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Confirmation of the Involvement of C₂₀-Carbonium Cation during the Hot
Acid Hydrolysis of Pregnanediol Disulfate¹⁾ (Clinical
Analysis on Steroids. XXIII²⁾)

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The formation of a C₂₀-carbonium cation during the hot acid hydrolysis of pregnanediol disulfate (**1b**) and the role of this cation in the rearrangement reactions were examined.

Hydrolysis of **1b** in boiling 3 N hydrochloric acid in oxygen-18 water gave 17 α -ethyl-17 β -methyl-18-nor-5 β -androst-13-en-3 α -ol (**2**) as the main product, together with pregnanediol (**1a**), 17 α -methyl-D-homo-5 β -androstane-3 α ,17 $\alpha\beta$ -diol (**3**), 17 α -methyl-17 $\alpha\beta$ -chloro-D-homo-5 β -androstan-3 α -ol (**4**), 5 β -pregn-20-en-3 α -ol (**5a**), 5 β -pregnane-3 α ,20 β -diol (**10**), and other minor degradation products. The isotope content of these products was determined by gas chromatography-mass spectrometry. Among them, **3** and **10** were quantitatively labeled with ¹⁸O at 17 $\alpha\beta$ - and 20 β -ol, respectively, and **1a** was 74% labeled at 20 α -ol. No uptake of ¹⁸O into 3 α -ol of the products was observed. These results confirm the formation of the C₂₀-carbonium cation from **1b**.

Hydrolysis of 5 β -pregn-20-en-3 α -ol sulfate (**5b**), which is considered to be the conjugated base of the C₂₀-cation, gave **2** as the main product accompanied with **1a**, **3**, **4**, **5a**, and **10**. This result suggests that the C₂₀-carbonium cation plays an important role as an intermediate to D-homosteroids as well as to the Δ^{13} -olefin **2**.

Keywords—carbonium cation from pregnanediol; pregnanediol disulfate; hydrolysis of pregnanediol sulfate; gas chromatography-mass spectrometry of C₂₁-steroid; mechanism of Δ^{13} -steroid formation; mechanism of D-homoannulation

In a recent investigation³⁾ of the hot acid hydrolysis of pregnanediol disulfate (**1b**), multiple degradation products (**2**—**4**, **5a**, **6**—**10**), including pregnanediol (**1a**), were identified, as listed in Table I. The isolation of **10**, even though in a trace amount, is very important, because the result implies the formation of a C₂₀-carbonium cation during the hydrolysis. If this is correct, the cation might act as an intermediate to D-homosteroids as well as to the

TABLE I. The Yields (%) of Hydrolysis Products of Pregnanediol Disulfate (**1b**) in Refluxing 3 N Hydrochloric Acid^{a)}

Hydrolysis product	Yield, %
5 β -Pregnane-3 α , 20 α -diol (1a)	18.8
17 α -Ethyl-17 β -methyl-18-nor-5 β -androst-13-en-3 α -ol (2)	45.8
17 α -Methyl-D-homo-5 β -androstane-3 α ,17 $\alpha\beta$ -diol (3)	11.4
17 α -Methyl-17 $\alpha\beta$ -chloro-D-homo-5 β -androstan-3 α -ol (4)	10.3
5 β -Pregn-20-en-3 α -ol (5a)	6.1
5 β -Pregn-17(20)-en-3 α -ol, <i>E</i> -isomer (6)	1.1
5 β -Pregn-17(20)-en-3 α -ol, <i>Z</i> -isomer (7)	0.01
5 β -Pregn-2-en-20 α -ol (8)	0.7
5 β -Pregn-3-en-20 α -ol (9)	1.3
5 β -Pregnane-3 α , 20 β -diol (10)	0.6
Others, dihydroxy steroid fraction	1.6
steroidal diene fraction	1.6

a) Results were obtained when 7.26 g of pregnanediol disulfate was refluxed in 3 N hydrochloric acid for 30 min. See ref. 3.

Δ^{13} -olefin **2**, although we have previously suggested a concerted mechanism for the formation of **2** from **1b**.⁴⁾

In this work, we have examined the acid hydrolysis of **1b**⁵⁾ in oxygen-18 water ($H_2^{18}O$) in order to detect the C_{20} -carbonium cation, and carried out product analyses by gas chromatography-mass spectrometry (GC-MS).

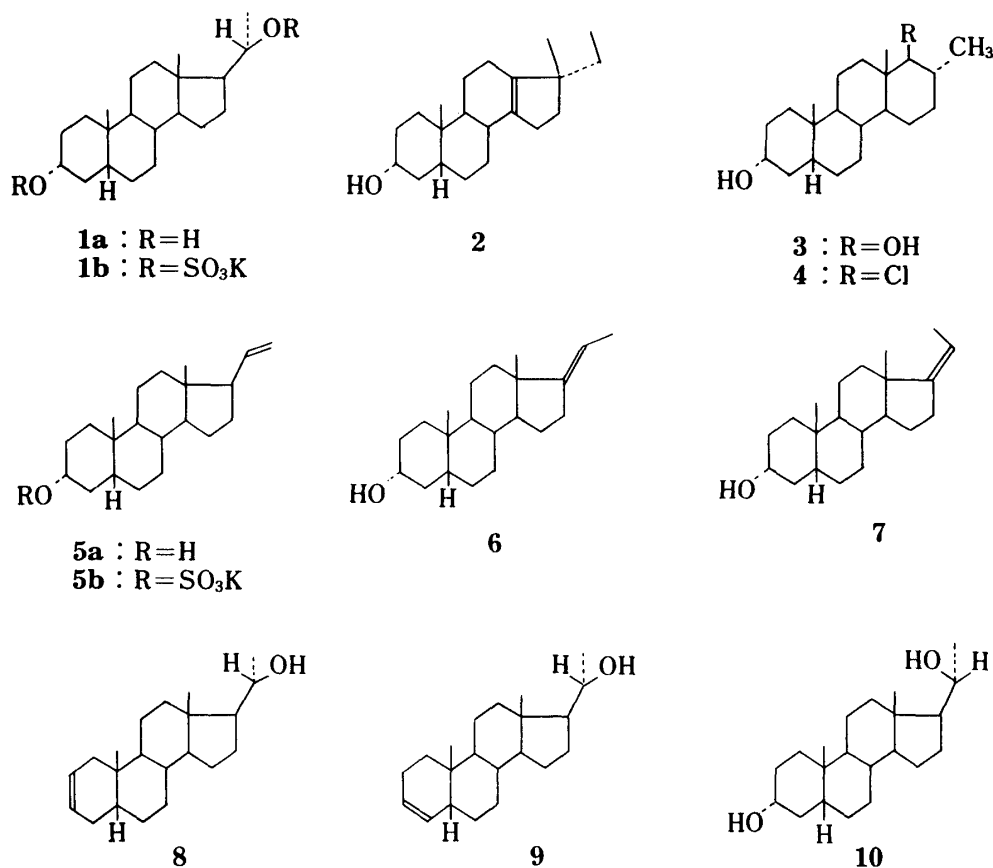


Chart 1

Experimental

Melting points were determined on a Kofler-type micro-hot stage (Mitamura) and are uncorrected. Nuclear magnetic resonance (NMR) spectra were measured on a JNM-PMX-60 spectrometer (JEOL, Tokyo) at 60 MHz and chemical shifts are expressed relative to 1% tetramethylsilane as an internal standard. Infrared (IR) spectra were recorded with a JASCO IR-2 spectrometer (Nihon Bunko, Tokyo). Gas liquid chromatography (GLC) was carried out on a 4 CM gas chromatograph (Shimadzu, Kyoto) using a glass column (2 m $\times$ 3 mm, i.d.) packed with 1.5% OV-1 on Shimalite W (80–100 mesh) with nitrogen as a carrier gas at a flow rate of 30 ml/min. The column temperatures employed were 210°C for the analyses of free

Compounds	Free	TMS ether
Estradiol 3-methyl ether	1.00 (6.85 min)	1.00 (8.20 min)
1a	1.48	1.82
2	0.53	0.49
3	1.43	1.54
4	1.70	1.53
5a	0.65	0.65
10	1.36	1.67

steroids, and 230°C for the analyses of the trimethylsilyl (TMS) ethers. Trimethylsilylation was done by using a commercial reagent, TMSI-H (a pyridine solution of hexamethyldisilazane and trimethylchlorosilane, Gaskuro Kogyo, Tokyo). As an internal standard, estradiol 3-methyl ether which had been prepared in this laboratory from estradiol (Teikoku Hormone Mfg., Tokyo) was used. Relative retention times of the principal hydrolysis products of **1b** and their TMS ethers were obtained as shown in the above Table.

Compounds **2**, **4**, and **5a** were determined as free steroids by using standard curves which were obtained beforehand. Compounds **1a**, **3**, and **10** were also determined as their TMS ethers by using standard curves. The peak areas were determined on the basis of peak width at half height. GC-MS was carried out with a 9000 B machine (Shimadzu, Kyoto) with the same column as described above; helium was used as a carrier gas at a flow rate of 25 ml/min. Other conditions employed were as follows: column temperature, 270°C; ionization potential, 30 eV. The mass spectra of authentic steroids were obtained by GC-MS under the same conditions as used for the hydrolysis products.

The steroidal materials, 5 β -pregnane-3 α ,20 α -diol and 5 β -pregnane-3 α ,20 β -diol were obtained from Steraloids Inc. (N.H., U.S.A.) and pregnanediol disulfate (**1b**),⁶⁾ the steroidal olefins (**2**, **5a**, **6**—**9**)³⁾ and D-homosteroids (**3** and **4**)⁷⁾ were obtained according to the method described previously. Oxygen-18 water was from B.O.C. Ltd. (England) and the purity was 98.3 atom %. Elemental analyses were done by the staff of the Analytical Center in Hokkaido University (Sapporo), to whom our thanks are due.

Potassium 5 β -Pregn-20-en-3 α -yl Sulfate (5b**)**—Chlorosulfonic acid (0.05 ml) was added to dry pyridine (2 ml) under cooling, and the mixture was stirred for 15 min at 50°C. To this solution was added 12 mg of **5a**, and the whole was stirred at 50°C. After 1 h, the solution was cooled to room temperature and the pyridine was removed under reduced pressure at 50°C. The resultant residue was dissolved in 0.1 N KOH (10 ml), and the mixture was extracted with *n*-butanol (5 ml $\times$ 5). The combined extract was washed once with water and concentrated to a few ml under reduced pressure at 50°C. The concentrate was applied to a column (1.0 cm, i.d. $\times$ 10 cm) of Dowex 50 W ($\times$ 8, 200—400 mesh, K⁺ form) and eluted with water. The eluate was concentrated under reduced pressure below 50°C to give a white powder (17.5 mg), which was recrystallized from methanol to afford fine needles of **5b** (9.1 mg), mp 202—203°C. Anal. Calcd for C₂₁H₃₃KO₄S: C, 59.96; H, 7.51; S, 7.62. Found: C, 59.78; H, 8.04; S, 7.51. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 2940 and 2865 (CH), 1635 (C=C), 1240 and 1210 (OSO₃). NMR (methanol-*d*₄) δ : 5.8—5.4 (1H, m, C₂₀-H), 5.1—4.8 (2H, m, C₂₁-H), 4.3—4.1 (1H, m, 3 β -H), 0.95 (3H, s, 19-CH₃), 0.61 (3H, s, 18-CH₃).

Kinetics (Hydrolysis of **1b and **5b**)**—Boiling 6 N HCl (2 ml) was added to refluxing aqueous solutions (2 ml) of the sulfates (**1b** and **5b**, each 500 μ g) in reaction vessels, and the mixtures were refluxed. After 15, 30, 45, and 60 s, and after 2, 5, 10, 15, 20, and 30 min, the reactions were stopped by addition of ice-cooled water and 1 N KOH (12 ml). After addition of an internal standard (*ca.* 20 μ g), the mixtures were extracted with ether (5 ml $\times$ 5), and the combined extracts were washed with water, dried over anhydrous Na₂SO₄, and concentrated. Half of each product were used for GLC, and the other half was assayed by GLC as the TMS ether. The mean values of triplicate experiments were plotted (Figs. I and III).

Hydrolysis of **1b in 3 N HCl in H₂¹⁸O**—Prior to use, the apparatus was well dried and then flushed with dry nitrogen gas. The reaction was carried out with careful exclusion of moisture. **1b** (500 μ g) was dissolved in 2 ml of boiling 3 N HCl/H₂¹⁸O, and the mixture was immediately refluxed on the oil bath for 1 min. The reaction was stopped by adding ice-cooled water followed by 2 N KOH (2 ml). After addition of an internal standard (*ca.* 20 μ g), the mixture was extracted with ether (5 ml $\times$ 5), and the combined extract was washed with water, dried, and concentrated. GC-MS was carried out on the product or the TMS ether. The yields (%) of the products obtained in triplicate experiments were: **1a**; 11.4—14.5; **2**, 31.6—39.2; **3**, 3.6—9.4; **4**, 0.7—1.2; **5a**, 5.2—7.3; **10**, 0.4—0.6; others, 1.3—1.8. Table II gives the results of one experiment.

Decomposition of **10**—A methanolic solution of **10** (500 μ g in 2 ml) was added to 3 N HCl (2 ml), and the mixture was refluxed. The reaction was stopped by adding ice-cooled water and 1 N KOH (6 ml) to the reaction mixture at 1, 2, 5, 10, 15, 20, and 30 min. After addition of an internal standard (*ca.* 20 μ g), the mixtures were extracted with ether (5 ml $\times$ 5), and the combined extracts were washed, dried, and concentrated. GLC was carried out on these hydrolyzates or on the TMS ethers. The mean values of triplicate experiments were plotted (Fig. 2).

Results and Discussion

Prior to the ¹⁸O-experiment, it was important to select the suitable reaction time for the hydrolysis of **1b**, because the yield of **10** is extremely small. In Fig. 1, the time-courses of the yields (%) of **1a**, **2**, **3**, **4**, **5a**, and **10** are plotted. The yield of the olefin **2** reached the maximum at 10 min, then gradually decreased, probably as a result of decomposition to steroidal dienes as described previously.¹⁾

The yield of pregnanediol (**1a**) reached a maximum at 2 min, then gradually decreased with time. D-Homosteroids (**3** and **4**) were obtained in maximum yields at 10 min, and the yields remained constant thereafter.

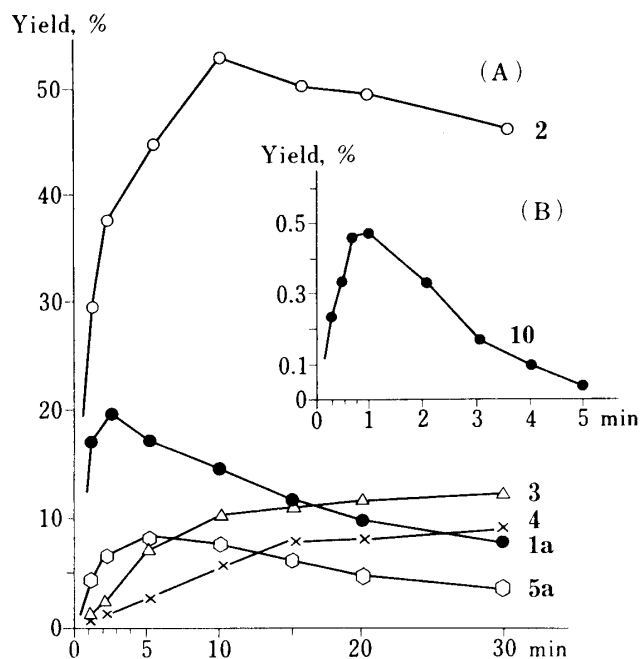


Fig. 1. Time-Courses of Products Formation from Pregnanediol Disulfate (**1b**) in Boiling 3 N Hydrochloric Acid

Mean values of triplicate experiments are plotted.

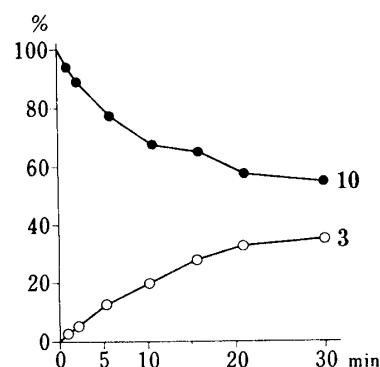


Fig. 2. Time-Courses of the Decomposition of **10** by Boiling in 50% Methanolic 3 N Hydrochloric Acid and of the Formation of **3**

Mean values of triplicate experiments are plotted.

As illustrated in Fig. 1 (B), the yield of **10** is extremely small. The maximum yield is about only 0.5% at about 1 min, and it disappears in a few minutes. Previously,³⁾ we had obtained a larger amount of **10** (0.6% as shown in Table I); this may have been because of the massive amount of **1b** used, so that the 20 β -ol **10** produced partly escaped decomposition owing to its insolubility in the aqueous medium.

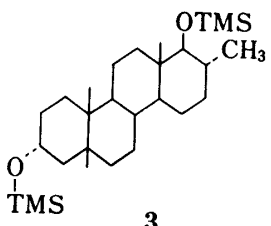
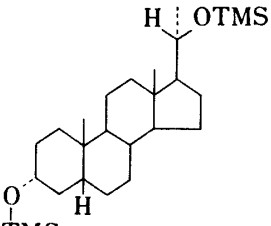
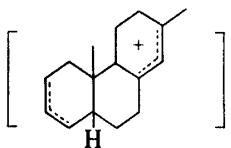
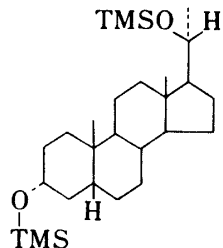
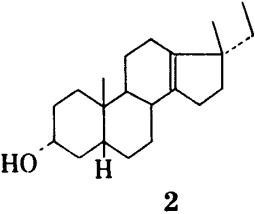
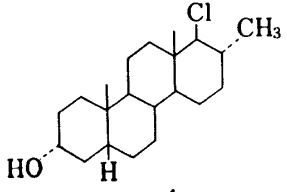
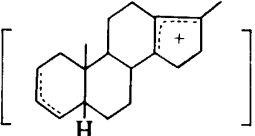
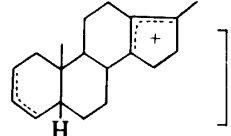
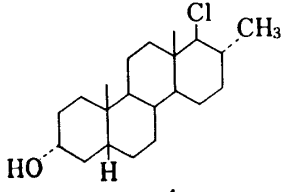
The rapid decomposition of **10** in Fig. 1 (B) might be due to the conversion to D-homosteroid **3**, in view of the quantitative D-homoannulation of a similar steroid 20 β -ol sulfate⁸⁾ or tosylate.⁹⁾ This was confirmed by the following experiment. When **10** was refluxed in 50% methanolic 3 N hydrochloric acid, it rapidly disappeared, and **3** was concomitantly formed (Fig. 2).

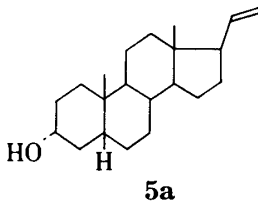
As the optimum reaction time was 1 min, the acid hydrolysis of **1b** in H₂¹⁸O was carried out for 1 min. In Table II, the principal ions in the mass spectra of the main hydrolysis products obtained in the ¹⁸O-experiment are compared with those of authentic specimens.

The spectral patterns of **2**, **4**, and **5a** obtained in the ¹⁸O-experiment are identical with those of corresponding authentic steroids. It is apparent from these results that no incorporation of ¹⁸O into 3 α -ol occurred, although the hydroxyl group is conjugated with sulfuric acid. The result is in conflict with the previous report by Ramseyer and Hirschmann, who detected a C₃-carbonium cation during the hydrolysis of androsterone sulfate.¹⁰⁾ The divergence between these results may be attributable to the steric difference at C-3 of the substrates studied: the configuration of the reported sulfate is axial, whereas that of pregnanediol is equatorial.

As can be seen from Table II, the TMS ether of the authentic 20 β -ol **10** shows the molecular ion at m/e 464 and fragment ions at m/e 374, m/e 284, m/e 269, together with a diagnostic fragment ion of pregnanediol TMS ether at m/e 117.¹¹⁾ In contrast to the authentic steroid, the TMS ether of **10** obtained in the ¹⁸O-experiment gave some new fragment ions having greater mass by 2 units than those of the authentic steroid at m/e 464, m/e 374, and m/e 117. These are attributable to the molecular ion [M^+ , containing ¹⁸O], [M^+ (466)–90], and [$(CH_3)_3Si-^{18}O^+=CH-CH_3$], respectively. The ratio of the intensities at m/e 466 and m/e 464,

TABLE II. Mass Spectral Ions and Their Peak Intensities of **2**, **4**, and **5a**, and Those of the Trimethylsilyl Ethers of **1a**, **3**, and **10**: Comparison of Authentic Steroids and the Hydrolysis Products obtained from Pregnanediol Disulfate in Refluxing 3 N Hydrochloric Acid in Oxygen-18 Water

Compounds ¹⁾	Authentic		¹⁸ O-Experiment	
	<i>m/e</i>	Intensity, %	<i>m/e</i>	Intensity, %
 3	464	[M ⁺] 50.6	466	62.3
			464	1.6
	374	[M ⁺ - 90 (TMSOH)] 100	376	100
	284	[M ⁺ - 2 × 90] 44.9	374	15.3
	269	[M ⁺ - 2 × 90 - 15 (CH ₃)] 28.9	284	52.3
			269	30.4
 1a	215	[] 49.7	215	61.9
	143	[(CH ₃) ₃ Si-O ⁺ =CH-C<CH ₃ /CH ₂] 56.5	145	73.0
			143	1.9
	464	[M ⁺] 2.3	466	4.0
 10			464	1.4
	374	[M ⁺ - 90] 13.3	376	2.2
	284	[M ⁺ - 2 × 90] 39.3	374	12.8
	269	[M ⁺ - 2 × 90 - 15] 22.7	284	39.0
			269	18.2
	117	[(CH ₃) ₃ Si-O ⁺ =CH-CH ₃] 100	119	100
 2			117	36.0
	464	[M ⁺] 8.3	466	7.1
			464	0.3
	374	[M ⁺ - 90] 30.7	376	12.5
	284	[M ⁺ - 2 × 90] 62.8	374	12.4
	269	[M ⁺ - 2 × 90 - 15] 33.3	284	40.4
 4			269	25.6
	117	[(CH ₃) ₃ Si-O ⁺ =CH-CH ₃] 100	119	100
			117	4.1
	302	[M ⁺] 6.9	302	5.9
	287	[M ⁺ - 15 (CH ₃)] 2.1	287	2.1
	273	[M ⁺ - 29 (C ₂ H ₅)] 100	273	100
	255	[] 30.8	255	24.2
 5a	340	[M ⁺] 2.3	340	2.4
	338	[M ⁺] 5.7	338	5.8
	322	[M ⁺] 31.5	322	30.8
	320	[M ⁺ - 18 (H ₂ O)] 100	320	100
	307	[M ⁺] 12.3	307	16.9
	305	[M ⁺ - 18 - 15] 32.7	305	49.8

Compounds ¹⁾	Authentic		¹⁸ O-Experiment	
	<i>m/e</i>	Intensity, %	<i>m/e</i>	Intensity, %
 5a	302	[M ⁺]	100	302
	287	[M ⁺ - 15]	22.2	287
	284	[M ⁺ - 18]	48.1	284
	269	[M ⁺ - 15 - 18]	34.3	269
				100
				16.0
				50.3
				24.9

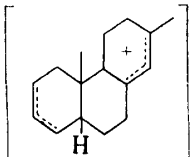
TMS: trimethylsilyl [(CH₃)₃Si-].

of fragment ions at *m/e* 119 and *m/e* 117 were both 96:4. Because no uptake of ¹⁸O into 3 α -ol was apparent, and the ¹⁸O-content of the water employed was 98.3 %, it may be concluded that the 20 β -ol **10** produced was labeled quantitatively at C-20.

The results with **10** led us to consider that the 20 α -ol **1a** may also contain a heavy oxygen at C-20. The TMS ether of standard **1a** has the molecular ion at *m/e* 464, and fragment ions at *m/e* 374, *m/e* 284, *m/e* 269, and *m/e* 117. The TMS ether of **1a** obtained in the ¹⁸O-experiment has common fragment ions with the authentic specimen at *m/e* 284 and *m/e* 269. However, ¹⁸O-labeled **1a** has two ion pairs, namely at *m/e* 466 [M⁺(¹⁸O)] and *m/e* 464 (M⁺), and *m/e* 119 [(CH₃)₃Si-¹⁸O=CH-CH₃] and *m/e* 117, and the ratios of both pairs were 74:26. Thus, as the hydroxyl group at C-3 is not labeled with ¹⁸O, it became evident that about 74% of **1a** was produced *via* the C₂₀-carbonium cation, in other words, the amount of **1a** produced by normal cleavage of the oxygen-sulfur bond at the C-20 sulfooxy group was only 26%.

Because the fragment ions at *m/e* 376 and *m/e* 374 are produced by elimination of trimethylsilanol (TMSOH) from C-3 and of TMS¹⁸OH from C-20, we speculate that the 20- α trimethylsiloxy group is more easily eliminated as trimethylsilanol than the 20 β group, by comparison of the peak intensities at *m/e* 374 of **1a** and **10** obtained in the ¹⁸O-experiment. In the TMS ether of the 20 β -ol **10**, the ratio of *m/e* 376 to *m/e* 374 is 12.5:12.4, that is, essentially equal. In contrast, the ratio of those peaks in **1a** is 2.2:12.8, *i.e.*, the peak height of *m/e* 374 is about six times that of *m/e* 376, which means that the 20 α -trimethylsiloxy group is more easily eliminated as TMSOH than the 20 β group. As the ¹⁸O-content of **1a** was 74%, the real ratio of this elimination of the 20 α -trimethylsiloxy group can be calculated as about eight times that of the 20 β group. These results may reflect differences of steric environment of the C-20 hydroxyl groups. To be eliminated as TMSOH, the oxygen atom of the 20-trimethylsiloxy group may abstract a hydrogen from C-16 β , as indicated by analysis of the fragmentation patterns of sterically crowded trialkylsilyl ether derivatives of the same steroid.¹²⁾ In the case of the 20 α -trimethylsiloxy compound, the C-20 oxygen can approach to abstract 16 β -hydrogen, whereas this approach is difficult in the 20 β -derivative owing to steric interaction between the 18- and 21-methyl groups.

Finally, the ¹⁸O-content of the D-homosteroid **3** was examined. The TMS ether of authentic **3** has the molecular ion at *m/e* 466, and fragment ions at *m/e* 374, *m/e* 284, *m/e* 215,

and *m/e* 143; the last two ions are considered to have the structures  and

$[(\text{CH}_3)_3\text{Si}-\text{O}^+=\text{CH}-\text{C}\begin{smallmatrix} \nearrow \text{CH}_3 \\ \searrow \text{CH}_2 \end{smallmatrix}]$, respectively, as reported in the fragmentation of a similar D-homosteroid.¹³⁾ The TMS ether of **3** obtained from the ^{18}O -experiment, on the other hand, has peaks in common with the authentic steroid at m/e 284, m/e 269, and m/e 215. Two other ions are observed as pairs; a pair of molecular ions at m/e 466 and m/e 464, and a pair of fragment ions at m/e 145 and m/e 143. The ions at m/e 466 and m/e 145 correspond to ^{18}O -molecular ion and $[(\text{CH}_3)_3\text{Si}-^{18}\text{O}^+=\text{CH}-\text{C}\begin{smallmatrix} \nearrow \text{CH}_3 \\ \searrow \text{CH}_2 \end{smallmatrix}]$, respectively. The ratios of these pairs were both 96:4, which indicates a quantitative uptake of ^{18}O into the $17\alpha\beta$ -ol. In this case, the ratio of m/e 376 to m/e 374, corresponding to the fragments $(\text{M}^+-\text{TMSOH})$ and $(\text{M}^+-\text{TMS}^{18}\text{OH})$, respectively, was 100:15. This suggests that the 3α -trimethylsiloxy group is more easily eliminated than the $17\alpha\beta$ group.

From the above elimination results observed in TMS ethers of the ^{18}O -products (**1a**, **3**, and **10**), it is clear that the sequential elimination of TMSOH from polyhydroxy steroid TMS ether is dependent on the structural environment of the trimethylsiloxy function, as has been reported in similar steroid.¹⁴⁾

As the (alcoholic) oxygen of the alcohol produced from the sulfate is no longer exchangeable with oxygen of the solvent in the hydrolysis of alkyl sulfate in acidic medium,¹⁵⁾ oxygen-18 might be incorporated directly into the C_{20} -carbonium cation to give **1a**, **3**, and **10**. Thus, the possibility arose that the C_{20} -carbonium cation might be a precursor to **2**. To examine this, the following experiment was undertaken.

As the olefin **5a** is considered to be the conjugated base of the C_{20} -carbonium cation, **5a** may yield the cation in an acidic medium and this cation, once produced, may give alcohols by solvation or may suffer rearrangements to D-homosteroids such as **3** and **4** as well as to Δ^{13} -steroid **2**. Because **5a** was not soluble in aqueous solution, it was converted to the corresponding sulfate, potassium 5β -pregn-20-en- 3α -yl sulfate (**5b**), which was used in the experiment.

Figure 3 shows the time-course on the production of the principal products from **5b** under reflux in 3N hydrochloric acid. The following results were obtained.

1) The 20α - and 20β -ols were definitely produced from the C_{20} -carbonium cation.—This supports the observation on the production of **10** from **1b**.

2) Once the C_{20} -carbonium cation is produced, this cation acts as a precursor to the Δ^{13} -olefin **2**.—Two possible mechanisms for the production of **2** can be considered: one is a concerted mechanism, as described previously;^{1,4)} protonation occurs initially on the alcoholic oxygen of C-20 followed by removal by sulfuric acid with simultaneous hydride shift from C- 17α to C-20, to which the 18-methyl group migrates by 1,2-shift, and finally loss of a proton at C-14. The other mechanism is a stepwise one; the positive charge at C-20

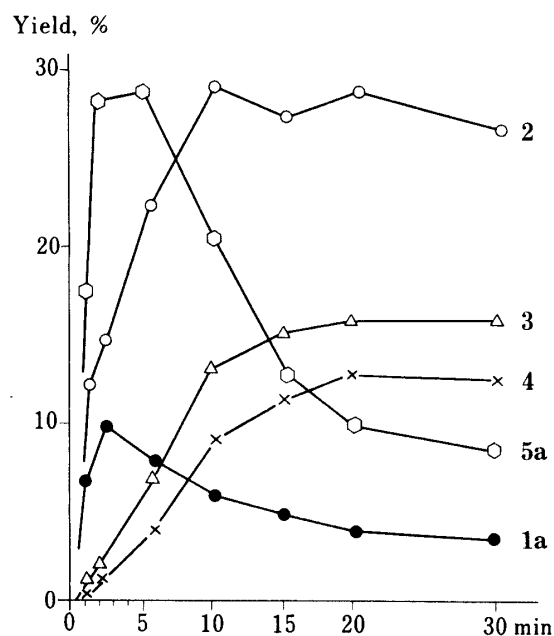


Fig. 3. Time-Courses of Products Formation from 5β -Pregn-20-en- 3α -ol Sulfate (**5b**) in Boiling 3 N Hydrochloric Acid

Mean values of triplicate experiments are plotted. The maximum amount of **10** is 0.65% at 1 min, which is not shown in the figure, because the amount is too small.

migrates to C-17 to form a C₁₇-carbonium cation, which is converted to **2**. The actual mechanisms concerned are currently under investigation.

3) A substantial portion of the C₂₀-carbonium cation is converted to D-homosteroids.—This result suggests that the ring-enlargement reaction may occur at the C₂₀-carbonium cation with a migration of the C₁₃-C₁₇ or C₁₆-C₁₇ bond to C-20. Another mechanism, the concerted one, may also participate, as speculated previously.⁷⁾ This remains to be investigated.

As discussed above, the mechanism of the rearrangement reactions of **1b** is very complex, especially as regards the 20 β -ol sulfate. It is clear, however, that there is a big difference in reactivity between 20 α - and 20 β -ol sulfates. The 20 β -ol sulfate, upon acid hydrolysis, gave the uranediol-type D-homosteroid quantitatively as reported previously.^{7,8)} Thus, this stereospecific D-homoannulation may proceed *via* a concerted mechanism as speculated by Hirschmann *et al.*⁸⁾ In contrast to the 20 β -ol, the 20 α -ol sulfate gave the Δ^{13} -olefin as the main product with simultaneous formation of many other kinds of degradation products as described above and previously.³⁾ This may be a result of C₂₀-carbonium cation formation (from) the 20 α -ol sulfate. In other words, the rearrangement reactions of **1b** proceed mainly *via* a stepwise mechanism involving the C₂₀-cation.

It is known for an isomeric difference at the C-20 hydroxyl group to influence the product formation, *e.g.*, in the case of the 20-tosyloxy derivatives of 5 α -pregnanes,¹⁶⁾ boron trifluoride-catalyzed rearrangement reaction of 20-acetoxy 5 α -pregnanes,¹⁷⁾ and dichlorobis(benzonitrile)palladium-catalyzed reaction of similar steroids.¹⁸⁾ In any case, multiple kinds of degradation products, including Δ^{13} -olefin, were formed from the 20 α -ol derivatives, in contrast to the stereospecific D-homoannulation observed in the 20 β derivatives. The sharp contrast between the reactions of 20 α - and 20 β -ol steroids may be attributable to steric interactions at the side chain and D-ring, as described by Altona and Hirschmann.¹⁹⁾

The present and also the previous results³⁾ are in accord with the results of Metcalf, who investigated kinetically the degradation of conjugated pregnanediol by hydrochloric acid at elevated temperature.²⁰⁾ In the determination of urinary or serum steroids, solvolysis or enzymatic hydrolysis have been used under moderate conditions. Indeed, hot acid hydrolysis still remains popular, largely for reasons of speed and cost. However, the degradation of the steroids during such procedures can not be neglected as shown in the present paper.

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