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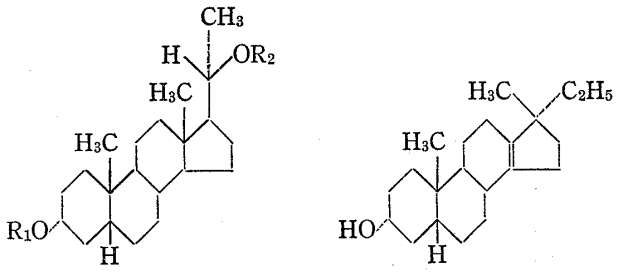
The Rearranged Product of Pregnanediol 20-Sulfate by Hot Acid Hydrolysis

In acid catalized hydrolysis of steroid sulfate the cleavage occurs generally at oxygen-sulfur bond resulting to give a steroidal alcohol, whose configuration is retained. Abnormal carbon-oxygen bond rupture, however, happens in some case when hydrolysis is carried out in drastic condition such as in boiling acid. On such hydrolysis various artifacts such as steroidal olefins which were formed by elimination of sulfuric acid, and epimeric and/or isomeric alcohols which were derived by consequent hydration to carbonium ion(s) formed are obtained. For example, androsterone sulfate gave a large amount of Δ^2-olefin accompanied with minor alcohols, by heating in 1N hydrochloric acid.[1]



Ia : R_1=H, R_2=SO_3H
Ib : R_1=SO_3H, R_2=SO_3H
Ic : R_1=SO_3H, R_2=H

Chart 1

In the present study, the authors describe that pregnanediol 20-sulfate (Ia) was almost quantitatively rearranged to olefin (II) by hot acid hydrolysis.

5β-Pregnane-3α,20α-diol 20-sulfate (Ia) obtained by the same method as described by Rajagopalan, *et al.*[2] was refluxed in 4N hydrochloric acid (100 mg sulfate per 50 ml acid) for 30 min and then extracted with ether. The residue of the extract was subjected to preparative thin-layer chromatography (ethyl acetate: cyclohexane=1: 3) to give a main product (yield; 93%), mp 200—202°, pregnanediol (4%), and other minor products (3%). This principal product was also obtained from pregnanediol 3,20-disulfate (Ib)[2] in the yield of 89%, but not from pregnanediol 3-sulfate (Ic),[2] from which only an intact aglycone was obtained by the same condition. The structure of this major hydrolysate was assigned as 17α-ethyl-17β-methyl-18-nor-5β-androst-13-en-3α-ol (II) by the following examinations.

This rearranged product has an empirical formula $C_{21}H_{34}O$ (mol. weight; 302.48) which was supported by the elemental analysis (Calcd: C, 83.38; H, 11.23. Found: C, 83.01; H, 11.56) and mass spectrum (M^+, *m/e* 302). Although there was no indication of the presence of vinyl proton(s) in the nuclear magnetic resonance (NMR) spectrum of II, this compound gave an yellow color with tetranitromethane, supporting a tetra-substituted double bond. The NMR signals at δ 3.62 (septet, J_1=J_1'=10 cps, J_2=J_2'=5 cps, 1H) are attributable to the 3β-axial proton and two singlet signals at δ 0.93 (3H) and 0.90 (3H) are assigned as methyl groups at C-17 and C-10, respectively. Signals at δ 0.79 (triplet, J=7 cps, 3H) are attributed to the methyl protons of 17α-ethyl group. Structure of II was further substantiated by mass spectral analysis. The spectrum exhibited the molecular ion peak at *m/e* 302, a peak at *m/e* 287 (M-CH_3), an intense fragment at *m/e* 273 due to the loss of 29 mass unit (C_2H_5), and a fairly intense fragment peak at *m/e* 255 derived from the loss of water and ethyl group. These NMR and mass spectra of II are in quite agreement with those of similar rearranged olefins formed by the solvolysis of 5α-pregnan-20α-ol 20-tosylate.[3]

The mechanism of the formation of II from Ia and Ib is suggested as follows: protonation occurred initially on the alcoholic oxygen of C-20 followed by removal of sulfuric acid with

1) J. Ramseyer, J.S. Williams, and H. Hirschmann, *Steroids*, **9**, 347 (1967).
2) M.S. Rajagopalan and A.B. Turner, *J. Chem. Soc.* (*C*), **1969**, 1858; *idem*, *ibid.*, **1970**, 2266.
3) M. Leboeuf, A. Cave, and R. Gountarel, *Bull. Soc. Chim. Fr.*, **1969**, 1624, 1628.

simultaneous hydride shift from C-17α to C-20, to which 18-methyl group migrates by 1,2-shift, and finally loss of a proton at C-14. The present result is in contrast with the previous report by Hirschmann, *et al.*[4] in that 5α-pregnane-3β,20β-diol 20-sulfate was converted by hot acid hydrolysis to uranediol: 17α-methyl-D-homo-5α-androstane-3β,17aβ-diol. It may be concluded, therefore, that 20α-sulfate of pregnane derivatives are converted in boiling acid to rearranged olefin as shown in the present study and that 20β-sulfate is, on the other hand, envisaged to D-homoannulation resulting to give uranediol-type alcohol.

Because the present hydrolysis condition is widely used in clinical laboratories by reasons of its convenience and its speedy procedure especially for the determination of 17-keto steroids, it was predicted as a matter of course that II should be contained in the urinary hydrolysate obtained under this condition. So urines were collected from healthy women and each 5—10 ml was tested. Urine was incubated with β-glucuronidase followed by extraction with ether to give an enzyme hydrolysate. The aqueous phase was submitted to the above hydrolysis followed by extraction with ether to give an acid hydrolysate. Gas–liquid chromatography of these two hydrolysates was then carried out (1.5% OV-17 on chromosorb-W, temp., programed from 200° to 250°).

In the glucuronide fraction, pregnanediol was observed in the chromatogram, but II was not observed. In the sulfate fraction, the peaks at the retention times 10.6, 11.6, 13.2, 15.0, 19.0, 20.0, and 23.8 min were identified as being derived from pregnanediol 3- or 3,20-sulfate by comparison with authentic hydrolysates of Ia and Ib. The first peak at 10.6 min corresponding to II occupied over 90% of the sum of these peak areas and the last one at 23.8 min corresponding to pregnanediol was less than 3%.

The present result is well agreeable to the early report by Okamura, *et al.*[5] who reported that a substantial portion of urinary pregnanediol was excreted as the sulfate and is suggestive of introducing the precise and convenient method of analysis of urinary pregnanediol and various metabolites of 20-hydroxylated progestogens.

The nature of the minor products obtained from Ia and Ib are now under investigation and a new analytical method of urinary pregnanediol will be reported in the near future.

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4) H. Hirschmann and J.S. Williams, *J. Biol. Chem.*, **238**, 2305 (1963).
5) Y. Okamura and H. Tateyama, *Folia Endocrinol.*, **41**, 137 (1965).