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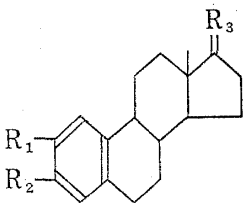
Occurrence of "NIH Shift" during Hydroxylation of Aromatic Steroid

It has recently been discovered that during enzymatic hydroxylation of aromatic substrates the substituent (^{2}H, ^{3}H, Cl, Br, *etc.*) displaced by the entering hydroxyl group migrates to an adjacent position. These phenomena called "NIH shift" have been demonstrated with enzymes derived from animal and plant sources.[1,2] We have previously reported that hydroxylation does take place at C-2 and C-3 when 3-deoxyestrone (I) is orally given to rabbit.[3] Therefore it seemed to be of considerable interest to us to elucidate whether the aromatic steroid would similarly undergo "NIH shift" or not. We now wish to report the *in vivo* hydroxylation of the specifically deuterated 3-deoxyestrone.



I : $R_1=R_2=H$, $R_3=O$
II : $R_1=OH$, $R_2=H$, $R_3=\overset{OH}{H}$
III : R_1=1-phenyl-5-tetrazolyloxy-, $R_2=H$, $R_3=\overset{OH}{H}$
IV : $R_1=D$, $R_2=H$, $R_3=O$
V : $R_1=H$, $R_2=OH$, $R_3=O$
VI : $R_1=H$, R_2=1-phenyl-5-tetrazolyloxy-, $R_3=O$
VII : $R_1=H$, $R_2=D$, $R_3=O$

First, 2-deuteriosteroid was prepared as a substrate from 2-hydroxy-3-deoxyestradiol (II) in three steps. Condensation of II with 1-phenyl-5-chlorotetrazole in the presence of potassium carbonate[4] gave the 2-(1-phenyl-5-tetrazolyl) ether (III), mp 140—141°, as colorless needles (from aq. acetone). Catalytic reduction over palladium–on–barium carbonate under a stream of deuterium gas followed by oxidation with Jones reagent furnished the desired 2-deuterio-3-deoxyestrone (IV), mp 141—142°, as colorless needles (from ether). Likewise 3-deuterio-3-deoxyestrone (VII) was also synthesized starting from estrone (V) by way of 3-(1-phenyl-5-tetrazolyl) ether (VI), mp 204—206° (from aq. acetone). The distribution and quantity of the isotope in these selectively labelled steroids were determined by means of nuclear magnetic resonance and mass spectrometries.

A single dose of suspension of 2-deuterio-3-deoxyestrone (IV) (475 mg) in Tween 80 was orally given to a male rabbit weighing about 2.3 kg. The urine was collected for the following

1) G. Guroff, C. Reifsnyder, and J.W. Daly, *Biochem. Biophys. Res. Commun.*, **24**, 720 (1966); G. Guroff, M. Levitl, J. W. Daly, and S. Udenfriend, *ibid.*, **25**, 253 (1966); J. Renson, J. W. Daly, H. Weissbach, B. Witkop, and S. Udenfriend, *ibid.*, **25**, 504 (1966); G. Guroff, K. Kondo, and J. W. Daly, *ibid.*, **25**, 622 (1966); S. Udenfriend, P. Zaltzman–Nierenberg, J. W. Daly, C. Chidsey, and B. Witkop, *Arch. Biochem. Biophys.*, **120**, 413 (1967); J. W. Daly, D. Jerina, and B. Witkop, *ibid.*, **128**, 517 (1968).
2) D.W. Russell, E.E. Conn, A. Sutter, and G. Grisebach, *Biochim. Biophys. Acta*, **170**, 210 (1968).
3) *a*) T. Nambara and M. Numazawa, *Chem. Pharm. Bull.* (Tokyo), **16**, 383 (1968); *b*) T. Nambara, M. Numazawa, and H. Takahashi, *ibid.*, **16**, 1148 (1968); *c*) T. Nambara and M. Numazawa, *ibid.*, **17**, 1200 (1969).
4) W.J. Musliner and J.W. Gates, Jr., *J. Am. Chem. Soc.*, **88**, 4271 (1966).

72 hr, and hydrolysis of the glucuronides was processed with beef–liver β-glucuronidase. The hydrolyzate was extracted with ethyl acetate, which in turn was subjected to solvolysis. Separation and purification of the metabolites were carried out in the manner as described in the preceding paper.[3c] Thus two phenolic metabolites, 2-hydroxy-3-deoxyestrone and 17α-estradiol, were isolated in the crystalline state.

Measurement of mass spectrum revealed 47% retention of the labelled deuterium with 2-hydroxy-3-deoxyestrone. Inspection of the nuclear magnetic resonance spectrum of the aromatic proton region[5] was indicative of the localization of deuterium at C-3 but not at C-1 (see Fig. 1). These results evidently tell us that the isotope migrates toward C-3 during hydroxylation at C-2. As for another phenolic metabolite, that is 17α-estradiol, deuterium was found to be retained at the initially labelled position with the extent of 81%.

These results prompted us further to examine the metabolic fate of 3-deuterio-3-deoxyestrone(VII). Administration of the labelled steroid followed by isolation of the urinary metabolites was performed in the manner as mentioned above. As might be expected the intramolecular shift of the labelled deuterium did actually take place with 17α-estradiol, in which the isotope retentions at C-2 and C-4 were found to be *ca.* 50 and 20%, respectively. As for the remaining metabolite, 2-hydroxy-3-deoxyestrone, C-2 hydroxylation exerted no significant influence on the labelled position resulting in 81% deuterium retention. This result seems to be incompatible with the previous finding involving the 1,2-shift of deuterium from C-2 to C-3. However, the apparent discrepancy may be explained in terms of the isotope effect, with which deuterium-carbon bond would be more stable than that of hydrogen against breaking in the stage of metabolic intermediate.

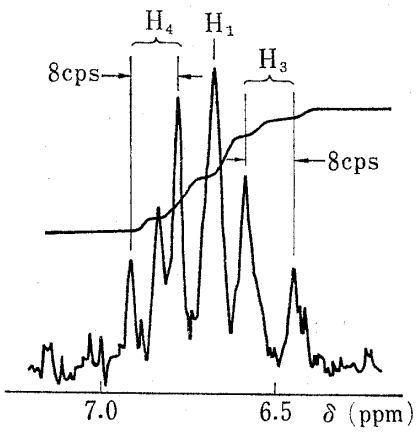


Fig. 1. NMR Spectrum of Labelled 2-Hydroxy-3-deoxyestrone (Metabolite of 2-Deuterio-3-deoxyestrone) in DMSO

It is thus concluded that during the enzyme–catalyzed hydroxylation the labelled isotope of 2- and 3-deuterio-3-deoxyestrones transfers to the position next to the entering oxygen. So far as we learn this apears to be the first example of "NIH shift" in the field of steroids. The present finding is also of interest in suggesting the close similarity in hydroxylation mechanism between aromatic steroids and drugs. In addition it is to be noted that the labelled deuterium migrates preferentially to C-3 or C-2 rather than to C-1 or C-4 implying the participation of the arene oxide as a probable metabolic intermediate.[6] It is hoped that the present observations may provide a clue to clarify the hydroxylation mechanism of the aromatic steroid. Further studies on these problems are being conducted in this laboratory and the details will be reported in near future.

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6) D.M. Jerina, J.W. Daly, and B. Witkop, *J. Am. Chem. Soc.*, **90**, 6523 (1968); D.M. Jerina, J.W. Daly, B. Witkop, P. Zaltzman–Nierenberg, and S. Udenfriend; *ibid.*, **90**, 6525 (1968).