5α-PREGNANE-3α, 6α, 20α-TRIOL, A METABOLITE OF PROGESTERONE IN THE RABBIT

B. A. Knights, A. W. Rogers and G. H. Thomas
Department of Anatomy, University of Birmingham, England.

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When the conjugated metabolites from the urine of rabbits injected with labelled progesterone, were hydrolysed and fractionated as previously described (Thomas, 1962), it was found that a significant proportion of the radioactivity was extracted from light petroleum-benzene (1:1) into water. The water extractable metabolites are referred to henceforth as the water fraction. Table I gives the percentage of the radioactivity recovered in this fraction for male and female rabbits. In the experiment where a mixture of 4-C^{14} and 16α-H^3-progesterone was injected, the H^3/C^{14} ratio for the water fraction was very close to that of the injected material. A similar agreement has been found for the H^3/C^{14} ratios in the acid and neutral fractions in this experiment (Rogers and Thomas, 1962). It must be concluded that 16α-hydroxylation is quantitatively not an important catabolic pathway for progesterone in the rabbit.

Evidence for 6-hydroxylated metabolites followed from examination of the oxidation products of the water fraction. The main oxidation product had the same chromatographic mobility as 5α- and 5β-pregnane-3,6,20-trione* in the systems A, B3 and B4 of Bush (1952) and had the properties expected for a 3,6-diketone, i.e. the substance did not adsorb in the UV, but

*5α-Compound supplied by L. Lights & Co. Ltd. (Colnbrook, England); 5β-isomer obtained by oxidation of 3α,6α-dihydroxy-5β-pregnan-20-one (Canada Packers Ltd., Toronto).

Table I Water extractable metabolites from the urine of rabbits injected subcutaneously with labelled progesterone.

Injected material[a]			% Radioactivity in water fraction.[b]	
Female rabbits.				
50 mg.	H^3-Progesterone		25.9	
380 μg.	4-C^{14}-Progesterone		10.8	
210 μg.	H^3-Progesterone[c]	(5.86)	7.8	(5.00)
	4-C^{14}-Progesterone		6.0	
85 μg.	16α-H^3-Progesterone[c]	(3.96)	7.1	(3.33)
	4-C^{14}-Progesterone		8.0	
462 μg.	4-C^{14}-Progesterone		6.9	
Male rabbits.				
50 mg.	H^3-Progesterone		6.9	
380 μg.	H^3-Progesterone		13.2	

a) H^3-Progesterone refers to generally labelled material (The Radiochemical Centre, Amersham, England). b) Results are expressed as a percentage of the total radioactivity recovered in the conjugated fraction. c) Values in brackets give H^3/C^{14} ratios for injected material and water fraction.

gave a sodium hydroxide-fluorescence reaction typical of a Δ^4-3-ketone (see Neher, 1959).

Alumina chromatography of the non-ketonic, digitonin non-precipitable (α) products of the pooled water fractions yielded 5α-pregnane-3α,6α, 20α-triol, m.p. 219-221°, $(\alpha)_D$ + 36.8° (c 0.47 in $CHCL_3$) (Found: C, 74.58; H, 10.7. $C_{21}H_{36}O_3$ requires C, 74.95; H, 10.8%). The infra-red spectrum in Nujul showed OH bands at 3325, 1041 and 1009 cm^{-1}. The Rf values in the systems B3, B4 and C of Bush (1952) were 0.05, 0.26 and 0.49 respectively, and in the EB2 system of Eberlein and Bongiovani (1955), 0.73. Two methods

were used for detecting the triol on paper chromatograms: (i) The paper was treated with chromium trioxide-acetic acid; the oxidized triol could then be detected by the sodium hydroxide-fluorescence reaction (see above). (ii) The compound exhibited a yellow fluorescence under UV light after treatment of the paper with $SbCl_3$ -$CHCl_3$. Using these methods 5α-pregnane-3α,6α, 20α-triol has been detected in the urine of both male and female rabbits injected with progesterone (dose level 50 mg.).

The proposed structure for the triol was consistent with the following observations:

1) Oxidation gave 5α-pregnane-3,6,20-trione.

2) Non-precipitation with digitonin was indicative of a 3α-hydroxyl group.

3) The observed molecular rotation (+124) was in good agreement with the value (+127) calculated from the data of Klyne (1957). Further, the molecular rotation difference between a 6α- and 6β -hydroxyl in the 5α-series is sufficiently large (-105) to exclude the possibility of the triol having a 6β -hydroxyl group.

4) The rabbit excretes 20α- in preference to 20β -hydroxy metabolites. Thus, both 20β -hydroxypregn-4-en3-one and 5β -pregnane-3α, 20β -diol undergo oxidation-reduction in vivo to give 5β -pregnane-3α,20α-diol (Knights and Thomas, 1962).

Synthesis

3α,6α-Dihydroxy-5β -pregnan-20-one was converted into the 20-ethylene ketal which was then oxidized to give 5β -pregnane-3,6,20-trione 20-ethylene ketal. Selective reduction with sodium borohydride in pyridine yielded 3α-hydroxy-5β -pregnane-6,20-dione 20-ethylene ketal. Inversion

of configuration at C-5 was effected by treatment with alkali, giving 3α-hydroxy-5α-pregnane-6,20-dione 20-ethylene ketal. Reduction with lithium in liquid ammonia-methanol, followed by hydrolysis of the ketal group, gave 3α,6α-dihydroxy-5α-pregnan-20-one. This compound and its 5β-epimer were treated separately with reducing agents differing in their stereospecificity towards reduction of 20-ketones. The resultant mixtures were then analysed by gas chromatography using the fluoralkyl silicone polymer QF-1-0065 as the stationary phase. The relative proportions of the C-20 epimers formed in these reductions are given in Table II. The structural assignments for the C-20 alcohols thus could be made with assurance based on the known stereospecificity of the reducing agents used. The assigned configurations also conformed to the general rule that, for substances differing only in their configuration at C-20, the 20β-ol is more mobile than its 20α-epimer on QF-1-0065 (Knights and Thomas, unpublished work). The relative retention times for the reduction products, their triacetates and tripropionates are given in Table III. Also included in the table are the relative retention times for the triol metabolite and its acyl derivatives, and it can be seen that their mobilities are consistent with the proposed structure, 5α-pregnane-3α,6α,20α-triol.

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Table II Proportions of 20α- and 20β-ols formed on reduction of the 5α- and 5β-epimers of 3α,6α-dihydroxypregnan-20-one.

Reducing agent	Reduction products [a]			
	5α-series		5β-series	
	20β-ol	20α-ol	20β-ol	20α-ol
Sodium borohydride/methanol	89	11	86	14
Lithium aluminium hydride/ether	77	23	82	18
Sodium/methanol	41	59	33	67

a) Calculated from the peak areas in gas chromatograms of the propionylated reduction products. Results are expressed as percentages. Chromatographic conditions are given in Table III.

Table III Comparison of the chromatographic mobilities of the progesterone metabolite and the reduction products of 3α,6α-dihydroxy-5α-pregnan-20-one and its 5β-epimer.

	Relative retention times*		
	Triol	triacetate	tripropionate
Triol metabolite.			
5α-pregnane-3α,6α,20α-triol	5.4	13.1	19.0
Reduction products.			
5α pregnane-3α,6α,20α-triol	5.4	12.9	19.1
5α-pregnane-3α,6α,20β-triol	4.9	12.2	17.2
5β-pregnane-3α,6α,20α-triol	6.0	11.5	16.6
5β-pregnane-3α,6α,20β-triol	5.3	11.3	14.6

* Chromatographed on a Pye argon chromatograph with Sr^{90} ionization detector. Column conditions: 6% QF-1-0065 on acid washed celite 545(100-120 mesh) at 250°; inlet pressure 21 p.s.i. argon, flow rate 55 ml/min.; column length, 3'3". Retention times relative to cholestane (4.2 min.).

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