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Glucosiduronation of 2-Hydroxyestrone with Guinea Pig Liver Homogenate

The major metabolite of estradiol in man is catechol estrogen 2-hydroxyestrone (I) which has two "indistinguishable" phenolic groups.[1] This catechol estrogen is further transformed in man to 2-methoxyestrone[2] by specific O-methylation just observed in the case of important catecholamines[3] in which the O-methylation leads nearly exclusively to the *meta*-methyl ether. In sharp contrast with these results in the living animal, *in vitro* O-methylation of catechols by the liver O-methyltransferase enzyme is relatively indiscriminate and yields mixtures of monomethyl derivatives, often in approximately equivalent amounts.[1,4] This divergence between the results *in vitro* and those in the living animal is very interesting problem.

Although 2-hydroxyestrone (I) is excreted into urine as glucosiduronate, the point of attachment of conjugating group to the steroidal aglycone is different by species; namely at the 3-position in human being[5] and at the 2-position in the rat as well as in the hamster.[6] Therefore, it is important to establish which phenolic group of catechol estrogen is conjugated *in vitro* by UDPGA transferase of guinea pig. This report represents evidence that the conjugation mechanism is also specific. 2-Hydroxyestrone is exclusively conjugated at the C-2 phenolic group by UDPGA transferase of guinea pig liver homogenate.

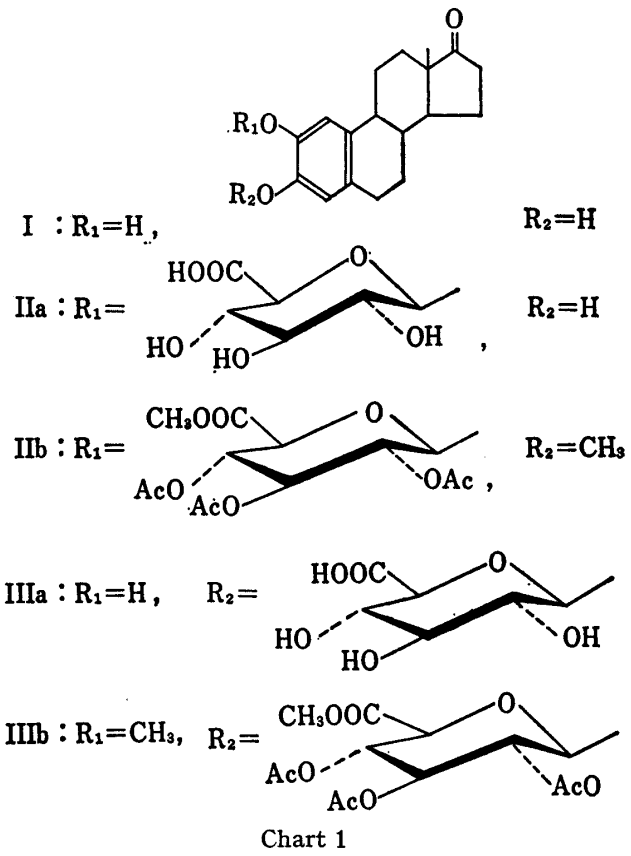

Chart 1

2-Hydroxyestrone-6,7-^{3}H was obtained by the enzymatic hydroxylation of estrone-6,7-^{3}H with mushroom tyrosinase (MILES) described by Jellinck, *et al.*[7] Aliquot of 2-hydroxyestrone-6,7-^{3}H was incubated with guinea pig liver homogenagete in phosphate buffer in the presence of UDPGA (triammonium salt, SIGMA) at 37°. In each experiment, 30 μg of substrate (8.40×10^5 cpm) was incubated in 5 ml of 4% homogenate in phosphate buffer (0.05M, pH 7.4) and the molar ratio of UDPGA to substrate was seven. The reaction was

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2) J. Fishman, R.I. Cox, and T.F. Gallagher, *Arch. Biochem. Biophys.*, **90**, 318 (1960).
3) J. Axelrod, "Transmethylation and Methionine Biosynthesis," ed. by S.K. Shapiro and F. Schlenck, Univ. Chicago Press, 1965, pp 71—84.
4) R. Knuppen and H. Breuer, *Z. Physiol. Chem.*, **346**, 114 (1966).
5) I. Yoshizawa and J. Fishman, *J. Clin. Endocr. Metab.*, **29**, 1123 (1969).
6) K.I.H. Williams, *Steroids*, **15**, 105 (1970).
7) P.H. Jellinck and B.J. Brown, *Steroids*, *17*, 133 (1971).

stopped by adding ethanol to incubation media and followed by an addition of 2-hydroxyestrone and its two isomeric conjugates; 3-hydroxy-17-oxo-estra-1,3,5(10)-trien-2-yl-β-D-glucopyranosiduronic acid (IIa) and 2-hydroxy-17-oxo-estra-1,3,5(10)-trien-3-yl-β-D-glucopyranosiduronic acid (IIIa). The synthesis[8] of these conjugates made present studies possible. Supernatant fraction separated at 6000 rpm was then extracted with ethyl ether to remove unreacted substrate diluted with carrier. Ether extracted fraction was acetylated and recrystallized to constant specific activity. The radioactivity of free steroid fraction was less than 10% of the substrate incubated for 30 min.

The aqueous layer was then treated with Amberlite XAD-2 resin as described by Bradlow[9] to give a conjugate fraction. The radioactivity of this fraction was about 80% or over of the substrate incubated for 30 min. To investigate this aqueous layer as glucosiduronate of 2-hydroxyestrone, half of the conjugate fraction was hydrolyzed with β-glucuronidase (SIGMA). Acetylation of this hydrolyzate and recrystallization of the acetate to constant specific activity were carried out and over 97% of radioactivity of conjugate fraction was retained in 2-hydroxyestrone diacetate.

Another half of the aqueous layer was evaporated under reduced pressure at 40° to give a residue which was treated with diazomethane in methanol and then acetylated. The mixture of these isomeric acetylated methyl glucuronides, (IIb) and (IIIb), were separated by preparative thin layer chromatography on silica gel by multiple running in 2% acetone in benzene. Two isomers separated were then recrystallized to constant specific activity. The radioactivity of conjugate fraction was contained only in 2-hydroxyestrone-2-glucuronide derivative (IIb) over 98% and negligible in another isomer (IIIb).

The results of these studies suggest that the UDPGA transferase of guinea pig liver homogenate is highly specific for C-2 phenolic function with catechol steroid substrate.

Further studies on a detailed enzymatical works and also on another conjugate species, sulfation of catechol estrogens, are now under active investigation.

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9) H.L. Bradlow, *Steroids*, **11**, 265 (1968).