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On the Structure of the Hot Acid Hydrolysis Products of 3α,20α-Disulpho-oxy-5α-pregnane[1]

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The hydrolysis of 3α,20α-disulpho-oxy-5α-pregnane (5α-pregnane-3α, 20α-diol disulfate, VII) in boiling 3 N hydrochloric acid gave Δ^{13}-steroid as a main product, which was completely identical with the synthetic one (Va) prepared by the dehydration of 5α-pregnane-3α,17α-diol (IVa). Other minor products obtained and identified were the E-isomer of 17-ethylidene compound (IIb) and intact aglycone (VI).

Keywords——rearrangement reaction; steroid; hot acid hydrolysis of sulfate; 17α-ethyl-17β-methyl-5α-androst-13-en-3α-ol; NMR; mass spectroscopy

It was reported previously that the unknown steroidal olefin was isolated from a female urine when hydrolyzed in boiling 3 N hydrochloric acid and that the structure was proposed as 17α-ethyl-17β-methyl-18-nor-5β-androst-13-en-3α-ol, although the absolute configuration remained obscure.[3] It was also reported that this abnormal steroid was the artifact of the urinary pregnanediol 20-sulfate.[3] Because there have been no examples of such a rearrangement reaction, the structure of the product should be confirmed.

In the present paper, we describe that the Δ^{13}-steroid derived from pregnane steroid 20-sulfate was completely identical with the synthetic one prepared by the different procedure.[4]

It is well known that 17α-hydroxy steroids having 17β-alkyl side chain are easily converted to Δ^{13}-olefins, the configuration of which is arranged as 17α-alkyl and 17β-methyl.[5] This reaction is classified as one of the Wagner–Meerwein rearrangements. From this point of view, such materials as IVa might be a suitable substrate for preparing the authentic Δ^{13}-steroid.

The Wittig reaction[6] of *cis*-androsterone (I) yielded two isomeric 17-ethylidene compounds, IIa and IIb in the approximate ratio of 95: 5,[7] the former of which was treated with *m*-chloroperbenzoic acid to give 17α,20α-epoxide (III). The epoxide was then converted to 3α,17α-diol (IVa) by reduction with lithium aluminum hydride. For the purpose of nuclear magnetic resonance (NMR) study as described below, the deuterated material (IVb) was also prepared from III with deuteride reagent. Acid treatment of these 17α-hydroxy steroids gave easily and quantitatively the desirable authentic Δ^{13}-steroids, Va and Vb, respectively.

As 20-monosulfate of 5α-pregnane-3α,20α-diol (VI) is difficult to prepare, easily obtainable disulfate (VII) was used for the following experiment. Hydrolysis of VII was carried

1) This paper constitutes Part III of the series entitled "Clinical Analysis on Steroids"; Part II: I. Yoshizawa, R. Oh'uchi, A. Nakagawa, N. Kawahara, T. Miura, M. Kimura, K. Anzai, and S. Matsuda, *Yakugaku Zasshi*, **98**, 215 (1978).

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3) I. Yoshizawa, T. Miura, M. Kimura, K. Anzai, and S. Matsuda, *Chem. Pharm. Bull.* (Tokyo), **21**, 1622 (1973).

4) Although pregnanediol was studied in the previous paper,[1,3] *cis*-androsterone (5α-steroid) was selected as a starting material instead of 5β-one because of its expensive price.

5) N.L. Wendler, "Molecular Rearrangements," ed. by P. de Mayo, John Wiley and Sons, New York, 1964, Chapter 16.

6) E.P. Oliveto, "Organic Reactions in Steroid Chemistry," Vol. 2, ed. by J. Fried and J.A. Edwards, Van Nostrand Reinhold Co., New York, 1972, pp. 131—132.

7) A.M. Krubiner and E.P. Oliveto, *J. Org. Chem.*, **31**, 24 (1966).

out as described previously,[1,3] and the hydrolyzate was afforded to preparative thin-layer chromatography. The corresponding Δ^{13}-steroid was obtained as a major product in the yield of 51%. The remaining minor products identified were IIb (22%) and VI (15%).

The Δ^{13}-steroid derived from sulfate was compared with Va by instrumental analyses. NMR spectrum of each olefin was completely identical and these are shown in Fig. 1 (a and b).

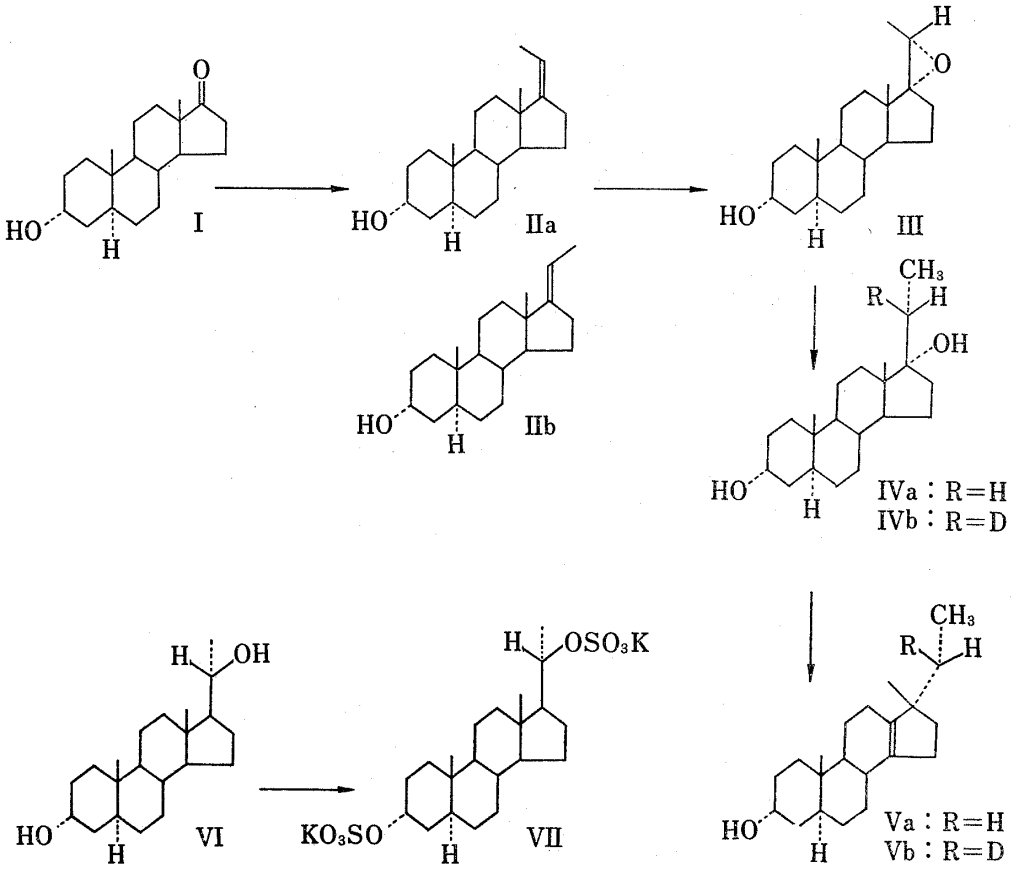

Chart 1

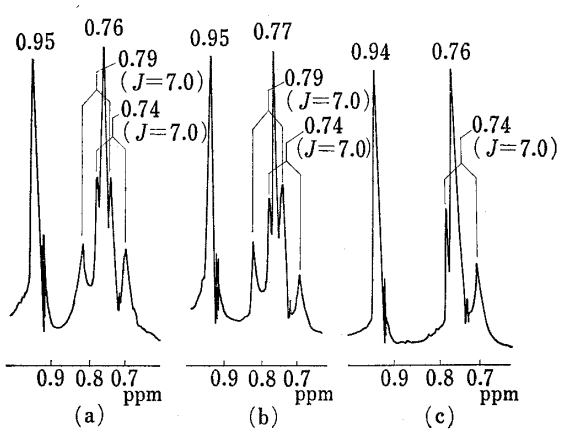

Fig. 1. Nuclear Magnetic Resonance Spectra of Hydrolysis Product of VII (a), Va (b), and Vb (c)

Signals at 0.95 and 0.76 (0.77 in b) ppm are corresponding to 17β- and 19-methyl protons. Signals of 21-methyl protons, on the other hand, are splitted into a pair of doublets (0.79 and 0.74 ppm, respectively) by coupling with two unequal protons at C-20. The signal at down field (0.79 ppm) is attributed to the methyl protons coupled with 20β-proton and the upper field signal at 0.74 ppm with 20α-one. These were assigned by the following result of the deuterated material (Vb), in the NMR spectrum of which only an upper field signal at 0.74 ppm is observed (Fig. 1, c). These results are showing that 21-methyl protons coupled with 20β-proton are resonating in the down field than with 20α-one.

Other instrumental analyses including mass spectra, infrared absorption spectra, and melting point determinations showed that Δ^{13}-olefin obtained from sulfate (VII) was completely identical with Va.

It was confirmed, therefore, that the hydrolysis of 20α-sulfate of pregnane steroid in hot acid medium gave the rearranged Δ^{13}-steroid having such an absolute configuration at C-17 as shown in Va. The present result is in sharp contrast with the previous result[8] in that 20β-sulfate of similar steroid was converted to D-homo steroid, which is known as uranediol rearrangement.[9] This divergence between the results in 20α- and 20β-sulfate is the problem to be investigated which is now under progress.

Experimental

Melting points were determined on a micro hot-stage (Mitamura) and are uncorrected. NMR spectra were recorded on a JEOL-JNM-PS-100 spectrometer (Nihon Denshi) by using 5% solutions containing tetramethylsilane as an internal standard. The infrared absorption (IR) spectra were taken with JASCO-IR-A-2 (Nihon Bunko). Mass spectra (MS) were taken with RMU-6E (Hitachi) by direct insertion method. The steroid compounds, *cis*-androsterone and 5α-pregnane-3α,20α-diol, were purchased from Teikoku Hormone Mfg. Co., Tokyo.

5α-Pregn-17(20)-en-3α-ol (IIa, b)——A solution of I (7.7 g) dissolved in 150 ml of DMSO is added rapidly to 170 ml of DMSO solution of ethylenetriphenylphosphorane (5.0 g). After heating at 60° overnight under N_2 stream, the reaction mixture was poured into ice-water, and extracted with three portions of ether, back-washed with three portions of water and ether removed. The crude product (6.0 g) dissolved in a mixture of *n*-hexane and benzene (3: 1) was filtered through 240 g of alumina (Merck, grade III). From the eluates with *n*-hexane–benzene (3: 1 and 1: 1), crystalline material (5.23 g) was obtained. Recrystallization from MeOH gave IIa as fine needles, mp 196—197°. *Anal.* Calcd. for $C_{21}H_{34}O$ (302.48): C, 83.38; H, 11.33. Found: C, 83.25; H, 11.34. IR ν_{max}^{KBr} cm^{-1}: 3200—3050 (OH). MS, *m/e*: 302 (M^+), 287 (M^+-15), 269 [$M^+-33(H_2O+CH_3)$]. NMR ($CDCl_3$) δ: 4.98 (1H, multiplet, C_{20}–H), 4.03 (1H, broad singlet, 3β-H), 2.33—2.10 (2H, multiplet, 16-CH_2), 1.63 (3H, doublet, J=7.0 Hz, 21-CH_3), 0.96 (3H, singlet, 19-CH_3), 0.77 (3H, singlet, 18-CH_3).

The crystalline powder obtained from the mother liquor was shown to contain an isomeric material by NMR. The mixture was then submitted to preparative thin-layer chromatography using SiO_2 impregnated with 10% $AgNO_3$ (developed 5 times with the solvent system: cyclohexane–acetone, 20: 1). The isomeric mterial was obtained (yield: *ca.* 5%) from the upper zone of the two separated bands. Recrystallization from MeOH gave IIb as fine needles, mp 190°. *Anal.* Calcd. for $C_{21}H_{34}O$ (302.48): C, 83.38; H, 11.33. Found: C, 83.62; H, 11.50. IR ν_{max}^{KBr} cm^{-1}: 3200—3050 (OH). MS, *m/e*: 302 (M^+), 287, 269. NMR ($CDCl_3$) δ: 5.04 (1H, multiplet, C_{20}–H), 4.06 (1H, multiplet, 3β-H), 2.38—2.10 (2H, multiplet, 16-CH_2), 1.54 (3H, doublet, J=7.0 Hz, 21-CH_3), 0.78 (3H, singlet, 19-CH_3), 0.72 (3H, singlet, 18-CH_3).

Epoxidation of IIa——To a $CHCl_3$ solution (200 ml) of IIa (1.13 g) was added *m*-chloroperbenzoic acid (850 mg). After allowing to stand at room temperature for 3 hr, the mixture was washed with 10% Na_2SO_3, then with 5% $NaHCO_3$, and finally with water and dried over anhydrous Na_2SO_4. Recrystallization of crude material (1.09 g) from a mixture of *n*-hexane and ether gave III as colorless fine needles, mp 159—161°. *Anal.* Calcd. for $C_{21}H_{34}O_2$ (318.48): C, 79.19; H, 10.76. Found: C, 79.08; H, 10.64. IR ν_{max}^{KBr} cm^{-1}: 3250 (OH). MS, *m/e*: 318 (M^+), 303, 300, 285, 274. NMR ($CDCl_3$) δ: 4.01 (1H, broad singlet, 3β-H), 2.97 (1H, quartet, J=6.0 Hz, C_{20}–H), 1.33 (3H, doublet, J=6.0 Hz, 21-CH_3), 0.87 (3H, singlet, 19-CH_3), 0.76 (3H, singlet, 18-CH_3).

Reduction of Epoxide (III)——(i) With $LiAlH_4$: The ether solution (200 ml) containing 526 mg of III and $LiAlH_4$ (730 mg) was refluxed for 3 hr. After cooling, to the reaction mixture was added EtOAc to decompose an excess of reagent. The solution was washed with water and dried over anhydrous Na_2SO_4. Crude material (530 mg) obtained was recrystallized from a mixture of *n*-hexane and ether to give IVa as fine needles, mp 197—197.5°. *Anal.* Calcd. for $C_{21}H_{36}O_2$ (320.48): C, 78.69; H, 11.32. Found: C, 78.49; H, 11.21. MS, *m/e*: 320 (M^+), 302, 291. NMR ($CDCl_3$) δ: 4.03 (1H, broad singlet, 3β-H), 0.96 (3H, triplet, J=7.0 Hz, 21-CH_3), 0.80 (3H, singlet, 19-CH_3), 0.69 (3H, singlet, 18-CH_3).

(ii) With $LiAlD_4$: By the same procedure as described above, 201 mg of corresponding product (IVb) was obtained from III by using deuteride reagent, mp 197° (EtOAc). *Anal.* Calcd. for $C_{21}H_{35}DO_2$ (321.48): C, 78.45; H, 11.60. Found: C, 78.43; H, 11.56 (calcd. as D=2×H). MS, *m/e*: 321 (M^+), 303, 291. NMR ($CDCl_3$) δ: 4.03 (1H, broad singlet, 3β-H), 0.96 (3H, doublet, J=7.0 Hz, 21-CH_3), 0.81 (3H, singlet, 18-CH_3), 0.70 (3H, singlet, 19-CH_3).

Acid Treatment of IVa and IVb——(i) The compound (IVa, 150 mg) was dissolved in 25 ml of MeOH containing 1 ml of 1 N HCl, and the solution was warmed at 50° for 3 hr. The solvent was evaporated to give an oil which was dissolved in ether. The ether solution was washed with water and dried over anhydrous Na_2SO_4. Crude material (130 mg) obtained was recrystallized from ether to afford Va as fine needles, mp

8) H. Hirschmann and J.S. Williams, *J. Biol. Chem.*, **238**, 2305 (1963).
9) H. Hirschmann, F.B. Hirschmann, and A.P. Zala, *J. Org. Chem.*, **31**, 375 (1966).

121—121.5°. *Anal.* Calcd. for $C_{21}H_{34}O$ (302.48): C, 83.38; H, 11.33. Found: C, 83.36; H, 11.28. MS, *m/e*: 302 (M^+), 284, 273. IR ν_{max}^{KBr} cm^{-1}: 3600—3400 (OH). NMR ($CDCl_3$) δ: 4.01 (1H, broad singlet, 3β-H), 1.25 (2H, quartet, J=7.0 Hz, 20-CH_2), 0.95 (3H, singlet, 17β-CH_3), 0.79 (3/2 H, doublet, J=7.0 Hz, 21-CH_3 coupled with 20β-H), 0.77 (3H, singlet, 19-CH_3), 0.74 (3/2 H, doublet, J=7.0 Hz, 21-CH_3 coupled with 20α-H).

(ii) By the same procedure as in (i), 36 mg of Vb was obtained from 40 mg of IVb, mp 121° (ether). MS, *m/e*: 303 (M^+), 273. NMR ($CDCl_3$) δ: 4.02 (1H, broad singlet, 3β-H), 0.94 (3H, singlet, 17β-CH_3), 0.76 (3H, singlet, 19-CH_3), 0.74 (1H, doublet, J=7.0 Hz, 21-CH_3).

3α,20α-Disulpho-oxy-5α-pregnane (VII)——To the pyridine solution (40 ml) of VI (160 mg) was added SO_3-pyridine (850 mg), and the mixture was stirred at room temperature for 2 days. Pyridine was removed under reduced pressure at 45° to give a residue, which was dissolved in 2% K_2CO_3 (50 ml). The solution was extracted with ether to remove an unreacted substrate, then with *n*-BuOH. The latter extracts were combined and the solvent was removed under reduced pressure at 45° to give a white powder (185 mg), which was recrystallized from MeOH, mp 167—169.5°. *Anal.* Calcd. for $C_{21}H_{34}K_2O_8S_2$ (524.74): C, 48.06; H, 6.53; S, 6.11. Found: C, 47.80; H, 6.47; S, 5.84.

Hot Acid Hydrolysis of VII——To a refluxing aqueous solution (120 ml) of VII (160 mg) was added a warmed 6 N HCl (120 ml), and the mixture was refluxed for 20 min. After cooling, the mixture was extracted with ether. The combined extract was washed with water, then dried over Na_2SO_4. Filtration of Na_2SO_4 and removal of ether gave a syrup (104 mg), which was then submitted to preparative thin-layer chromatography (solvent system: cyclohexane–acetone, 20: 1). The corresponding band was shown to be a mixture by NMR. The mixture was again submitted to $AgNO_3$ impregnated preparative thin-layer chromatography as described. Two different materials were obtained. From the upper band of the two separated bands, crystalline material (46 mg) was obtained, mp 121° (ether). *Anal.* Calcd. for $C_{21}H_{34}O$ (302.48): C, 83.38; H, 11.33. Found: C, 83.29; H, 11.40. IR ν_{max}^{KBr} cm^{-1}: 3600—3400 (OH). MS, *m/e*: 302 (M^+), 284, 273. NMR ($CDCl_3$) δ: 4.01 (1H, broad singlet, 3β-H), 1.25 (2H, quartet, J=7.0 Hz, 20-CH_2. Each four signal is further splitted to doublet when the scale is expanded ×4), 0.95 (3H, singlet, 17β-CH_3), 0.76 (3H, singlet, 19-CH_3), 0.79 (3/2 H, doublet, J=7.0 Hz, 21-CH_3 coupled with 20β-H), 0.74 (3/2 H, doublet, J=7.0 Hz, 21-CH_3 coupled with 20α-H). This material was identified as Va by mixed melting point determination. From the lower band of the two separated bands, another compound (20 mg) was obtained as colorless fine needles, mp 189° (MeOH), which was completely identical with IIb.

Beside these artifacts, intact aglycone (VI) was also obtained (14 mg).