THE STRUCTURE OF A URINARY METABOLITE OF PROSTAGLANDIN E_2 IN THE GUINEA PIG

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The excretory products of a number of prostaglandins in the rat have been characterized. Thus, one of the urinary metabolites of 5,6-$^{3}H_2$-$PGF_{1\alpha}$ [1] was identified as dinor-$PGF_{1\alpha}$ (Granström et al. 1965), whereas a urinary metabolite formed from 9β-^{3}H-$PGF_{2\alpha}$ was tetranor-$PGF_{1\alpha}$ (Grēen and Samuelsson 1968). The structures of the urinary metabolites of PGE_1 have not been determined. However, the labeled material excreted in the urine after administration of 5,6-$^{3}H_2$-PGE_1 consisted exclusively of compounds more polar than PGE_1 (Samuelsson 1964) and experiments with 3-^{14}C-PGE_1 indicated that the formation of these metabolites involved degradation of the carboxyl side chain of PGE_1 by four carbon atoms (Samuelsson 1965). In accordance with these findings the ^{14}C label of 1-^{14}C-PGE_1 administered to rats was mainly recovered in the expired carbon dioxide (Miller and Krake 1968).

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1 The following trivial names and abbreviations are used: Prostaglandin E_1 (PGE_1), 11α,15-dihydroxy-9-ketoprost-13-enoic acid; prostaglandin E_2 (PGE_2), 11α,15-dihydroxy-9-ketoprosta-5,13-dienoic acid; prostaglandin B_2 (PGB_2), 15-hydroxy-9-ketoprosta-5,8(12),13-trienoic acid; prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$), 9α,11α,15-trihydroxyprost-13-enoic acid; prostaglandin $F_{1\beta}$ ($PGF_{1\beta}$), 9β,11α,15-trihydroxyprost-13-enoic acid; and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 9α,11α,15-trihydroxyprosta-5,13-dienoic acid.

The present communication describes experiments which have led to identification of the major urinary metabolite of PGE_2 in the guinea pig as 5β,7α-dihydroxy-11-ketotetranor-prostanoic acid.

METHODS AND RESULTS

17,18-$^3H_2$-PGE_2 (1.5-1000 µg), prepared as previously described (Änggård et al. 1965) and with a specific activity of 420 or 0.42 µC/µmole, was injected subcutaneously to male guinea pigs. Of the injected radioactivity 35-40% was recovered in the urine within 12 hours. Of the labeled material present in the urine 85-90% could be extracted with diethyl ether after acidification. The extracted material was fractionated by reversed phase partition chromatography (column 9 g of hydrophobic Hyflo Super-Cel, solvent system C-36, c.f. Hamberg 1968). The material forming the main peak of radioactivity (Metabolite I, 96-120 ml effluent) was subjected to silicic acid chromatography (eluted with ethyl acetate - benzene, 80:20). The methyl ester, prepared by treatment with diazomethane solution, was again subjected to silicic acid chromatography (eluted with ethyl acetate - benzene, 60:40). This procedure yielded material which behaved homogenously upon thin layer chromatography (solvent system M II, c.f. Gréen and Samuelsson 1964, R_f = 0.75; reference PGE_1 methyl ester, R_f = 0.83) and gas-liquid chromatography (see below).

On treatment of the methyl ester of Metabolite I with sodium hydroxide no absorption band at 237 mµ or 278 mµ developed. Under the conditions used, PGE_2 was transformed into PGB_2 (λ_{max}^{EtOH} = 278 mµ) and 11α,15-dihydroxy-9-ketoprostanoic acid was transformed into 15-hydroxy-9-ketoprost ¬8(12)-enoic acid (λ_{max}^{EtOH} = 237 mµ) (c.f. Bergström et al. 1963).

Gas chromatographic-mass spectrometric analysis of four derivatives of the methyl ester of Metabolite I (c.f. the Table and Fig. 3) indicated the following structural features: carbon chain of 16 carbon atoms, two hydroxyl groups and one keto group. The two hydroxyl groups were

Table. Retention times on gas-liquid chromatography[x].

Derivative	1% SE 30			1% EGSS-X		
	(1)[xx]	(2)[xx]	(3)[xx]	(1)[xx]	(2)[xx]	(3)[xx]
TMSi	21.0	20.7	20.7	23.3	22.8	22.8
TMSi-MO	21.1	20.8	20.8	22.7	22.3	22.3
Acetate	21.9	21.8	21.8			
Acetate-MO	22.0	21.9	21.9			

TMSi, trimethylsilyl ether; MO, O-methyl oxime.

x) C-values, see Hamberg 1968

xx) (1) methyl 5α,7α-dihydroxy-11-ketotetranor-prostanoate; (2) methyl 5β,7α-dihydroxy-11-ketotetranor-prostanoate; (3) methyl ester of Metabolite I.

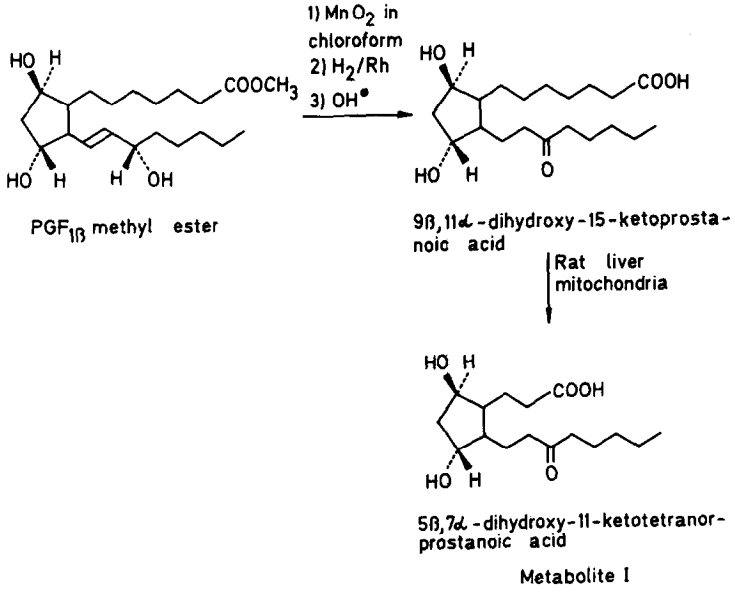

Fig. 1. Reactions used to prepare 5β,7α-dihydroxy-11-ketotetranor-prostanoic acid.

most likely located in the five-membered ring and the keto group in the side chain, since ultraviolet spectrometry indicated that the β-ketol system of PGE_2 was not retained in Metabolite I. The retention times(expressed as C-values, c.f. Hamberg 1968) of the trimethylsilyl ether, trimethylsilyl ether- O-methyl oxime, acetate, and acetate-O-methyl oxime derivatives prepared of the methyl ester of Metabolite I were identical with those of the corresponding derivatives of methyl 5β,7α-dihydroxy-11-ketotetranor-prostanoate using 1% SE 30 and 1% EGSS-X (see the Table).

For the preparation of 5α,7α-dihydroxy-11-ketotetranor-prostanoic acid and 5β,7α-dihydroxy-11-ketotetranor-prostanoic acid, $PGF_{1\alpha}$ and $PGF_{1\beta}$ were oxidized and hydrogenated (c.f. Änggård and Samuelsson 1964) to give 9α,11α-dihydroxy-15-ketoprostanoic acid and 9β,11α-dihydroxy-15-keto-prostanoic acid. These compounds were then converted into the corresponding C_{16} homologues using the β-oxidation system of rat liver (Hamberg 1968) (Fig. 1).

The mass spectra of the four derivatives of the methyl ester of Metabolite I were identical with those of the corresponding derivatives of methyl 5β,7α-dihydroxy-11-ketotetranor-prostanoate. The mass spectra of the trimethyl-silyl ether derivatives are shown in Fig. 2 and Fig. 3. Ions of high intensity are present at m/e 443 (M-15), 427 (M-31), 368 (M-90), 353 (M-(15+90)), 281 (M-(87+90)), 278 (M-2x90), 254 (M-(114+90)), 241 (M-(127+90)), 217, 191 (M-(87+2x90)) and 179 (M-(99+2x90)) (c.f. Fig. 2).

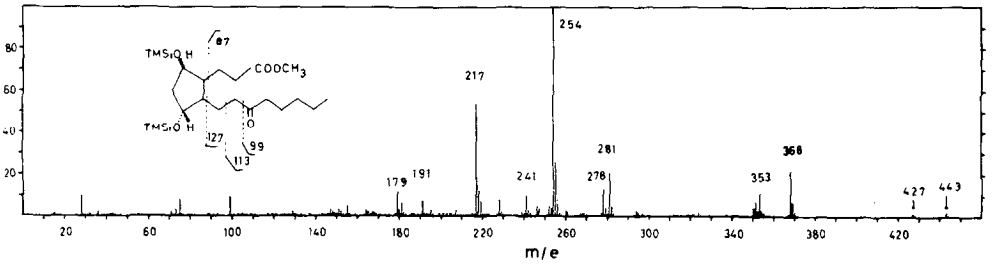

Fig. 2. Mass spectrum of trimethylsilyl ether derivative of methyl 5β,7α-dihydroxy-11-ketotetranor-prostanoate.

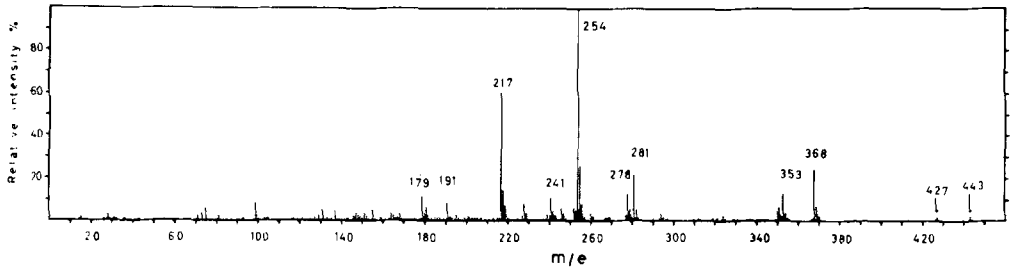

Fig. 3. Mass spectrum of trimethylsilyl ether derivative of the methyl ester of Metabolite I.

DISCUSSION

The formation of 5β,7α-dihydroxy-11-ketotetranor-prostanoic acid from PGE_2 involves oxidation of the secondary alcohol group in the side chain, reduction of the trans double bond, β-oxidation of the carboxyl side chain and reduction of the keto group in the five-membered ring. The first two reactions were originally discovered in guinea pig lung and were also demonstrated to occur in other tissues and species (Änggård and Samuelsson 1964, Änggård and Samuelsson 1967). Degradation of the carboxyl side chain of prostaglandins by β-oxidation has been demonstrated previously both in vivo (Granström et al. 1965, Grēen and Samuelsson 1968) and in vitro (Hamberg 1968). However, reduction of the keto group in the five-membered ring has not previously been observed. The configuration (β) of the hydroxyl group formed is opposite to that found in "primary" prostaglandins. Studies on the enzyme system catalyzing this reaction as well as the sequence of the reactions involved in the transformation of PGE_2 into 5β,7α-dihydroxy-11-ketotetranor-prostanoic acid are in progress in our laboratory.

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