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# Mechanism of the D-Homoannulation of Pregnanediol Disulfate in Refluxing 3 N Hydrochloric Acid[1)]

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In order to elucidate the mechanism of D-homoannulation of pregnanediol 20-sulfate, solvolysis of [$^{13}$C-20]-5$\beta$-pregnane-3$\alpha$,20$\alpha$-diol disulfate (**3**) in refluxing 3 N hydrochloric acid was investigated.

The resulting D-homosteroids, 17$\alpha$-methyl-D-homo-5$\beta$-androstane-3$\alpha$,17a$\beta$-diol (**8**) and 17$\alpha$-methyl-17a$\beta$-chloro-D-homo-5$\beta$-androstan-3$\alpha$-ol (**10**), contained a quantitative amount of $^{13}$C only at C-17, indicating that the ring-enlargement reaction of the 20$\alpha$-ol sulfate proceeded with stereospecific migration of the $C_{16}$–$C_{17}$ bond. The same result was obtained from isomeric [$^{13}$C-20]-5$\beta$-pregnane-3$\alpha$,20$\beta$-diol disulfate (**6**).

Based on these results, the D-homoannulation of pregnanediol 20-sulfate was concluded to proceed by a stepwise mechanism through the C-20 carbocation. The stereochemistry of this Wagner–Meerwein type rearrangement reaction is also discussed.

**Keywords**——5$\beta$-pregnane-3$\alpha$,20$\alpha$-diol (pregnanediol); pregnanediol disulfate; 5$\beta$-pregnane-3$\alpha$,20$\beta$-diol; D-homoannulation; stereochemistry; steroidal sulfate; acid hydrolysis; $^{13}$C-NMR

Previously, we reported the isolation of 17$\alpha$-ethyl-17$\beta$-methyl-18-nor-5$\beta$-androst-13-en-3$\alpha$-ol (**7**) as a urinary steroid,[2)] and showed that the compound was an artifact formed from pregnanediol 20-sulfate by heating in 3 N hydrochloric acid.[3)] Recently, we isolated two kinds of D-homosteroids as the main products after **7** from the hydrolyzate of pregnanediol disulfate (**3**) and determined their structures as 17$\alpha$-methyl-D-homo-5$\beta$-androstane-3$\alpha$,17a$\beta$-diol (**8**) and 17$\alpha$-methyl-17a$\beta$-chloro-D-homo-5$\beta$-androstan-3$\alpha$-ol (**10**).[4)]

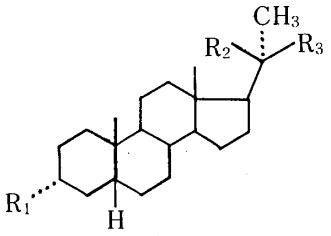
In the most recent paper,[1)] we clarified the involvement of the C-20 carbocation by carrying out the hydrolysis of **3** in 3 N hydrochloric acid in $H_2{}^{18}O$. At the same time, it was shown that the C-20 cation acts as an intermediate for the formation of D-homosteroids (**8** and **10**) as well as the $\Delta^{13}$-olefin (**7**). These results mean that the D-homoannulation of the 20$\alpha$-ol sulfate proceeds *via* the C-20 carbocation. However, the question arose, which bond (either $C_{13}$–$C_{17}$ or $C_{16}$–$C_{17}$) migrated in this ring-enlargement reaction?

The present paper deals with an experiment designed to elucidate the above problem. For this purpose, [$^{13}$C-20]-pregnanediol disulfate (**3**) is considered to be a suitable material. If the D-homoannulation proceeds with migration of the $C_{13}$–$C_{17}$ bond, the isotope of the product might be located at C-17a, while if the $C_{16}$–$C_{17}$ bond is involved, the isotope might be located at C-17.

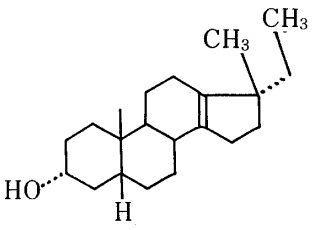
A similar D-homoannulation of 20$\beta$-ol sulfate of 5$\alpha$-pregnane steroid has been reported by Hirschmann and Williams,[5)] who investigated the hot acid hydrolysis of 5$\alpha$-pregnane-3$\beta$,20$\beta$-diol 20-sulfate, yielding uranediol (17$\alpha$-methyl-D-homo-5$\alpha$-androstane-3$\beta$,17a$\beta$-diol) quantitatively. They speculated that this rearrangement reaction proceeds with migration of the $C_{16}$–$C_{17}$ bond by a concerted mechanism. Later, this interesting D-homoannulation was named the uranediol rearrangement based on the accumulation of similar examples.[6)]

This time, we prepared [$^{13}$C-20]-5$\beta$-pregnane-3$\alpha$,20$\beta$-diol disulfate (**6**), and analyzed the

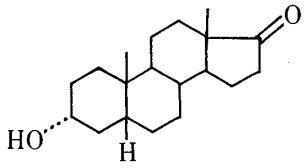
acid hydrolysis products to confirm the occurrence of the uranediol rearrangement.



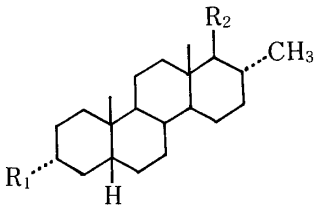
**1**: $R_1=OH$, $R_2=H$, $R_3=OH$
**2**: $R_1=OAc$, $R_2=H$, $R_3=OAc$
**3**: $R_1=OSO_3K$, $R_2=H$, $R_3=OSO_3K$
**4**: $R_1=OH$, $R_2=OH$, $R_3=H$
**5**: $R_1=OAc$, $R_2=OAc$, $R_3=H$
**6**: $R_1=OSO_3K$, $R_2=OSO_3K$, $R_3=H$



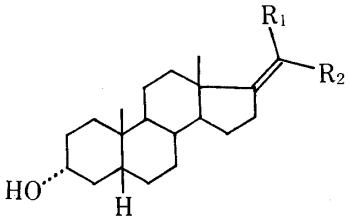
**7**



**11**



**8**: $R_1=OH$, $R_2=OH$
**9**: $R_1=OAc$, $R_2=OAc$
**10**: $R_1=OH$, $R_2=Cl$



**12**: $R_1=CH_3$, $R_2=H$
**13**: $R_1=H$, $R_2=CH_3$

Chart 1

## Experimental

Melting points were determined on a Kofler-type hot stage (Mitamura, Tokyo) and are uncorrected. The synthetic $^{13}C$-labeled compounds are all known materials, and their identities were confirmed by melting point (mp) and mixed mp determination, and by comparison of infrared, proton nuclear magnetic resonance, and mass spectra with those of authentic specimens. Gas chromatography (GLC) was performed under the same conditions with the same apparatus as described previously.[1] The results of elemental analyses of synthetic compounds are not shown, because the analytical values obtained for carbon, hydrogen, and sulfur of each steroid were all within the range of the calculated value $\pm 0.3\%$. Thin-layer chromatography (TLC) was performed with Merck precoated Silica gel 60 $F_{254}$ plates. Preparative TLC was done with similar commercial plates ($20 \times 20$ cm) with a thickness of 0.25 mm. A mixture of cyclohexane and acetone (3 : 1) was used as a mobile phase. For column chromatography, silica gel (Kiesel gel, 60—230 mesh, Merck) was used.

The steroidal materials, etiocholanolone and pregnanediol (20α- and 20β-ols), were obtained from Sigma (St. Louis, U.S.A.) and Steraloids (N.H., U.S.A.), respectively. Other steroids were prepared in this laboratory by the reported methods.[1-4,7] [$^{13}C$-1]-Iodoethane (90 atom%) was obtained from Merck and was diluted with non-labeled iodoethane to 11.0 atom% prior to use. $^{13}C$-Labeled ethyltriphenylphosphonium iodide $[(Ph)_3P^{13}CH_2CH_3]^+I^-$ was prepared from triphenylphosphine and the above diluted labeled reagent according to the reported method.[8]

Quantitative analyses of $^{13}C$ were done with a $^{13}C$-nuclear magnetic resonance ($^{13}C$-NMR) spectrometer (JNM FX-100, JEOL, Tokyo) at 22.5 MHz in the decoupling mode without nuclear Overhauser effect (NOE), with an irradiation time of 0.8192 s in a cycle of 3.000 s at the frequency of 5000 Hz.

**Wittig Reaction of 3α-Hydroxy-5β-androstan-17-one (11)**——About 2.4 g of sodium hydride (50% dispersion in mineral oil) was washed 3 times with *n*-hexane and blown dry with nitrogen gas. Dry dimethyl sulfoxide (DMSO, 40 ml) was added and the mixture was heated at 75—85 °C with stirring under nitrogen. After 1 h, the solution was cooled to room temperature and 24 g of [$^{13}C$-1]-ethyltriphenylphosphonium iodide in 100 ml of DMSO was added rapidly. The mixture was heated at 55—65 °C with stirring under nitrogen. After 1 h, the solution was cooled to room temperature, and a solution of **11** (10.0 g) in 240 ml of dry DMSO was added. The whole was again heated at 60 °C under nitrogen. After 5 h, the mixture was cooled, poured into ice-water, and extracted with ether and then with ethyl acetate. The combined organic layer was washed with water, dried over anhydrous $Na_2SO_4$, and evaporated to give a wettish crystalline material (12.5 g), which was chromatographed on a column (2.5 cm, i.d.) packed with 100 g of silica gel. A fraction eluted with a mixture of benzene and *n*-hexane (1 : 3) gave a crystalline material (10.5 g), which was shown by GLC to contain **12** and **13** in a ratio of *ca.* 95 : 5 (relative retention times: 0.79 and 0.73). The relative retention times of the authentic steroids were 0.79 and 0.74, respectively (internal standard; estradiol 3-methyl ether = 1.00, 11.85 min).

**Separation of *Z*-(12) and *E*-(13)-Isomers of [$^{13}C$-20]-5β-Pregn-17(20)-en-3α-ol**——Repeated crystallization of the

above mixture (10.5 g) from methanol gave the *Z*-isomer of [$^{13}$C-20]-5$\beta$-pregn-17(20)-en-3$\alpha$-ol (**12**) as fine needles (8.67 g), mp 192.5—193.5 °C (lit.,[9] 185—187 °C).

A portion of the mother liquor (1.53 g) was chromatographed on a column (2.5 cm, i.d.) packed with 20% $AgNO_3$–silica gel (100 g) and eluted with a mixture of benzene and *n*-hexane (3 : 7). Fractions (50 ml) were collected automatically and monitored by GLC. Eluates were divided into three fractions: Fr. 1 (81 mg, impurity), Fr. 2 (608 mg, **13** as the main product), and Fr. 3 (704 mg, mainly **12** containing a small amount of **13** and others). Repeated crystallization of Fr. 2 (608 mg) from *n*-hexane gave the *E*-isomer of [$^{13}$C-20]-5$\beta$-pregn-17(20)-en-3$\alpha$-ol (**13**) as fine needles (391 mg), mp 131.0—132.5 °C (lit.,[9] 128—131 °C).

**Hydroboration of the [$^{13}$C-20]-*Z*-Isomer (12)**——A well-stirred suspension of 2.6 g of sodium borohydride in 500 ml of diglyme containing 5.2 g of the [$^{13}$C-20]-*Z*-isomer (**12**) was treated with 33 ml of boron trifluoride etherate (13 ml) in diglyme. The mixture was kept at room temperature for 1 h and excess hydride was decomposed with water. The organoborane was oxidized at 35 °C by addition of 10% NaOH (80 ml), followed by dropwise addition of 80 ml of 30% hydrogen peroxide. The whole was stirred at room temperature for 1 h, then a large amount of water was added. The precipitate was filtered, washed well with water, and dried (5.24 g). Crystallization of the product from methanol gave [$^{13}$C-20]-pregnanediol (**1**) as fine needles (3.92 g), mp 241 °C (lit.,[10] 239 °C). $^{13}$C-NMR measurement was carried out on the diacetate, 3$\alpha$,20$\alpha$-diacetoxy-5$\beta$-pregnane (**2**).

**Hydroboration of the [$^{13}$C-20]-*E*-Isomer (13)**——By the same procedure as described for the preparation of **12**, crude product (411 mg) was obtained from 380 mg of the [$^{13}$C-20]-*E*-isomer (**13**). Crystallization of the product from methanol gave [$^{13}$C-20]-5$\beta$-pregnane-3$\alpha$,20$\beta$-diol (**4**) as fine needles (340 mg), mp 232.0—234.0 °C (lit.,[11] 231—234 °C). $^{13}$C-NMR measurement was carried out on the diacetate, [$^{13}$C-20]-3$\alpha$,20$\beta$-diacetoxy-5$\beta$-pregnane (**5**).

**Potassium [$^{13}$C-20]-5$\beta$-Pregnane-3$\alpha$,20$\alpha$-diyl-sulfate (3)**——Chlorosulfonic acid (7.2 g) was added to a stirred solution of [$^{13}$C-20]-pregnane-diol (**1**) (1.0 g) in dry pyridine (50 ml) under cooling. The mixture was then warmed to about 60 °C and stirred for 1 h. Pyridine was removed under reduced pressure to give a residue, to which 0.1 N KOH was added until the solution became alkaline (pH about 9.5). The mixture was dissolved in 500 ml of *n*-butanol, and washed with KCl-saturated water, then once with water, and evaporated under reduced pressure below 60 °C. The crude material (1.67 g) was placed on a column of Dowex 50 W (×8, 200—400 mesh, $K^+$ form) and eluted with water. The eluate was evaporated under reduced pressure below 60 °C to give a white powder (1.47 g), which was crystallized from aq. acetone to afford fine needles of [$^{13}$C-20]-pregnanediol disulfate (**3**) (840 mg), mp 191.0—193.0 °C (lit.,[7] 193.0—193.5 °C).

**Potassium [$^{13}$C-20]-5$\beta$-Pregnane-3$\alpha$,20$\beta$-diyl-sulfate (6)**——By the same procedure as described above, a crude sulfate (330 mg) was obtained from 235 mg of [$^{13}$C-20]-5$\beta$-pregnane-3$\alpha$,20$\beta$-diol (**4**). Crystallization of the product from aq. acetone gave [$^{13}$C-20]-5$\beta$-pregnane-3$\alpha$,20$\beta$-diol disulfate (**6**) as fine needles (270 mg), mp 198.0—200.5 °C (lit.,[4] 197—199 °C).

**Acid Hydrolysis of the [$^{13}$C-20]-Sulfate (3)**——An equal volume of boiling 6 N HCl was added to a boiling aq. solution of the [$^{13}$C-20]-sulfate (**3**) (700 mg in 50 ml), and the whole was refluxed for 15 min. After cooling, the oily precipitate was removed, and the aq. layer was extracted with ethyl acetate. The precipitate and the extract were combined, and the combined mixture was washed with water, dried and evaporated. The residue (370 mg) was chromatographed on a column (1 cm, i.d.) packed with 20 g of silica gel. Fractions (20 ml) were collected automatically and monitored by TLC. Eluates were divided into the following four fractions according to their compositions:

| Fraction No. | Solvent | Volume (l) | Weight (mg) | Composition by TLC |
|---|---|---|---|---|
| 1 | Benzene/*n*-hexane (70 : 30) | 1.2 | 176 | Mainly **7** |
| 2 | Benzene/*n*-hexane (70 : 30) | 0.6 | 68 | **7** and others |
| 3 | Benzene/*n*-hexane (70 : 30) | 2.5 | 25 | Mainly **10** |
| 4 | Chloroform | 1.0 | 74 | Mainly **1** and **8** |

Crystallization of Fr. 3 (25 mg) from acetone gave **10** (16 mg) as fine needles, mp 208.5—209.5 °C (lit.,[4] 207—209 °C).

Preparative TLC of Fr. 4 (74 mg) in multiple runs afforded a crude material (44 mg) corresponding to **8**. Crystallization of the material from acetone gave fine needles of **8**, mp 268.5—269.0 °C (lit.,[4] 268—271 °C).

17$\alpha$-Methyl-D-homo-5$\beta$-androstane-3$\alpha$,17a$\beta$-diol Diacetate (**9**): The above D-homosteroid (**8**) obtained from the [$^{13}$C-20]-sulfate (**3**) was acetylated in the usual way to give the diacetate (**9**), mp 189.0—190.0 °C (crystallized from methanol) (lit.,[4] 189—191 °C); its $^{13}$C-NMR spectrum was measured.

**Acid Hydrolysis of [$^{13}$C-20]-5$\beta$-Pregnane-3$\alpha$,20$\beta$-diyl-sulfate (6) and Isolation of the D-Homosteroid**——Under the conditions described above, 53 mg of an oily product was obtained from 100 mg of the [$^{13}$C-20]-sulfate (**6**) in 5 ml of boiling water and the same volume of boiling 6 N HCl. The product was subjected to preparative TLC in multiple runs. The separated bands corresponding to authentic **8** and **10** were scraped off and extracted with ethyl acetate. The product corresponding to **10** was not obtained in sufficient amount for characterization.

Crude material (34 mg) corresponding to **8** was crystallized from acetone to give pure **8** as fine needles (25 mg), mp 267.0—270.0 °C.

Diacetate: The above D-homosteroid was acetylated in the usual way to give the corresponding diacetate, mp 188.0—190.0 °C (crystallized from methanol); its $^{13}$C-NMR spectrum was determined.

## Results

### (1) Preparation of $^{13}$C-Enriched Pregnanediol Disulfates (3 and 6)

For the preparation of [$^{13}$C-20]-isomeric steriod, etiocholanolone (**11**) was selected as a starting material. The synthetic procedures are the same as described for the synthesis of **12** and **13**,[9] exeept for use of [$^{13}$C-1]-ethyltriphenylphosphonium iodide.

The isotope content of synthetic compound was determined by $^{13}$C-NMR in the decoupling mode without NOE. The amount of $^{13}$C at C-20 was expressed with respect to the natural abundance of the isotope at C-3 as 1.00%. For example, the isotope contents at C-20 and C-3 of the standard olefin **12** were obtained as 1.04 and 1.00%, respectively, whereas the amounts of $^{13}$C at the same positions of the [$^{13}$C-20]-*Z*-isomer (**12**) were 12.20 and 1.00%, respectively. Thus, the ratio of $^{13}$C-amount at C-20 of the labeled steroid to that of the standard steroid becomes 11.7. This result means that the C-20 position of the steroid was quantitatively labeled, because the isotope amount of the reagent used was 11.0 atom%.

Table I showed comparisons of the isotope contents between normal and $^{13}$C-enriched steroids. It can be seen that the isotope contents of the [$^{13}$C-20]-steroid (**12**) and its subsequent

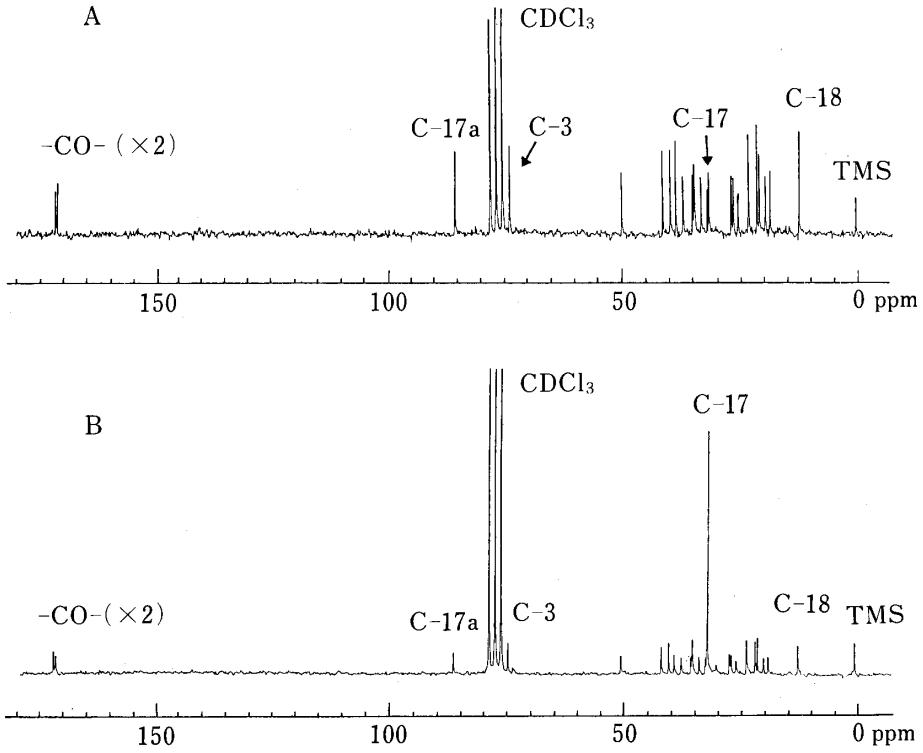



Fig. 1. $^{13}$C-Nuclear Magnetic Resonance Spectra of 17$\alpha$-Methyl-D-homo-5$\beta$-androstane-3$\alpha$,17a$\beta$-diol Diacetate (A) and the Hydrolysis Product (as the acetate) of [$^{13}$C-20]-Pregnanediol Disulfate (B)

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

TABLE I. Amounts (%) of $^{13}C$ at C-3 and C-20 of the Standard and Synthetic [$^{13}C$-20]-Steroids Determined by $^{13}C$-Nuclear Magnetic Resonance[a]

| Compound | | **12** ($CDCl_3$) | | **2** ($CDCl_3$) | | **3** (DMSO-$d_6$) | |
|---|---|---|---|---|---|---|---|
| Source | | Standard | Enriched | Standard | Enriched | Standard | Enriched |
| $^{13}C$ at | C-3 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | C-20 | 1.04 | 12.20 | 1.06 | 12.18 | 1.13 | 12.48 |
| Ratio at C-20 | | 11.7 | | 11.5 | | 11.0 | |
| Compound | | **13** ($CDCl_3$) | | **5** ($CDCl_3$) | | **6** (DMSO-$d_6$) | |
| Source | | Standard | Enriched | Standard | Enriched | Standard | Enriched |
| $^{13}C$ at | C-3 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | C-20 | 1.01 | 10.01 | 1.06 | 9.87 | 1.05 | 9.92 |
| Ratio at C-20 | | 9.91 | | 9.31 | | 9.45 | |

a) Isotope content at C-3 of each steroid was expressed as 1.00%. Parentheses indicate the solvents used for measurement; tetramethylsilane was used as an internal standard.

Ratio at C-20: $\frac{\text{amount (\%) of } ^{13}C \text{ at C-20 of synthetic } ^{13}C\text{-enriched compound}}{\text{amount (\%) of } ^{13}C \text{ at C-20 of standard compound}}$

TABLE II. Comparison of the Amounts (%) of $^{13}C$ at C-3, C-17, and C-17a of the Standard D-Homosteroids and of Hydrolysis Products of 5$\beta$-Pregnane-3$\alpha$,20$\alpha$-diol Disulfate (**3**) and Its 20$\beta$-Isomer (**6**)[a]

| Compound | | **9** | | | **10** | |
|---|---|---|---|---|---|---|
| Source | | Standard | [$^{13}C$-20]-**3** | [$^{13}C$-20]-**6** | Standard | [$^{13}C$-20]-**3** |
| Amount (%) of $^{13}C$ at | C-3 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | C-17 | 1.03 | 12.10 | 9.33 | 1.01 | 11.02 |
| | C-17a | 0.89 | 0.86 | 0.81 | 0.93 | 0.86 |
| Ratio at | C-17 | — | 11.7 | 9.06 | — | 10.9 |
| | C-17a | — | 0.97 | 0.94 | — | 0.92 |

a) $^{13}C$-Nuclear magnetic resonance spectra were measured in $CDCl_3$ with tetramethylsilane as an internal standard. The isotope content at C-3 of each steroid was expressed as 1.00%.

Ratio at C-17 (or C-17a): $\frac{\text{amount (\%) of } ^{13}C \text{ at C-17 (or C-17a) of hydrolysis product}}{\text{amount (\%) of } ^{13}C \text{ at C-17 (or C-17a) of standard steroid}}$

products (**2** and **3**) are about 12%, while the 20-isomers (**13**, **5**, and **6**) have about 10% contents. The NOE of each compound was suppressed sufficiently, and the difference (*ca.* 2%) between the two groups cannot be explained at the present time.

## (2) $^{13}C$-NMR of the D-Homosteroids

Hot acid hydrolysis of the [$^{13}C$-20]-sulfate (**3**) gave D-homosteroids, **8** and **10**, in yields of about 11 and 4%, respectively. The same steroid **8** was obtained from the isomeric [$^{13}C$-20]-sulfate (**6**) in about 60% yield. The chlorine-containing product (**10**), however, could not be isolated in the crystalline state from **6**, because of its small amount.

In Fig. 1, the $^{13}C$-NMR spectrum (A) of authentic D-homosteroid diacetate (**9**) is compared with that (B) of the product of [$^{13}C$-20]-pregnanediol disulfate (**3**). In spectrum A, two close peaks at about 170 ppm correspond to the carbonyl carbon of the two ester groups. The peaks at 85.9 and 74.2 ppm are assigned to the carbons at C-17a and C-3, respectively.[4] The peak at 31.67 ppm can be assigned to the skeletal carbon at C-17, since this singlet peak became a doublet when measured in the single-frequency off-resonance mode.

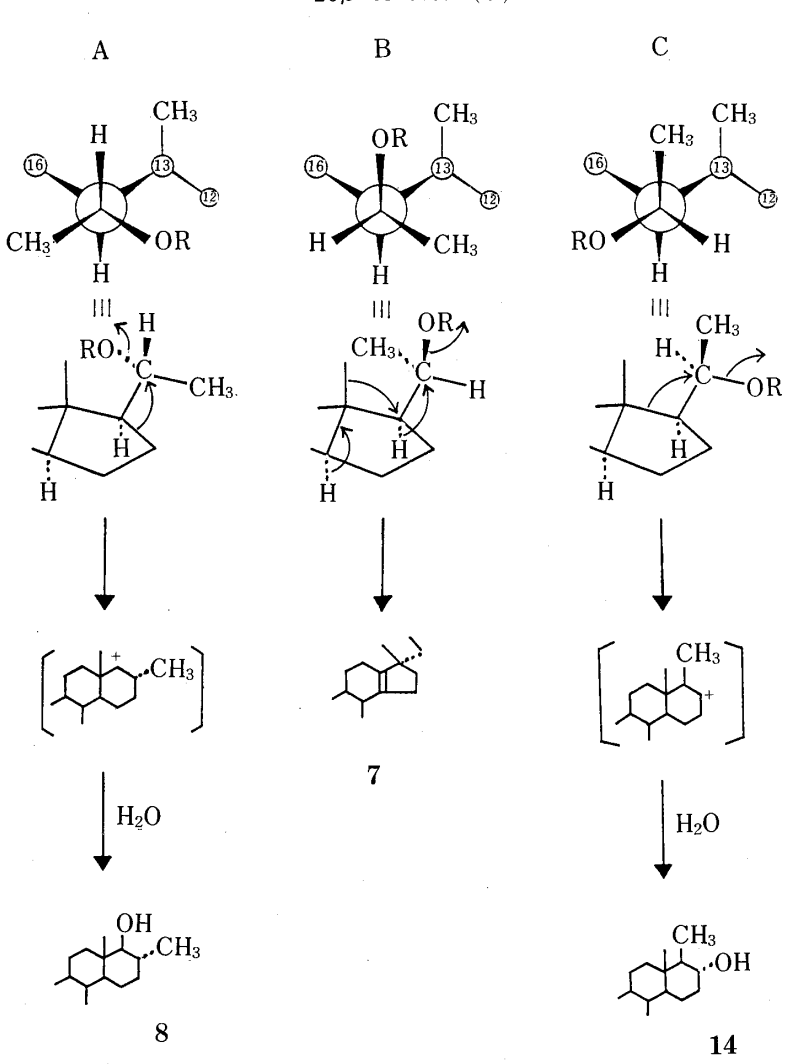



Fig. 2 (continued)

As shown in spectrum B, the D-homosteroid obtained from the [$^{13}$C-20]-sulfate (**3**) is enriched with $^{13}$C only at position 17 of the molecule. Similarly, the chlorine-containing D-homosteroid (**10**) obtained from the [$^{13}$C-20]-sulfate (**3**) was shown to be labeled only at C-17.

The D-homosteroid (**8**) derived from the C-20 isomeric [$^{13}$C-20]-sulfate (**6**) was also shown to be labeled only at C-17.

Table II shows comparisons of the isotope abundances at C-17 and C-17a with that at C-3 for the D-homosteroids obtained from the [$^{13}$C-20]-sulfates and the natural compounds, where the natural abundances of the isotope at C-3 are expressed as 1.00%. For example, the $^{13}$C-abundances at C-17 and C-17a of the standard steroid **9** were 1.03 and 0.89, respectively, whereas those of the product of the [$^{13}$C-20]-sulfate (**3**) were 12.10 and 0.86, respectively. Thus, the ratios of isotope content at C-17 and C-17a between $^{13}$C-enriched and standard steroids can be calculated as 11.7 and 0.97, respectively. The ratio at C-17 of the product is almost equal to those at C-20 of the original [$^{13}$C-20]-steroids (**12**, **2** and **3**). On the other hand, the ratio at C-17a is about 1, indicating no enrichment of $^{13}$C at this position. Similar results were obtained for the chlorine-containing D-homosteroid (**10**) as shown in Table II, and also for the D-homosteroid (**9**) derived from [$^{13}$C-20]-sulfate (**6**) as shown in Table II. Therefore, D-homoannulation of the 20 β-ol sulfate (**6**) proceeds with migration of the $C_{16}$–$C_{17}$ bond to C-20, as has been postulated by Hirschmann and Williams.[5]

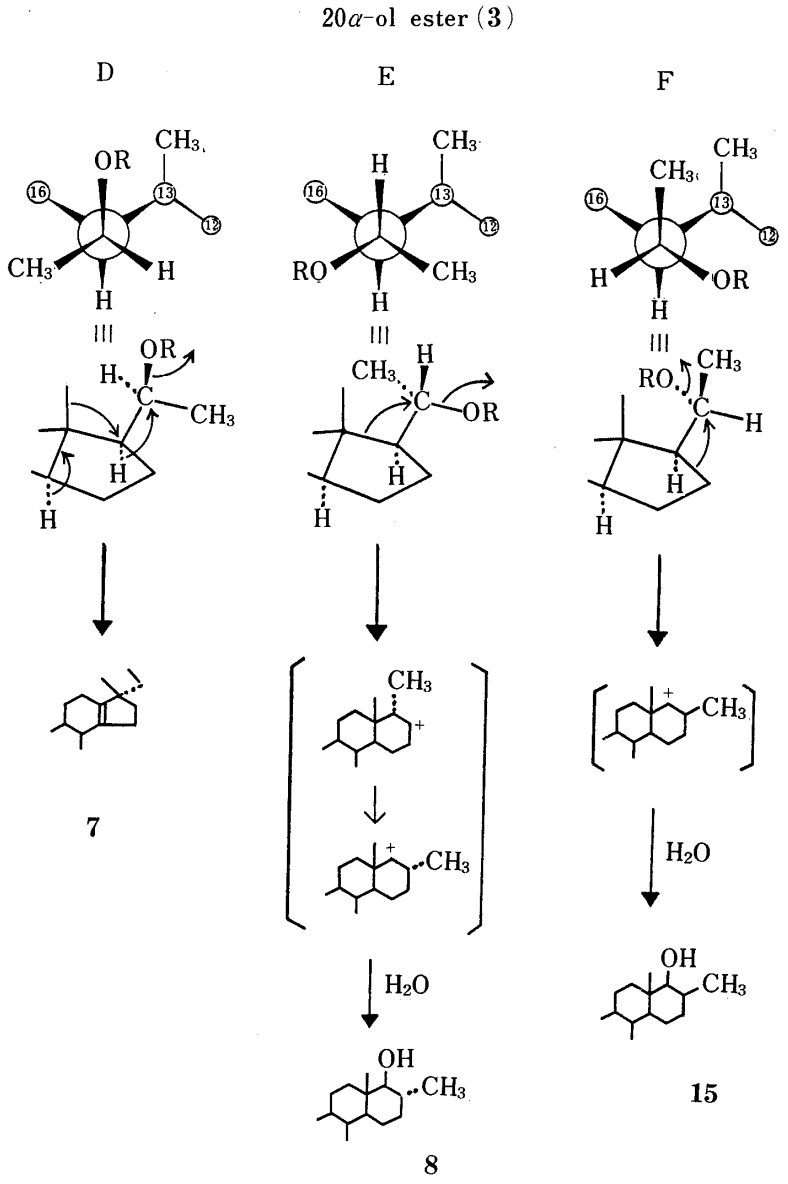



Fig. 2. Partial Structures of 5β-Pregnane-3α,20α-diol Disulfate (**6**) and 5β-Pregnane-3α,20β-diol Disulfate (**3**) and Their Newman's Projections along the $C_{17}$–$C_{20}$ Bond Visualized from C-21, Showing the Reaction Pathways Producing Rearranged Products $R = SO_3H$

## Discussion

The D-homosteroid (**8**) obtained from the [$^{13}$C-20]-sulfate (**3**) was identical with that from the 20-isomeric [$^{13}$C-20]-sulfate (**6**), and both products were shown to be enriched with isotope only at position C-17. This result means that the ring-enlargement reaction of these isomeric disulfates proceeded with migration of the $C_{16}$–$C_{17}$ bond to C-20.

For the D-homoannulation of the 20β-ol sulfate of a 5α-pregnane steroid, Hirschmann and Williams[5] postulated a concerted mechanism, as illustrated in Fig. 2 (conformation A→**8**). Based on this result, Wendler later predicted that the D-homoannulation of the 20α-ol sulfate might proceed with migration of the $C_{13}$–$C_{17}$ bond (Fig. 2, conformation E).[12]

The present result on the 20β-ol sulfate (**6**) demonstrates that the speculation by Hirschmann and Williams is correct. In contrast, the result obtained for the 20α-ol sulfate (**3**)

is completely different from the prediction by Wendler. There are at least two possible mechanisms for the D-homoannulation, the concerted and the stepwise mechanisms. Based on $^{13}$C-NMR data for the products, we could deduce which mechanism is involved in this D-homoannulation. These topics are discussed below.

**(1) Concerted Mechanism**

Figure 2 shows three possible Newman's projections of **6** and **3** (A, B, C, and D, E, F) along with the $C_{17}$–$C_{20}$ bond on the assumption that they are in staggered conformations.

According to the valence-force calculation method,[13] conformation A is the most stable among the three conformations of the 20$\beta$-ol sulfate. In conformation A, the leaving group (ester group) is situated in antiparallel relation to the $C_{16}$–$C_{17}$ bond. The D-homoannulation, thus, may proceed with migration of the $C_{16}$–$C_{17}$ bond to C-20, from which the ester group leaves simultaneously. The cation at C-17a of the D-homosteroid produced may readily yield the 17a$\beta$-ol (**8**) by solvation. From the similar considerations, we can expect the formation of the $\Delta^{13}$-olefin (**7**) by a concerted mechanism (conformation B→**7**), although Hirschmann and Williams have not reported the formation of **7**.[5] Conformation C may be neglected, because the interaction between the 18- and 21-methyl groups is severe. In fact, no product such as **14** which might be derived from conformation C could be detected in the reaction mixture.

Similarly, the most stable conformation for the 20$\alpha$-ol sulfate is considered to be D,[13] where the ester group and 17$\alpha$-hydrogen are arranged in antiparallel relation. Thus, **7** may be formed by a concerted mechanism by removal of the sulfooxy group with simultaneous hydride shift from C-17, to which the 18-methyl group migrates by 1,2-shift, followed by loss of the proton at C-14. In fact, the olefin **7** is always obtained as the main product of the 20$\alpha$-ol sulfate in 5$\beta$- (A/B-*cis*)[1,2,7] and also in 5$\alpha$-(A/B-*trans*) steroids.[14] Conformation E is the next most favorable one, having a slight steric interaction between the 21-methyl and 12-methylene groups. For the formation of the D-homosteroid **8** from conformation E, the $C_{13}$–$C_{17}$ bond must migrate to C-20 as illustrated in Fig. 2. If this mechanism is correct, the isotope would be enriched at C-17a instead of C-17 of the product. Conformation F may be neglected because of its excessive steric interactions: those between the 18- and 21-methyl groups, and between the ester and 12-methylene groups. Failure to detect a product such as **15** from **3** supports this assumption.

The present $^{13}$C-NMR spectra of the two D-homosteroids (**8** and **10**) obtained from the [$^{13}$C-20]-sulfate (**3**) clearly demonstrate that the ring-enlargement reaction of the 20$\alpha$-ol sulfate (**3**) occurred with migration of the $C_{16}$–$C_{17}$ bond. Thus, the D-homoannulation of **3** by the concerted mechanism as illustrated in Fig. 2 (conformation E→**8**) is unlikely.

**(2) Stepwise Mechanism**

Figure 3 shows Newman's projections of three stable rotationally isomeric C-20 cations (P, Q, and R) along with the $C_{17}$–$C_{20}$ bond. Cation P is produced from conformation A of the 20$\beta$-ol sulfate, whereas conformations Q and R are derived from conformations E and D of the 20$\alpha$-ol sulfate, respectively. The $sp^2$-planes at C-20 of P, Q, and R, are arranged perpendicular to the $C_{16}$–$C_{17}$ bond, $C_{13}$–$C_{17}$ bond, and 17$\alpha$-hydrogen, respectively. Based on reported results in similar steroids having such $sp^2$-planes (20-oxo-pregnanes,[15] or $\Delta^{20}$-olefins[16]) and also on the examination of a Büchi model, the relative stability of the three cations may be in the order of P>Q>R.

In the most stable cation P, hydration from the $\alpha$-side (the side of C-12) is considered to be difficult, because this side is sterically crowded.[17] Migration of the $C_{16}$–$C_{17}$ bond from the $\beta$-side (the side of C-16), on the other hand, may occur to product the D-homosteroid (**8**). Namely, the rearrangement reaction is preferred to the hydration in the cation P. The stereospecific D-homoannulation of the 20$\beta$-ol sulfate (**6**), thus, can be explained not only by the concerted mechanism as described above, but also by this stepwise mechanism.

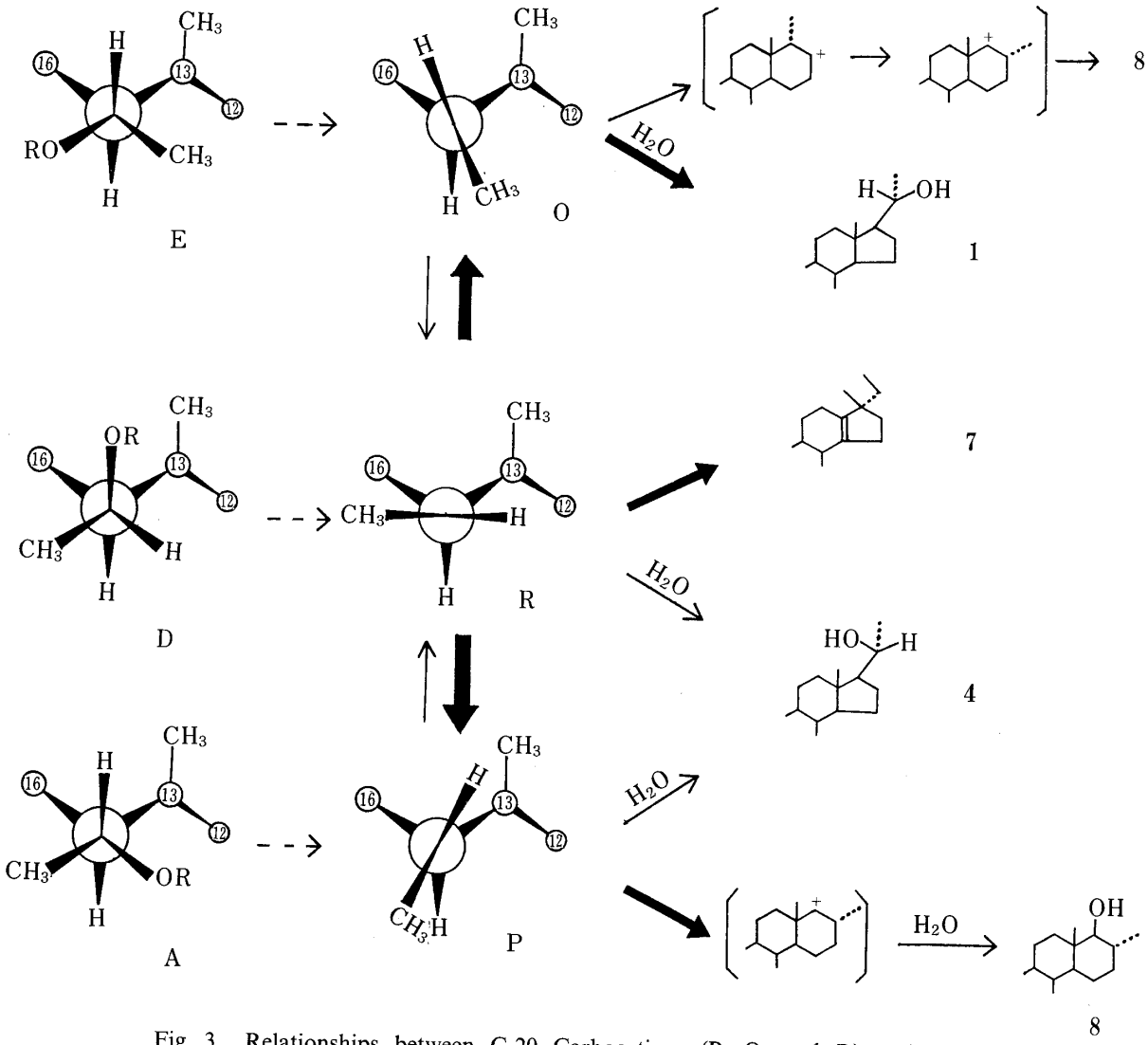



Fig. 3. Relationships between C-20 Carbocations (P, Q, and R) and Their Precursors Having $sp^3$-Conformations (A, E, and D, respectively), and Their Reactions

Conformations are arranged along the $C_{17}$–$C_{20}$ bond visualized from the C-21 side. Thick arrows (➔) mean that the reactions shown predominate over the reactions shown by fine arrows (→). R=$SO_3H$.

In cation Q, the D-homoannulation is expected to occur with migration of the $C_{13}$–$C_{17}$ bond to form the product **8**. This pathway, however, is contrary to the present $^{13}$C-NMR results. Therefore, if the C-20 cation produced from **3** acts as a precursor to the D-homosteroid, the conformation Q must rotate to the more favorable cation P, which then converts to **8**. Hydration of cation Q, however, may occur from the α-side to produce **1**, because solvolysis of **3** in 3 N hydrochloric acid in $H_2{}^{18}O$ gave isomeric 20-ols (**1** and **4**) labeled at C-20 with heavy oxygen in a ratio of 20:1.[1] Thus, hydration to Q is preferred to the rearrangement reaction. Further, a part of cation Q may be converted to the slightly more stable cation P by rotation of the side chain, since the D-homosteroid produced by migration of the $C_{16}$–$C_{17}$ bond is never obtained from cation Q.

Because cation R is considered to be unfavorable,[15,16] this cation may be forced to rearrange to the elimination product **7**, or may be converted to the more stable cations, P and/or Q. Hydration of R from the α-side is considered to be minor, since the formation of the 20β-ol (**4**) from **3** was extremely poor.[1] Thus, the elimination (more precisely, elimination-containing rearrangement reaction) in cation R may exceed the hydration.

Clearly, in the stepwise mechanism involving the C-20 carbocation, rearrangement and elimination reactions are always competing with hydration. The ratio between rearranged, eliminated, and hydrated products may depend on the feasibility of the three reactions, in a particular case.

In the 20$\beta$-ol sulfate (**6**), the C-20 cation produced from A may be arranged as cation P, where D-homoannulation occurs readily with migration of the $C_{16}$–$C_{17}$ bond, but hydration to cation P is difficult. Thus, **8** is always obtained as the main product from the 20$\beta$-ol sulfate (**6**).[4,5] On the other hand, in the 20$\alpha$-ol sulfate (**3**), the most stable conformation D is suitable for the production of **7** by the concerted mechanism. However, if cation R is produced from D, cation R may yield **7**, or be converted to the more stable cation P (and Q). Diversity of product formation from the 20$\alpha$-ol sulfate (**3**), *i.e.*, **7** (elimination), **8** (rearrangement), and **1** (hydration),[1,9] is thus explained. The reason why cation Q does not act as a precursor to the D-homosteroid may be as follows: since hydration of cation Q from the $\alpha$-side causes the positive charge at C-20 to decrease, the migration of the $C_{13}$–$C_{17}$ bond would become difficult.

In conclusion, the D-homoannulation of pregnanediol disulfate (**3**) by hot acid hydrolysis is considered to proceed by the stepwise mechanism involving the C-20 carbocation.

## References and Notes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