mmol) was stirred at room temperature. After 5 min, the mixture was diluted with water (200 ml) and extracted with chloroform (150 ml × 3). The combined organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give a residue (448 mg). The residue was chromatographed on a column (3 cm, i.d.) packed with silica gel (140 g). Fractions eluted with a mixture of hexane and chloroform (2:8, v/v) were concentrated *in vacuo* to give a crystalline material (307 mg), which was recrystallized from methanol to give needles (287 mg, 66%), mp 93—95 °C (**10a**: mp 93—95 °C). ^{13}C-NMR ($CDCl_3$): 170.8 ($CH_3\underline{C}OO$), 85.3 (C-17), 74.4 (C-3), 70.4 (C-20), 23.3 (C-19), 21.5 ($\underline{C}H_3COO$), 18.7 (C-21), 15.3 (C-18). The ratio of ^{13}C-abundance (**10b**/**10a**) at C-20 was 10.

Potassium [20-^{13}C]-5β-Pregnane-3α,17α,20β-triol 3-Acetate 20-Sulfate (11b) Using the same procedure as described for the preparation of **7a**, a crude product (505 mg) was obtained from **10b** (403 mg, 1.06 mmol). Recrystallization of the product from methanol gave fine needles (259 mg, 49%), mp 148—150 °C (**11a**: mp 150—151 °C). ^{13}C-NMR (DMSO-d_6): 169.7 ($CH_3\underline{C}OO$), 84.3 (C-17), 75.6 (C-20), 73.3 (C-3), 23.0 (C-19), 21.0 ($\underline{C}H_3COO$), 15.5 (C-21), 13.6 (C-18). The ratio of ^{13}C-abundance (**11b**/**11a**) at C-20 was 10.

Potassium [20-^{13}C]-5β-Pregnane-3α,17α,20β-triol 20-Sulfate (12b) Using the same procedure as described for the preparation of **8a**, a crude product (366 mg) was obtained from **11b** (429 mg, 0.86 mmol). Recrystallization of the product from a mixture of methanol and ethanol gave fine prisms (338 mg, 86%), mp 165—167 °C (**12a**: mp 166—167 °C). ^{13}C-NMR (DMSO-d_6): 84.3 (C-17), 75.7 (C-20), 69.8 (C-3), 23.2 (C-19), 15.5 (C-21), 13.7 (C-18). The ratio of ^{13}C-abundance (**12b**/**12a**) at C-20 was 10.

Hydrolysis of 12b and Isolation of D-Homosteroid Using the same procedure as described for the treatment of **8a**, hydrolysis of **12b** (334 mg, 0.73 mmol) gave a degradation product (211 mg), which was submitted to alumina column chromatography. fr. I (*Rf* 0.56, 174 mg) and fr. II (*Rf* 0.48, 45 mg) were obtained. Recrystallization of fr. II from a mixture of chloroform and hexane gave **17b** as needles (40 mg, 17%), mp 138—140 °C (**17a**: mp 139—140 °C). ^{13}C-NMR ($CDCl_3$): 217.4 (C-17a), 71.7 (C-3), 39.5 (C-17), 23.4 (C-19), 17.1 (C-18), 15.0 (17α-CH_3). The ratio of ^{13}C-abundance (**17b**/**17a**) at C-17 was 9, and that at C-17a was 1.

References

1) I. Yoshizawa, R. Oh'uchi, K. Nagata, S. Itoh, N. Kawahara, *Chem. Pharm. Bull.*, **30**, 2474 (1982).
2) I. Yoshizawa, K.Nagata, S. Itoh, *Yakugaku Zasshi*, **104**, 20 (1984).
3) H. Hirschmann, J. S. Williams, *J. Biol. Chem.*, **238**, 2305 (1963).
4) M. L. Lewbart, J. J. Schneider, *J. Org. Chem.*, **34**, 3505 (1969).
5) M. L. Lewbart, *J. Org. Chem.*, **33**, 1695 (1968).
6) C. Monneret, C. Tchernatinsky, Q. Khuong-Huu, *Bull. Soc. Chim.*

Fr., **1970**, 1520.
7) K. I. H. Williams, M. Smulowitz, D. K. Fukushima, *J. Org. Chem.*, **30**, 1447 (1965).
8) I. Yoshizawa, S. Itoh, K. Nagata, N. Kawahara, *Chem. Pharm. Bull.*, **31**, 3819 (1983).
9) A. J. Mancuso, S. L. Huang, D. Swern, *J. Org. Chem.*, **43**, 2480 (1978); M. Sugiyama, T. Sakamoto, Y. Kamigaki, H. Fukumi, K. Itoh, Y. Satoh, T. Yamaguchi, *Chem. Pharm. Bull.*, **41**, 882 (1993).
10) N. L. Wendler, "Molecular Rearrangements," Vol. 2, ed. by P. deMayo, Interscience Pub., New York, 1964, pp. 1084—1113.
11) T. H. Kritchevsky, T. F. Gallagher, *J. Biol. Chem.*, **179**, 507 (1949).
12) I. Yoshizawa, K. Nagata, R. Oh'uchi, S.Itoh, Y. Kanaiwa, T. Amiya, *Steroids*, **36**, 2629 (1980).
13) L. F. Fieser, M. Fieser, "Reagents for Organic Synthesis," Vol. 1, John Wiley and Sons, Inc., New York, 1967, p. 142.