Identification of metabolites from peroxisomal β-oxidation of prostaglandins

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Abstract We have recently shown that isolated rat liver peroxisomes can chain-shorten prostaglandin $F_{2\alpha}$ and prostaglandin E_2 to tetranor-metabolites. In the present report dinor-metabolites of these two prostaglandins were also identified, suggesting that the peroxisomal chain-shortening reaction of prostaglandins is a β-oxidation reaction. Furthermore, an intermediate containing an extra double bond was isolated from incubates of prostaglandin $F_{2\alpha}$ with peroxisomes. This intermediate was tentatively assigned the structure 2,3-dehydro-prostaglandin $F_{2\alpha}$. Prostaglandin E_1 and a major circulating prostaglandin $F_{2\alpha}$ metabolite were also metabolized to chain-shortened products by peroxisomes. The accumulation of the 2,3-dehydro-metabolite and the dinor-metabolites suggest that the peroxisomal β-oxidation sequence is not tightly coupled, in contrast to mitochondrial fatty acid oxidation. — **Diczfalusy, U., and S. E. H. Alexson.** Identification of metabolites from peroxisomal β-oxidation of prostaglandins. *J. Lipid Res.* 1990. **31:** 307–314.

Supplementary key words prostaglandin $F_{2\alpha}$ • prostaglandin E_1 • chain-shortening • rat liver peroxisomes

In 1976 Lazarow and De Duve (1) described a peroxisomal fatty acid oxidation system in rat liver. Characterization of these reactions indicated the presence of a β-oxidation sequence (2), although the enzymes involved were shown to be different from the corresponding mitochondrial enzymes (3). Peroxisomes seem to have a function in the oxidation of very long-chain fatty acids (4) and long-chain polyunsaturated fatty acids (5, 6). β-Oxidation of dicarboxylic acids (7, 8) and the oxidative side-chain cleavage in the biosynthesis of bile acids (9, 10) seem to occur almost exclusively in peroxisomes.

We have shown that rat liver peroxisomes can chain-shorten prostaglandin $F_{2\alpha}$ and prostaglandin E_2 to their tetranor-metabolites (11, 12). Schepers et al. (13) have shown that the carboxylic side-chain of prostaglandin E_2 is metabolized to acid-soluble products mainly by peroxisomes.

It is generally assumed that the peroxisomal chain-shortening of fatty acids is a β-oxidation reaction. Previous experiments supporting the presence of a β-oxidation system in peroxisomes have been performed by measuring the individual enzymatic reactions in the β-oxidation sequence by spectrophotometry (2, 6). In addition, Osmundsen, Neat, and Borrebaek (14) detected chain-shortened metabolites by radio gas–liquid chromatography. The only β-oxidation intermediates isolated and characterized so far are 3α,7α,12α,24-tetrahydroxy-5β-cholestanoic acid and 3α,7α,12α-trihydroxy-5β-cholest 24-enoic acid, intermediates in the formation of cholic acid from 3α,7α,12α-trihydroxy-5β-cholestanoic acid (THCA) (15, 16). It should be noted that the β-oxidation of THCA to cholic acid appears to be catalyzed, at least in part, by an enzyme system different from the β-oxidation of fatty acyl CoAs, as the formation of cholic acid was not induced by clofibrate treatment (17). The peroxisomal β-oxidation of prostaglandins resembles that of the fatty acyl CoAs in that it can be induced by di(2-ethylhexyl)phthalate and clofibrate (12, 13). In the present article we describe the formation of and characterization of the dinor-intermediates in the peroxisomal conversion of prostaglandins E_2 and $F_{2\alpha}$ to their tetranor-metabolites. We have also isolated and tentatively identified a peroxisomal β-oxidation intermediate, 2,3-dehydro-prostaglandin $F_{2\alpha}$.

MATERIALS AND METHODS

Chemicals

Prostaglandin $F_{2\alpha}$ (Tris salt), prostalgandin E_1, and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ were purchased from Sigma (St. Louis, MO). [9β-^{3}H]Prostaglandin $F_{2\alpha}$, [5,6,8,11,12,14,15-$^3H_7$]prostaglandin E_2, and [5,6-$^3H_2$]prostaglandin E_1 were purchased from NEN Research Products (Dreieich, FRG) and [5,6,8,9,11,12,14(n)-$^3H_7$] 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ was obtained from

Abbreviations: DEHP, di(2-ethylhexyl)phthalate; HPLC, high performance liquid chromatography; THCA, 3α,7α,12α-trihydroxy-5β-cholestanoic acid.

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Amersham (Buckinghamshire, England). All other chemicals were of analytical grade.

Preparation of rat liver peroxisomes

Male Sprague-Dawley rats were treated with di(2-ethylhexyl)phthalate (DEHP) for 1-2 weeks in order to induce peroxisomes. Rat liver peroxisomes were isolated by isopycnic sucrose gradient centrifugation of a light mitochondrial fraction as described earlier (12), with the exception that the rats were not injected with Triton WR-1339.

Incubations of prostaglandins with DEHP-peroxisomes

Incubations of prostaglandins in an analytical scale were performed in 0.085 M Tris buffer, pH 8.0 (total volume 1.0 ml). The incubation medium was supplemented with the following cofactors (final concentrations): 2.5 mM ATP, 0.5 mM CoA, 10 mM $MgCl_2$, and 35 μM FAD. The substrate concentration was 60 μM and 2 μCi tritium-labeled prostaglandin was added to each incubation. One hundred μg peroxisomal protein was added to the incubation mixture which was preincubated for 15 min at 37°C before the reaction was started by addition of 10 μl 20 mM NAD. The incubation was allowed to proceed for 30 min. Hydrolysis of CoA-esters was accomplished by treatment with 350 μl 0.5 M KOH at 60°C for 60 min.

Preparative incubations were performed to isolate the metabolites for structural identification by gas-liquid chromatography-mass spectrometry. These incubations were scaled up ten times compared to the analytical incubations with the exception of peroxisomal protein which was increased to 2 mg per incubation. Prostaglandins other than prostaglandin $F_{2\alpha}$ (Tris salt) were dissolved in an α-cyclodextrin solution as described in reference 18.

Extraction of reaction products

The reaction mixtures were extracted by means of a Sep Pak C_{18} cartridge (Waters Associates, Milford, MA) as previously described (12).

HPLC of incubation extracts

Analytical incubations were analyzed by HPLC using a Waters Radial-Pak Nova Pak C_{18} 4μ-column (8 mm × 10 cm). The mobile phase used for analysis of prostaglandin $F_{2\alpha}$ metabolites was methanol-water-acetic acid 60:40:0.01 (v/v/v) and the flow rate was 1 ml/min. The UV absorption was monitored at 205 nm. Sixty fractions of 0.5 ml were collected, mixed with 7 ml OptiPhase HiSafe II scintillation cocktail (LKB, Bromma, Sweden), and analyzed for radioactivity in an LKB 1217 Rackbeta liquid scintillation counter. For other prostaglandins, the methanol-water ratio of the mobile phase was altered so that the unmetabolized prostaglandin should have the same retention time as prostaglandin $F_{2\alpha}$ in methanol-water-acetic acid 60:40:0.01.

Preparative scale incubations were chromatographed on a Polygosil 60-10μ C_{18}-column (10 mm × 50 cm, Macherey-Nagel, Düren, FRG) using methanol-water-acetic acid mixtures as mobile phase.

Preparation of derivatives for gas-liquid chromatography

Carboxyl groups were converted to methyl esters using etheral diazomethane. Hydroxyl groups were derivatized to trimethylsilyl ethers by treatment with trimethylchlorosilane-hexamethyldisilazane-pyridine 1:2:3 (v/v/v). Keto groups were converted to methoximes using O-methoxyamine hydrochloride in pyridine.

Catalytic hydrogenation

Twenty-50 μg of the substance to be hydrogenated was esterified with etheral diazomethane. The methyl ester was dissolved in 1 ml of methanol. One mg of platinum oxide (Adams catalyst) was added and a slow stream of hydrogen was allowed to bubble through the solution for 90 sec. The catalyst was removed by filtration.

Radio gas-liquid chromatography and gas-liquid chromatography-mass spectrometry

Radio gas-liquid chromatography was performed as previously described (19). The column (1% OV-101 on Chromosorb WHP, 80-100 mesh, 150 cm × 2 mm) was operated isothermally. Retention times were converted to C-values as described earlier (20), using methyl esters of saturated fatty acids as reference compounds. Gas-liquid chromatography-mass spectrometry was performed either on a LKB 2091 gas chromatograph-mass spectrometer, equipped with a 30-m capillary column (DB-1301, 0.25 mm × 30 m, J&W Scientific, Folsom, USA) or a Hewlett Packard 5890 gas chromatograph/5970 mass selective detector. This instrument was equipped with an HP-1 column (0.2 mm × 12 m, methyl silicone gum).

RESULTS

Incubation of prostaglandin $F_{2\alpha}$ with peroxisomes

Incubation of prostaglandin $F_{2\alpha}$ with peroxisomes isolated from livers of DEHP-treated rats yielded several products. **Fig. 1** shows a reversed phase HPLC chromatogram of an extract from a prostaglandin $F_{2\alpha}$ incubation with peroxisomes. The major peak in Fig. 1 (peak IV) is the unconverted substrate (prostaglandin $F_{2\alpha}$). The material in peak I has earlier been identified by gas-liquid chromatography-mass spectrometry as tetranor-prostaglandin $F_{1\alpha}$ (11). The material corresponding to peak II was isolated by HPLC from a preparative incubation.

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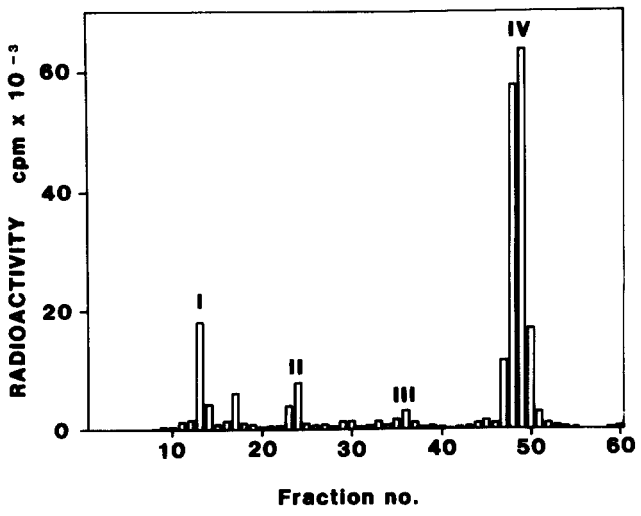

Fig. 1. Reversed phase HPLC chromatogram of an extract from an incubation of prostaglandin $F_{2\alpha}$ with peroxisomes. Peak IV is unconverted prostaglandin $F_{2\alpha}$ and peaks I, II, and III are polar metabolites. Peaks I, II, and III contained 9.8%, 5.6%, and 2.7%, respectively, of the recovered radioactivity.

After treatment with diazomethane and silylating reagents, the material was analyzed by radio gas–liquid chromatography. Peak II showed only one radioactive component with a C-value of 22.7 on an OV-101 column (**Fig. 2**). Prostaglandin $F_{2\alpha}$ (methyl ester, trimethylsilyl ether) had a C-value of 24.4 on the same column. Gas–liquid chromatography–mass spectrometry of peak II (methyl ester, trimethylsilyl ether) yielded a C-value of 22.2 on a DB-1301 column and a mass spectrum with ions present at *m/z* 556 (molecular ion, M), 541 (M–15, loss of $\cdot CH_3$), 485 (M–71, loss of $\cdot(CH_2)_4CH_3$), 466 (M–90, loss of trimethylsilanol), 451 (M–(90 + 15)), 443 (M–113, loss of C_1–C_5), 395 (M–(90 + 71)), 376 (M–(2 × 90)), 369 (M–187,(186 + 1), loss of C_{14}–C_{20}), 305 (M–(2 × 90)–71), 280 (M–276(186 + 90)), 279 (M–277(186 + 1 + 90)), 237, 217 ($(CH_3)_3SiO^+=CH$–$CH=CH$–$OSi(CH_3)_3$), 207, 199 (methyl end chain), 191, 173 ($(CH_3)_3SiO^+=CH$–$(CH_2)_4CH_3$), 129, 75 and 73. The mass spectrum is identical to the mass spectrum of the trimethylsilyl derivative of the methyl ester of 9,11,15-trihydroxy-2,3-dinorprosta-5,13-dien-1-oic acid (dinor-prostaglandin $F_{2\alpha}$) (21). Additional evidence for this structure was obtained from the catalytically hydrogenated material in peak II. The mass spectrum of the hydrogenated compound (methyl ester, trimethylsilyl ether) did not show any molecular ion, but ions were present at *m/z* 545 (M–15), 489 (M–71), 470 (M–90), 399 (M–(90 + 71)), 380 (M–(2 × 90)), 355, 309, 217, 191, 173, 129, 75, and 73. A shift of four mass units (e.g., *m/z* 545, 489, 470, 399, 380, 309) compared to the ions of the nonhydrogenated sample clearly indicated the presence of two double bonds in the parent molecule.

Incubation of prostaglandin E_2 with peroxisomes

In a previous study (12) we have shown that prostaglandin E_2 is chain-shortened to its tetranor-metabolite by incubation with peroxisomes. **Fig. 3** shows a reversed phase HPLC chromatogram of an extract from an incubation of PGE_2 with peroxisomes. Prostaglandins of the E-type are converted to B-type prostaglandins under the alkaline conditions used to hydrolyze the CoA-esters after the incubation (20). The reaction products from incubations with prostaglandin E_2 were therefore analyzed as prostaglandin B_2-metabolites. The major peak (III) in Fig. 3 corresponds to unconverted substrate (prostaglandin E_2), here analyzed as prostaglandin B_2. The most polar peak in the chromatogram, I, has earlier been shown to be a tetranor-metabolite (12). Peak II was isolated by reversed phase HPLC. The substance was dissolved in ethanol and a UV spectrum was recorded. An absorption maximum was seen at 278 nm, typical of prostaglandins of the B-type. After derivatization to methyl ester-O-methyloxime-trimethylsilyl ether, the material in

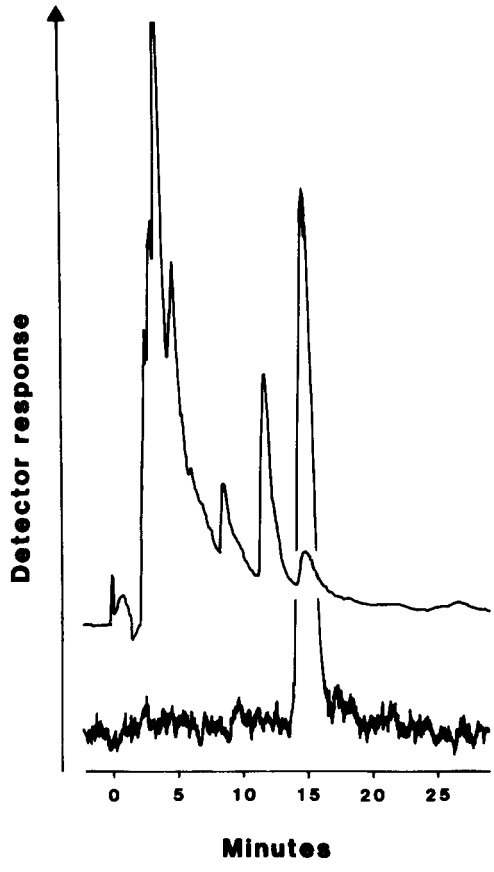

Fig. 2. Radio gas–liquid chromatogram of peak II in Fig. 1 (methyl ester-trimethylsilyl ether derivative). Upper trace: flame ionization detector. Lower trace: radioactivity detector.

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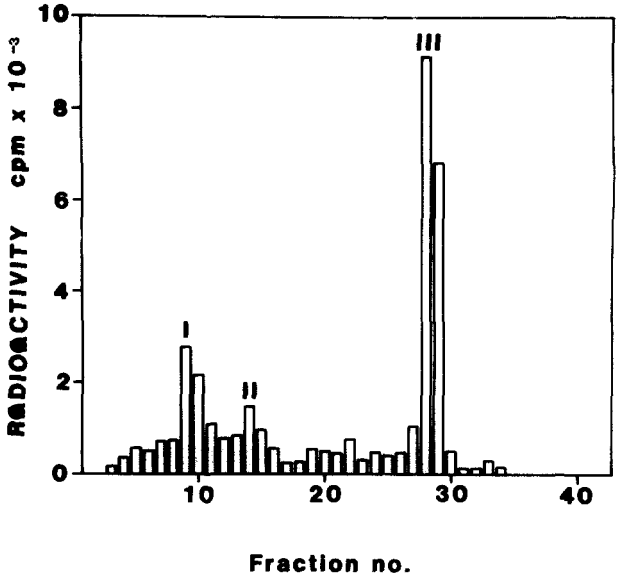

Fig. 3. Reversed phase HPLC chromatogram of an extract from an incubation of prostaglandin E_2 with peroxisomes. Peak III corresponds to unconverted prostaglandin E_2 (here analyzed as prostaglandin B_2) and peaks I and II are polar metabolites.

peak II was analyzed by radio gas-liquid chromatography. A single peak appeared with a C-value of 22.5 on an OV-101 column, indicating a C_{18} prostaglandin. Tetranor-prostaglandin B_1 has a C-value of 20.5 (22). A mass spectrum was recorded and ions were present at *m/z* 421 (M), 406 (M-15), 390 (M-31, loss of OCH_3), 350 (M-71), 331 (M-90), 322, 319 (M-(31 + 71)), 308, 300 (M-(90 + 31)), 173, 75 and 73. The UV absorption, the retention characteristics on HPLC, the C-value from radio gas-liquid chromatography, as well as the mass spectrum, suggest that peak II corresponds to dinor-prostaglandin B_2. Thus, the dinor-metabolite contained two double bonds, in analogy with the corresponding metabolite from prostaglandin $F_{2\alpha}$.

Incubation of prostaglandin E_1 with peroxisomes

In order to study whether the double bond in the carboxylic side chain of the prostaglandin affects the peroxisomal β-oxidation reaction, a prostaglandin without a 5,6-double bond, prostaglandin E_1, was incubated with peroxisomes. **Fig. 4** shows a reversed phase HPLC chromatogram of an extract from such an incubation. Peak III in Fig. 4 corresponds to unconverted substrate (prostaglandin E_1), here analyzed as prostaglandin B_1. The two peaks I and II were isolated by preparative HPLC. UV spectra were recorded for peaks I and II and both compounds had absorption maxima at 277 nm (in ethanol). Gas-liquid chromatography of peak I and II (as methyl ester-trimethylsilyl ether derivatives) gave C-values of 20.9 and 22.9, respectively, on a DB-1301 column. Mass spectrometry of peak I (methyl ester-O-methyloxime-trimethylsilyl ether) resulted in a mass spectrum identical to that of tetranor-prostaglandin B_1 as described by Green (22).

The mass spectrum of peak II (methyl ester-trimethylsilyl ether) showed a strong molecular ion at *m/z* 394 (M) and ions at *m/z* 379 (M-15), 362 (M-32, loss of methanol), 323 (M-71), 295 (M-(71 + 28)), 235, 191, 173 ($(CH_3)_3SiO^+ = CH\text{-}(CH_2)_4CH_3$), 75 and 73. Mass spectral analysis was also performed on the methyl ester-O-methyloxime-trimethylsilyl ether derivative of peak II. This mass spectrum showed ions at *m/z* 423 (M), 392 (M-31), 352 (M-71), 333 (M-90), 302 (M-(90 + 31)), 202, 75 (base peak) and 73. The HPLC retention characteristics, UV spectrum, C-value, and mass spectra suggest that peak II is identical to dinor-prostaglandin E_1.

There was no difference in the rate of β-oxidation of prostaglandin E_1 or prostaglandin $F_{2\alpha}$. (Peak I in Fig. 4 has not been corrected for the loss of the tritium in the 6-position of the substrate during the β-oxidation process.)

Incubation of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ with peroxisomes

The main circulating metabolite of prostaglandin $F_{2\alpha}$ in humans is 9α,11α-dihydroxy-15-oxoprost-5-enoic acid (13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$) (23). It was therefore of interest to study whether this metabolite could be further degraded by peroxisomal β-oxidation. An HPLC chromatogram of an extract from a preparative incubation of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ with peroxisomes is shown in **Fig. 5**. Peak II is unconverted substrate. The more polar peak I was isolated by preparative HPLC. Radio gas-liquid chromatography of peak I

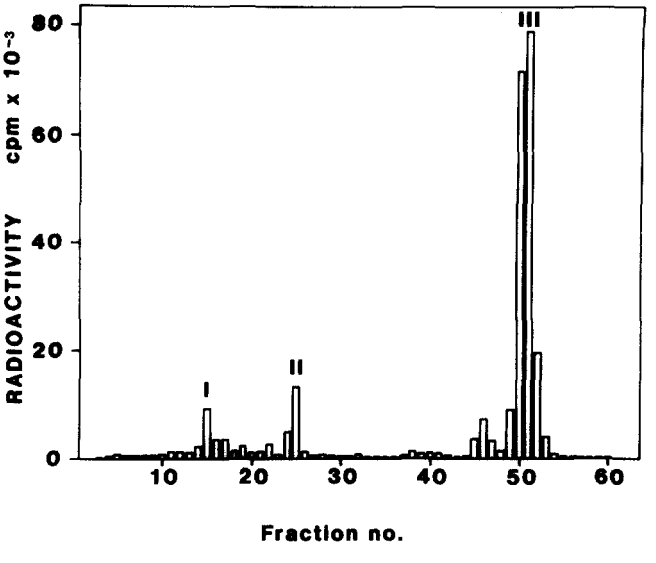

Fig. 4. Reversed phase HPLC chromatogram of an extract from an incubation of prostaglandin E_1 with peroxisomes. Peak III corresponds to unconverted prostaglandin E_1 (here analyzed as prostaglandin B_1) and peaks I and II are polar metabolites.

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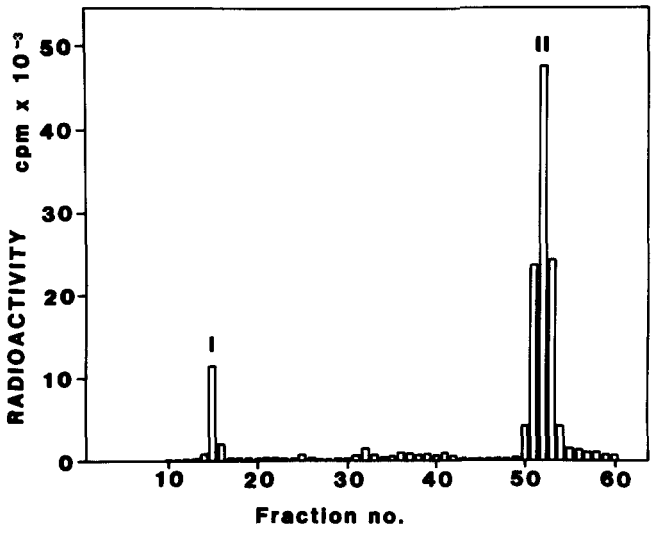

Fig. 5. Reversed phase HPLC chromatogram of an extract from an incubation of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ with peroxisomes. Peak II is unconverted 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ and peak I is a chain-shortened metabolite.

(as methyl ester-trimethylsilyl ether) on a Supelcowax 10 column (25 m × 0.25 mm) showed a single radioactive component with a C-value of 22.0. Mass spectrometry of this derivative gave a mass spectrum with ions at *m/z* 458 (M, weak), 443 (M-15, weak), 368 (M-90), 353 (M-(90 + 15)), 281, 278 (M-(2 × 90)), 254, 247 (M-(2 × 90)-31), 241, 228, 217 ($(CH_3)_3SiO^+=CH-CH=CH-OSi(CH_3)_3$), 191, 179 and 99. A C-value of 21.1 was obtained on a DB-1301 column. The C-value of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (methyl ester, trimethylsiyl ether) was 24.6. The mass spectrum was identical to the mass spectrum of the tetranor-metabolite of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ as described earlier by Granström and Samuelsson (24). The circulating metabolite 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ had thus been chain-shortened by four carbons through two cycles of peroxisomal β-oxidation. We were not able to detect a dinor-metabolite with the incubation conditions used.

Isolation of a β-oxidation intermediate

We have shown earlier that the conversion of prostaglandin $F_{2\alpha}$ to its tetranor-metabolite is dependent on exogenous NAD (12). When prostaglandin $F_{2\alpha}$ was incubated with peroxisomes in the absence of added NAD, we observed that the material corresponding to peak III (Fig. 1) accumulated and became the major metabolite. This is illustrated in **Fig. 6**. Peaks I and II correspond to the tetranor- and dinor-metabolites of prostaglandin $F_{2\alpha}$, respectively, the major products in the presence of added NAD. The material corresponding to peak III, from an incubation performed in the absence of added NAD, was isolated by HPLC and further characterized by radio gas-liquid chromatography. Peak III (methyl ester, trimethylsilyl ether) showed one radioactive component with a C-value of 24.7 on an OV-101 column. This indicated a C_{20} prostaglandin. Peak III was further analzyed by gas-liquid chromatography-mass spectrometry. As shown in **Fig. 7**, ions were present at *m/z* 582 (M), 567 (M-15), 511 (M-71), 492 (M-90), 421 (M-(90 + 71)), 402 (M-(2 × 90)), 376, 331 (M-(2 × 90)-71), 312 (M-(3 × 90)), 293, 241, 217 ($(CH_3)_3SiO^+=CH-CH=CH-OSi(CH_3)_3$), 199, 191, 173 ($(CH_3)_3SiO^+=CH-(CH_2)_4CH_3$, base peak), 129, 75 and 73. The mass spectrum was very similar to the mass spectrum of prostaglandin $F_{2\alpha}$ (methyl ester, trimethylsilyl ether), but with a shift of two mass units for many of the higher ions (e.g., 582, 567, 511, 492, 421, 376, 331) indicating an extra double bond in the molecule. The mass spectrum of catalytically hydrogenated peak III was identical to the mass spectrum of catalytically hydrogenated prostaglandin $F_{2\alpha}$ (both compounds as methyl esters, trimethylsilyl ethers) with ions at *m/z* 573 (M-15, very weak,), 517 (M-71), 498 (M-90), 427 (M-(90 + 71)), 408 (M-(2 × 90)), 355, 337 (M-(2 × 90)-71), 319, 310, 297, 247, 217 ($(CH_3)_3SiO^+=CH-CH=CH-OSi(CH_3)_3$), 199, 191, 173 ($(CH_3)_3SiO^+=CH-(CH_2)_4CH_3$), 129, 75 and 73. Both hydrogenated compounds had identical C-values, 24.6, on a packed OV-101 column. Peak III was also catalytically deuterated and analyzed by mass spectrometry as methyl ester-trimethylsilyl ether. The mass spectrum contained several ions with a shift of 6 mass units higher than the corresponding ions from the hydrogenated sample, e.g., *m/z* 523 (M-71), 504 (M-90), 494, 433 (M-(90 + 71)) and 414. This shows that peak III contained an extra double bond compared to prostaglandin $F_{2\alpha}$. The material in peak III did not have any

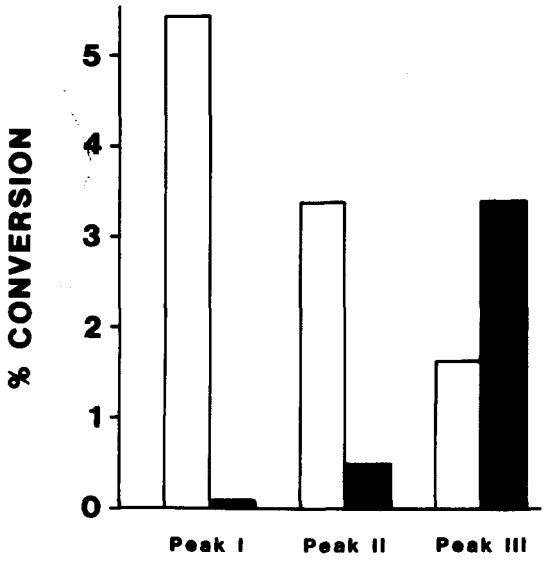

Fig. 6. Conversion of prostaglandin $F_{2\alpha}$ into compounds I, II, and III (Fig. 1) after incubation with peroxisomes in the presence (open bars) or absence (filled bars) of NAD.

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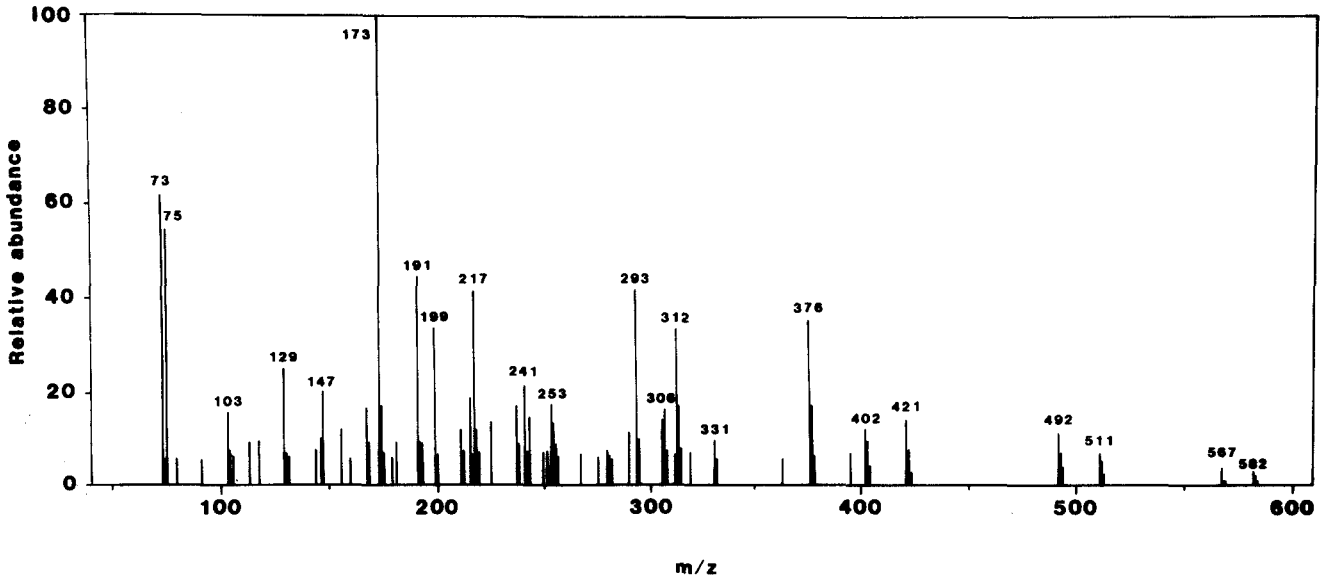

Fig. 7. Mass spectrum of methyl ester-trimethylsilyl ether derivative of peak III in Fig. 1. Ions with abundances of less than 5% have been omitted from the mass spectrum below *m/z* 550.

UV absorption corresponding to a conjugated diene. The structure was tentatively assigned 2,3-dehydro-prostaglandin $F_{2\alpha}$.

DISCUSSION

We have shown earlier that prostaglandins E_2 and $F_{2\alpha}$ can be chain-shortened to their tetranor-metabolites by peroxisomes (11, 12). As dinor-prostaglandins are normal excretion products in several species (21, 25, 26), it was of interest to study whether these compounds could be formed by peroxisomes. If the tetranor-metabolites are products of a β-oxidation process, one would expect the dinor-compounds to be formed as well. In an earlier study (12) we observed that several products were formed when prostaglandin $F_{2\alpha}$ was incubated with peroxisomes. Two of the previously unidentified products were isolated and characterized. One of these (peak II in Fig. 1) was identified as dinor-prostaglandin $F_{2\alpha}$. The corresponding dinor-metabolite was isolated from incubations of prostaglandin E_2. These results show that peroxisomes degrade prostaglandins by removal of acetyl units in analogy with the peroxisomal oxidation of long-chain fatty acids.

Dinor-prostaglandin $F_{2\alpha}$ was isolated from preparative incubations and incubated with peroxisomes to see whether it could be converted to the tetranor-metabolite. Although these incubations were performed in the same way as for the C_{20} prostaglandins, we could not detect any conversion at all. The reason for this is not clear, but one possible explanation could be that the dinor-prostaglandin, which was incubated in the free acid form, may be a bad substrate for the acyl-CoA synthetase reaction.

When prostaglandin E_1, which lacks the 5,6-double bond present in prostaglandin $F_{2\alpha}$, was incubated with peroxisomes, two main products were obtained. These were identified as the dinor- and tetranor-metabolites. Both the product pattern and the rate of conversion were very similar for prostaglandin E_1 and prostaglandin $F_{2\alpha}$, showing that the presence of the 5,6-double bond is not a rate-limiting factor in the oxidation of the carboxylic side-chain of prostaglandins of the 2-series.

Circulating prostaglandins are inactivated very rapidly to 15-keto-prostaglandins and subsequently to 13,14-dihydro-15-keto-prostaglandins (27). The latter is usually the major circulating metabolite which has lost most of its biological activity. As this inactivated prostaglandin is a likely substrate for β-oxidation in vivo, 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ was incubated with isolated peroxisomes in order to study its metabolism. This substrate was readily converted into the tetranor-metabolite but, surprisingly, no dinor-metabolite was formed (Fig. 5). The tetranor-metabolite appears in urine after administration of prostaglandin $F_{2\alpha}$ to humans (24).

As mentioned above, several products were formed upon incubation of prostaglandin $F_{2\alpha}$ with peroxisomes. One of these (peak III, Fig. 1) accumulated and became the major product in incubations where NAD was omitted (Fig. 6). This suggested that peak III could be an intermediate in the β-oxidation sequence, the most likely candidate being 3-hydroxy-prostaglandin $F_{2\alpha}$ since this is the substrate for the NAD-dependent dehydrogenase reaction. However, the result of the structural analysis

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showed that the material in peak III contained an extra double bond compared to prostaglandin $F_{2\alpha}$. Catalytic hydrogenation of the material in peak III gave a compound with a mass spectrum identical to that of catalytically hydrogenated prostaglandin $F_{2\alpha}$. Furthermore, catalytical deuteration clearly indicated the presence of an extra double bond. This ruled out the possibility that the double bond was formed by elimination of a hydroxyl group during analysis. The mass spectrum of the compound in peak III showed ions that indicated that the methyl end side-chain was identical to that of prostaglandin $F_{2\alpha}$ (e.g., *m/z* 173, 199). The ion *m/z* 217 indicated that the ring structure was unaltered, suggesting that the extra double bond was situated in the carboxylic side-chain. The material had no UV absorption corresponding to a conjugated diene, leaving only the possibility of a 2,3-position for the double bond. The structure was thus tentatively assigned 2,3-dehydro-prostaglandin $F_{2\alpha}$. It is surprising that it was not the 3-hydroxy-prostaglandin $F_{2\alpha}$ that accumulated in the absence of NAD. It has been reported, however, that the corresponding α,β-unsaturated metabolite was the major intermediate formed during peroxisomal oxidation of THCA in the absence of NAD (16). The relative amount of 2,3-dehydro-prostaglandin $F_{2\alpha}$ in incubations with peroxisomes from untreated rats was higher than in incubations with DEHP-peroxisomes. It is not likely that the decreased accumulation of 2,3-dehydro-prostaglandin $F_{2\alpha}$ found in incubations with peroxisomes after DEHP-treatment was a result of different degrees of induction of the β-oxidation enzymes as these have been shown to be induced in a parallel manner (28). When prostaglandin $F_{2\alpha}$ was incubated with microsomes from DEHP-treated rats, negligible amounts of tetranor-prostaglandin $F_{2\alpha}$ were formed (results not shown). Instead, a marked increase in the relative amounts of both dinor-prostaglandin $F_{2\alpha}$ and 2,3-dehydro-prostaglandin $F_{2\alpha}$ was observed. This may be explained by loss of enoyl-CoA isomerase (EC 5.3.3.8), an enzyme necessary for the β-oxidation of fatty acids containing an odd-numbered double bond, which has been shown to be present in peroxisomes (29). When microsomes from untreated rats were used no conversion could be detected at all.

In conclusion, peroxisomal β-oxidation of prostaglandins gives rise to both dinor-and tetranor-metabolites, suggesting that the β-oxidation system is not tightly coupled, in contrast to mitochondria. This could explain the occurrence of dinor-metabolites of prostaglandins in the urine.

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