Journal of Steroid Biochemistry, 1972, Vol. 3, pp. 43–44. Pergamon Press. Printed in Great Britain

SHORT COMMUNICATION

CONJUGATION OF 2-HYDROXYOESTRONE IN RAT BILE

HISAKO WATANABE, PETER TOFT and J. ALLAN MENZIES
Food and Drug Research Laboratories, Department of National Health and Welfare, Ottawa, Ontario K1A OL2, Canada

(*Received* 17 *August* 1971)

SUMMARY

2-Hydroxyoestrone is excreted in rat bile conjugated with "glucuronic acid" exclusively at C-2.

INTRODUCTION

2-HYDROXYOESTRONE "glucuronide" is a major urinary[1] and biliary[2] oestrogen metabolite in the rat. The conjugation with "glucuronic acid" occurs exclusively at the C-2 position in rat urine[3] whereas in humans[4] it occurs at the C-3 position.

A comparison of urinary and biliary oestrogens in the rat reveals marked differences in the metabolic products. Whereas in bile, besides 2-hydroxyoestrone "glucuronide", oestrone, 2-methoxyoestrone, oestradiol-17β, 2-hydroxyoestradiol and 2-methoxyoestradiol "glucuronides" have been found[2], none of the latter metabolites were detected in the urine[1]. It was therefore of interest to investigate the possibility of a dissimilarity in conjugation of the catechol oestrogen in the two biological fluids.

One μCi of carrier-free[^{3}H]-estradiol-17β (40 Ci/mM) was administered subcutaneously to adult female Wistar rats in 10% ethanolic saline, and bile samples were collected as previously described[2]. The bile was made 50% with respect to ethanol, and the supernatant obtained following centrifugation was processed in a manner essentially similar to that reported by others[3, 4].

An aliquot of the bile sample was methylated with methyl iodide and subsequently hydrolyzed with acid in the presence of 15 mg each of 2-hydroxyoestrone 2-methyl ether and 2-hydroxyoestrone 3-methyl ether. We have inferred from previous studies of hydrolysis by Ketodase and inhibition by saccharolactone that, in bile, the oestrogen metabolites are excreted mainly as "glucuronide" conjugates[2]. The aglycones obtained following hydrolysis were chromatographed on a thick layer of silica gel H by multiple runs in chloroform. The mono-methyl ethers were eluted from the plate and crystallized to constant specific activity (Table 1).

Since the rat excretes 2-methoxyoestrone "glucuronide" in bile[2], it was necessary to determine the quantities of this metabolite prior to methylation. An aliquot of the bile sample was therefore hydrolyzed with Ketodase (Warner–Chilcott) in the presence of the two mono-methyl ether carriers and the aglycones analyzed by thin-layer chromatography as before.

The results shown in Table 2 indicate that there was no net increase in 2-

Table 1. Crystallization of mono-methyl ethers of 2-hydroxyoestrone

Crystallizations	2-MeOE$_1$* (d.p.m./mg)	3-MeOE$_1$† (d.p.m./mg)
1	1902	2681
2	1772	2473
3	1922	2212
4	1762	2120
5	1778	2104
6	1810	2142

*2-methoxyoestrone.
†2-hydroxyoestrone 3-methyl ether.

Table 2. Quantitation of mono-methyl ethers of 2-hydroxyoestrone

	% of total radioactivity excreted	
	2-MeOE$_1$*	3-MeOE$_1$†
Unmethylated	28·9	0
Methylated	25·2	30·5

*2-methoxyoestrone.
†2-hydroxyoestrone 3-methyl ether.

methoxyoestrone in the methylated sample. Whereas the 3-methyl ether was not detectable in the control sample, upon methylation of the bile this compound was produced to the extent of 30·5% of the excreted dose. These results therefore demonstrate that, as in rat urine and in contrast to human urine, the catechol oestrogen in rat bile is conjugated with "glucuronic acid" exclusively in the 2 position.

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