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Studies on the Oxidation of ω-Hydroxyprostaglandins by an NAD-Dependent Dehydrogenase from Mammalian Liver Cytosol[1]

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The oxidation of 12-hydroxylauric acid methyl ester (12-OH-L-Me) and of ω-hydroxyprostaglandins (ω-OH-PGs) such as 20-OH-PGB_1 and 20-OH-PGE_1 was demonstrated with liver cytosol from rat, rabbit, and guinea pig in the presence of NAD; however, NADP did not support this oxidation. (ω-1)-Hydroxy-compounds (11-OH-laurate and 19-OH-PGB_1) and PGE_1, $PGF_{1\alpha}$, and PGB_1, all lacking the terminal (ω)-hydroxyl, did not reduce NAD. However, at pH 10, PGE_1 slightly enhanced NAD reduction, suggesting that at this pH PGE_1 could be a substrate for 15-hydroxy-PG dehydrogenase (PGDH). The oxidation products from incubations of 12-OH-L-Me, 20-OH-PGB_1-Me, and 20-OH-PGE_1 with guinea pig liver cytosol were isolated and identified by gas chromatography/mass fragmentation spectrometry as being the corresponding dicarboxylic acids. In contrast to the liver cytosol, guinea pig *kidney* cytosol had only a minimal effect on NAD reduction by 12-OH-L-Me but nevertheless did support the stimulation of NAD reduction by PGE_1, and $PGF_{1\alpha}$, but not by PGB_1, indicating the participation of kidney cytosolic PGDH in PGE_1 and $PGF_{1\alpha}$ oxidation and demonstrating that the oxidation of ω-OH to the carboxylic acid is not mediated by PGDH. Though the *in vivo* rate of oxidation of ω-OH-PGs has not been established, these results suggest that the urinary dicarboxylic-PG metabolites involve a multiple sequential-step oxidation of PGs involving ω-hydroxylation by an NADPH-cytochrome *P*-450 system in the endoplasmic reticulum and the subsequent oxidation of the ω-OH by an NAD-dependent dehydrogenase in the cytosol.

Prostaglandins (PGs)[4] have been shown to be metabolized *in vivo* in animals and man into ω-oxidized products, such as ω- and (ω-1)-hydroxy derivatives and dicarboxylic acid derivatives (1–6). Studies *in vitro* demonstrated that liver microsomes hydroxylate PGs (7–10) in the ω and ω-1 positions and that the enzymatic activity represents a typical monooxygenase (8, 9, 11, 12), suggesting that the hydroxylation of prostaglandins *in vivo* is mediated by the hepatic microsomal monooxygenase. However, the nature of the catalytic activity involved in the oxidation of ω-hydroxy-PGs (ω-OH-PGs) to the corresponding dicarboxylic acids has not been hitherto determined. The oxidation of various ω-hydroxy-fatty acids to dicarboxy acids by mammalian liver dehydrogenase has been previously demonstrated (13–15). The dehydrogenase(s) is present in the 100,000*g* supernate of liver homogenate and requires NAD as a cofactor for activity (15). It has been reported that the ω-hydroxy-fatty acid (ω-OH-FA) dehydrogenase does

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[4] Abbreviations used: PGs, prostaglandins; PGE_1, prostaglandin E_1; PGB_1, prostaglandin B_1; $PGF_{1\alpha}$, prostaglandin $F_{1\alpha}$; PG-Me, PG methyl ester; 20-OH-PG, ω-hydroxy-PG; 19-OH-PG, (ω-1)-hydroxy-PG; 12-OH-L, 12-hydroxy-laurate; 12-OH-L-Me, 12-OH-laurate methyl ester; hplc, high pressure liquid chromatography; GC, gas chromatography; MS, mass fragmentation spectrometry; AUFS, absorbance units full scale; ω-OH-FA, ω-hydroxy-fatty acid; ADH, alcohol dehydrogenase; PGDH, 15-OH-PG dehydrogenase.

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not appear to be alcohol dehydrogenase (ADH), since the isolated ω-OH-FA dehydrogenase was not active toward ethanol and ADH does not catalyze the oxidation of long-chain hydroxy-fatty acids (13, 14). By contrast, Björkhem (15, 16) ascribed the activity of ω-OH-FA dehydrogenase to ADH.

The present study examines whether a hepatic cytosolic (105,000*g* supernate) dehydrogenase catalyzes the oxidation of ω-OH-PGs to the corresponding dicarboxylic acids.

MATERIALS AND METHODS

Materials

NAD and NADP and horse liver alcohol dehydrogenase (ADH) were purchased from Sigma Chemical Company (St. Louis, Mo.). 12-Hydroxylauric acid methyl ester and 11-hydroxylauric acid were gifts from Dr. Sten Orrenius and Ms. Hjördis Thor; PGE_1 and $PGF_{2\alpha}$ were obtained from Upjohn Company (courtesy of Dr. John Pike); 19-OH-PGE_1 methyl ester was a gift from Dr. John Sih (Upjohn Co.); 20-OH-PGE_1 was a gift from Dr. Kazuo Sano (Ono Co., Japan); (5,6-^{3}H)-labeled PGE_1 was purchased from New England Nuclear (89.5 Ci/mmol) and from Amersham/Searle (40 Ci/mmol); acetonitrile (uv grade) was purchased from Burdick and Jackson (Muskegan, Mich.) or from Waters Associates (Milford, Mass.). "Fatty acid" columns for reversed phase hplc were purchased from Waters Associates.

Methods

Preparation of Prostaglandin Derivatives

20-OH-PGB_1 was prepared by base treatment of 20-OH-PGE_1 as previously described (9); 19-OH-PGB_1 was obtained by base treatment of human semen followed by extraction with organic solvents and purification with hplc (17). Methyl esters of the prostaglandins were prepared by esterification with diazomethane in a mixture of methanol–ether (9). The identity of the prostaglandin derivatives was established by coinjection with authentic compounds in hplc and GC and by GC/MS fragmentation patterns of the t-butyldimethylsilyl ether-methyl esters or of the corresponding trimethylsilyl derivatives of the respective PGB_1 methyl esters (9).

Preparation of 12-OH-Laurate (12-OH-L)

Hydrolysis of 12-OH-L-Me was carried out as follows: 2.5 mg of 12-OH-L-Me (melting point 33–34°C) was dissolved in 8 ml aqueous methanol containing 0.1 ml 10 N KOH and allowed to stand at room temperature for about 20 h. The solution was evaporated to 3 ml under a stream of N_2 and extracted with 3 × 2 vol chloroform. The aqueous phase was acidified to pH 2 with HCl and extracted with 3 × 2 vol ethyl acetate. The organic phase was evaporated to dryness under a stream of N_2. The residue (1.6 mg) had a melting point of 108–110°C and was assumed to be 12-OH-L. Structure was confirmed by GC/MS of a TMS derivative.

Preparation of Liver Cytosol

Animals were decapitated and livers were removed, rinsed in ice-cold water, and homogenized in 0.25 M sucrose (5 ml/g) with a Potter–Elvehjm glass-Teflon homogenizer. The homogenate was centrifuged at 10,000*g* for 20 min. The 105,000*g* supernate was prepared by centrifugation of the 10,000*g* supernate at 105,000*g* for 1 h and the supernate was carefully removed. In the initial studies freshly prepared 105,000*g* supernate was used; however once established that storage at −70°C retains activity, the supernate was stored frozen in small aliquots so that repeated freezing and thawing is not required, though freezing and thawing three times did not alter activity.

Assay for Dehydrogenase Activity

The substrates were dissolved so that the required amount was in 5–10 μl of acetone; usually less than 10 μl of the substrate solution was used per 0.96–1.0 ml of a final volume. Each cuvette contained 50 or 100 μl of the 105,000*g* supernate and 0.9 ml of the selected buffer (see text). The *blank* cuvette contained the liver supernate and buffer; the *control* cuvette contained supernate, buffer, NAD (3.0 or 4.0 mM; see tables) and acetone (5–10 μl); the *sample* cuvettes contained supernate, buffer, NAD, and the respective substrate in acetone (same volume as control). The reaction was started by addition of the substrate or NAD, and the change in absorbance at 340 nm versus time was recorded using a Gilford spectrophotometer. The difference between the absorbance in the presence of substrate versus that in the absence of substrate was an indication of substrate-mediated reduction of NAD.

Isolation of Metabolites from Incubations of 20-OH-PGB_1 and 20-OH-PGE_1

Incubations containing the respective substrates were carried out as described above using guinea pig liver supernate. When the rate of reduction of NAD in the sample cuvette leveled off, the reaction was assumed to approach completion; some increase in absorbance at 340 nm continued, probably due to oxidation of endogenous substrates (seen in the control cuvette lacking substrate). The metabolites were isolated from incubation of the following *substrates*.

20-OH-PGB_1-Me. Isolation of metabolite was carried out from two incubations containing 70–100 nmol of

20-OH-PGB_1 methyl ester/ml in 0.1 M phosphate buffer, pH 8.4. To each incubation was added 2 ml of 10% $NaHCO_3$ and extracted with 3 × 2 vol chloroform to remove residual substrate. The aqueous phase was acidified with HCl to pH 2.0 and extracted with 3 × 2 vol ethylacetate. The organic phase was rinsed once with H_2O (2 ml) and the ethylacetate phase was evaporated to dryness under a stream of nitrogen at room temperature. The residue was dissolved in methanol, the λ_{max} determined to be 278 nm as expected of PGB derivatives (18, 21), and an aliquot was subjected to reversed phase high pressure liquid chromatography (hplc) as previously described (9) using a linear gradient elution of increasing acetonitrile concentration versus water from 0 to 25% acetonitrile over 25 min; a peak (marked 1) with absorption higher at 280 nm than at 254 or 313 nm appeared at 16% acetonitrile: 84% H_2O (Fig. 1). The relatively high polarity of this compound and its solubility in bicarbonate solution indicated that the compound was most probably 20-carboxy-PGB_1-monomethyl ester. There was no residual substrate (20-OH-PGB_1 or 20-OH-PGB_1-Me) evident in the hplc scans. The residual methanolic solution which did not undergo hplc was treated with an ethereal solution of CH_2N_2 and an aliquot of the resulting product, dissolved in methanol, was subjected to hplc using a linear gradient of acetonitrile:H_2O (acetonitrile from 0–90%) over 65 min. A peak with PGB_1 spectral characteristics appeared with acetonitrile (40%):H_2O (60%). The low polarity of this product indicated that esterification had most probably occurred. Subsequently, the rest of the esterified product was purified with hplc isocratically, using acetonitrile (30%):H_2O (70%). The eluted peak was evaporated under N_2 and a small aliquot was analyzed by hplc to determine whether in the course of isolation the product remained intact. The product was dissolved in methanol and λ_{max} determined to be at 278 nm; quantitation using ϵ = 27.2 cm^{-1} mM^{-1} (9, 21) was obtained. The product was silylated with t-butyldimethylsilylchloride and subjected to GC/MS fragmentation analysis as previously described (9).

20-OH-PGE_1. After two incubations of 20-OH-PGE_1 (270 nmol/ml phosphate 0.1 M, pH 8.4), the reaction was stopped by addition of 1.5 ml 0.1 N NaOH and allowed to stand for 1 h to convert the substrate and products to PGB derivatives. The solution was extracted with chloroform (4 × 3 vol). The aqueous phase was acidified to pH 1–2 and extracted with ethyl acetate (4 × 2 vol) and the ethyl acetate phase was evaporated under a stream of N_2 at room temperature. The residue was dissolved in ether:methanol (1:1) and esterified with etheral CH_2N_2 and subjected to hplc analysis and purification using acetonitrile (30%):H_2O (70%) (Fig. 2). The major product (peak 1) had similar retention time in hplc as that of the esterified metabolite from 20-OH-PGB_1 methyl ester (see above); peak 2 represents residual esterified substrate (20-OH-PGB_1-Me). The eluted product containing the small more polar contaminant was isolated as above in incubations of 20-OH-PGB_1 methyl ester, dissolved in methanol, and the λ_{max} determined; the absorption peak was found at 278 nm. An aliquot (2% of total) was subjected to hplc analysis (Fig. 3); again two peaks (minor and major) were observed. The residual solution of the esterified metabolite was silylated with t-butyldimethylsilyl chloride and subjected to GC/MS.

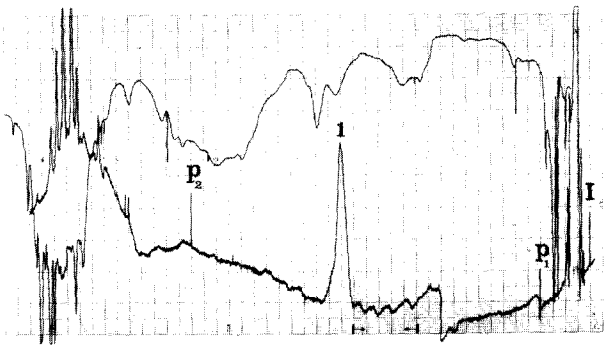

FIG. 1. High pressure liquid chromatography (hplc) of the oxidized metabolite from incubation of 20-OH-PGB_1-Me with guinea pig liver cytosol and NAD. The metabolite (peak 1), assumed to be 20-carboxy-PGB_1-1-methyl ester, was eluted with 16% acetonitrile:84% H_2O using a linear solvent gradient program (P_1) of 100 → 75% H_2O over 25 min. Conditions of hplc: Absorbance monitoring was at 280 nm (bottom scan) and 254 nm (upper scan); AUFS = 0.005; solvent flow = 2 ml/min; recording chart at 1 cm/min. P_2 = linear solvent gradient program from 25 to 90% acetonitrile in 10 min. The markings between arrows on the abscissa delineate 5 min of chromatography.

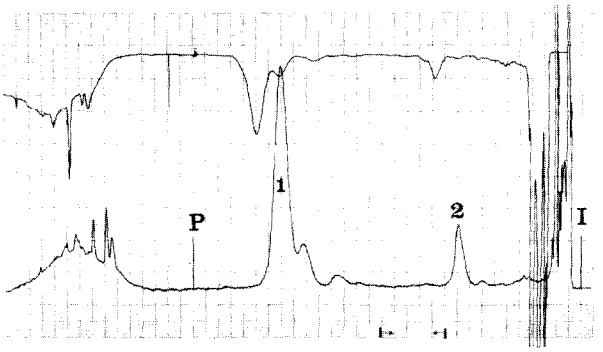

FIG. 2. High pressure liquid chromatography of esterified ethylacetate extract from incubation of 20-OH-PGE$_1$ with guinea pig liver cytosol and NAD. Peak 1 represents the metabolite of interest assumed as being the dimethyl ester of 9-keto-15-hydroxyprosta-8(12),13-diene-1,20-dioic acid. Peak 2 is assumed to be the residual substrate in the form of the methyl ester of 20-OH-PGB$_1$ by having identical retention time on hplc as a similar derivatized authentic compound. Conditions of hplc as in Fig. 1, except that the solvent system was composed of 30% acetonitrile:70% H_2O; AUFS = 0.02; P = linear solvent gradient program to 90% acetonitrile in 10 min.

RESULTS AND DISCUSSION

To establish optimal conditions for enzymatic activity, we utilized an indirect method which monitors, at 340 nm, the stimulation of reduction of NAD by a given ω-OH-FA. As a model substrate we selected 12-OH-L-Me, because 12-OH-L-Me was made available to us in sufficient amounts and because it has been previously assumed with rat liver cytosol that this compound is converted in the presence of NAD to a dicarboxylic acid (15). The stimulation of NAD reduction by 12-OH-L-Me was observed by us with a variety of buffers. Thus, 12-OH-L-Me elevated the rate of reduction of NAD at pH 8.4 (sodium phosphate), at pH 7.4 (Tris or sodium phosphate buffer), and at pH 10 (glycine buffer). Having established optimal conditions for 12-OH-laurate oxidation, we used 20-OH-PGB$_1$ as a model substrate for oxidation of ω-OH-PGs in most of the initial studies. The choice of PGB derivative instead of that of a "natural" ω-OH-PG was to avoid difficulties in interpretation of the cause of stimulation of NAD reduction since oxidation of the 15-hydroxyl by 15-OH-PG dehydrogenase (PGDH) would also be expected to stimulate NAD reduction. PGBs have been reported to be resistant to PGDH (18–20), and thus it was anticipated that stimulation of reduction of NAD by the addition of 20-OH-PGB$_1$

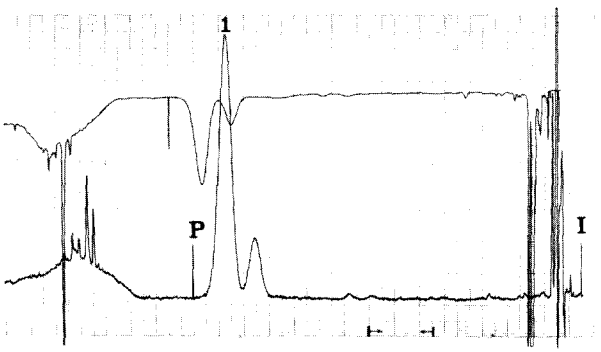

FIG. 3. High pressure liquid chromatography of an aliquot of isolated fraction (peak 1, Fig. 2) Conditions same as in Fig. 2. AUFS = 0.01; 2500 psi.

would indicate only the oxidation of the 20-hydroxyl group. In subsequent studies we observed that under certain conditions which permit the ω-oxidation to proceed, PGE_1 was totally resistant to oxidation and hence we also utilized 20-OH-PGE_1 as a substrate for the ω-OH-dehydrogenase.

In most experiments we used the methyl ester of 20-OH-PGB_1 (20-OH-PGB_1-Me) instead of the free acid. The choice of the methyl ester was for the following reasons: (a) 20-OH-PGB_1-Me is markedly more soluble than 20-OH-PGB_1 in acetone (the vehicle used in the enzymatic studies); (b) to provide a proper comparison with the methyl ester of 12-OH-laurate (12-OH-L-Me); and (c) to facilitate the differential extraction of the oxidized product from the residual substrate (see Isolation of Metabolites from Incubations of 20-OH-PGB_1 and 20-OH-PGE_1 under Materials and Methods).

Table I shows that the 105,000g supernate of liver homogenate from rat, rabbit, and guinea pig catalyzes the reduction of NAD by carboxyl containing compounds (lauric acid or PGs) possessing an ω-hydroxyl group[5]; there was no stimulation of NAD reduction when the hydroxyl was at the penultimate position (ω-1) [19-OH-PGB_1, 19-OH-PGB_1-Me, or 11-OH-L] or in the absence of an ω-hydroxyl group (PGE_1, $PGF_{1\alpha}$, PGB_1). NADP could not substitute for NAD, since 12-OH-L-Me and 20-OH-PGB_1 could not stimulate NADP reduction. Though the reaction with NAD proceeded at similar rates at pH values of 7.4 (Tris or phosphate), 8.4 (phosphate), and 10 (glycine), pH 8.4 was selected for subsequent studies. The reason for this choice was that at pH 10, PGEs might undergo conversion to PGBs and hydrolysis of the methyl esters may also occur; also at pH 10, PGE_1 stimulated NAD reduction to a small extent (ΔA/min = 0.007), probably due to slow oxidation of the 15-OH by the cytosolic PGDH. Finally, pH 8.4 was selected, in preference to pH 7.4, to facilitate solubility of nonesterified PGs.

By contrast to liver cytosol, guinea pig *kidney* cytosol oxidized PGE_1 at all pH values examined, with highest activity being at pH 10 (Table II), suggesting the action of PGDH.[6] However, there was

TABLE I

EFFECT OF 12-OH-LAURATE (12-OH-L) AND ITS METHYL ESTER (12-OH-L-Me), PROSTAGLANDINS, AND 19 OR 20-HYDROXYPROSTAGLANDINS ON THE RATE OF NAD REDUCTION BY RAT, RABBIT, AND GUINEA PIG LIVER CYTOSOL[a]

Species	Compound added (μg)		ΔA_{340}/min[b] pH 7.4	pH 8.4
Rat	12-OH-L-Me	(50)	0.021	ND[c]
	20-OH-PGB_1-Me	(30)	0.013	ND
Rabbit	12-OH-L-Me	(40)	0.261	0.295
	20-OH-PGE_1	(35)	0.097	0.143
	PGE_1	(45)	0.000	0.000
	11-OH-L	(40)	0.000	0.000
	19-OH-PGB_1	(20)	0.000	ND
Guinea Pig	12-OH-L-Me	(40)	0.060	0.045
	11-OH-L	(40)	0.000	0.000
	12-OH-L	(40)	0.034	ND
	20-OH-PGB_1-Me	(40)	0.052	0.050
	19-OH-PGB_1-Me	(40)	0.000	0.000
	20-OH-PGB_1[d]	(15)	ND	0.063
	19-OH-PGB_1	(12)	ND	0.000
	PGB_1	(13)	ND	0.000
	PGE_1[e]	(60)	0.000	0.000
	PGE_1	(100)	ND	0.000
	$PGF_{1\alpha}$	(60)	0.000	ND
	20-OH-PGE_1	(30)	0.013	0.015

[a] In the rat experiments, 3.0 mM NAD and 0.05 ml cytosol were used; in all other experiments, 4.0 mM NAD and 0.1 ml cytosol were used.

[b] Values of ΔA recorded over 5–10 min, corrected for ΔA in absence of compound added.

[c] Not determined.

[d] 20-OH-PGB_1 exhibited difficulties in solubility in acetone (the vehicle used), hence usually the methyl ester was used.

[e] At pH 10 (glycine buffer), a slight stimulation of NAD reduction (ΔA/min = 0.007) was observed suggesting action of 15-OH-PGDH.

[5] Though the rate of NAD reduction was usually higher with 12-OH-L-Me than with the 20-OH-PGs, the extent of NAD reduction (leveling off point) was dramatically higher with 20-OH-PGE_1 than with similar concentrations of 12-OH-L-Me, indicating marked differences in degrees of oxidation of the two substrates at equilibrium.

[6] Further evidence that the stimulation of NAD reduction by PGE_1 with kidney cytosol resulted in oxidation of the 15-hydroxyl was obtained by addition of several drops of 4 N NaOH to the reaction mixture

TABLE II

EFFECT OF 12-HYDROXYLAURATE METHYL ESTER (12-OH-L-Me) AND PROSTAGLANDINS ON THE RATE OF NAD REDUCTION BY GUINEA PIG KIDNEY CYTOSOL[a]

Compound (μg)		ΔA_{340}/min pH 7.4	8.4	10
PGE_1	(30)			0.017
PGE_1	(40)	0.003	0.003[b]	0.022[b]
PGE_1	(60)			0.034
PGB_1	(40)	0.000	0.000	0.001
12-OH-L-Me	(40)			0.006

[a] Conditions were as in Table I.

[b] When NADP was used instead of NAD, there was little or no evidence of stimulation of reduction at 340 nm by PGE_1 at pH 8.4 and 10.0.

only minimal stimulation of NAD reduction by 12-OH-L-Me at pH 10. Similarly, we observed that rat kidney cytosol supported only minimally the 12-OH-L-Me-mediated reduction of NAD (ΔA_{340} = 0.006/min). These results indicate that the ω-OH-FA dehydrogenase is low in the kidney and that PGDH and ω-OH-FA dehydrogenase are different enzymes.

There have been contrasting observations on whether the oxidation of hydroxy-fatty acids is catalyzed by alcohol dehydrogenase (ADH) or by a different enzyme(s) (13–16). Rat liver ADH catalyzes the oxidation of both the primary and secondary hydroxylated fatty acids (12), whereas our liver preparation catalyzed the oxidation of the primary alcohols but not of the secondary alcohols, suggesting that the catalytic activity was not due to ADH.

Also by contrast to previous observations that rat and horse liver ADH catalyze the oxidation of ω-OH-FAs (15, 16), we could not demonstrate a stimulation of NAD reduction by horse liver ADH in presence of 12-OH-L-Me, though the activity of the ADH toward ethanol was high (not shown).

To further explore the nature of the guinea pig liver ω-OH-FA dehydrogenase, we examined the effect of pyrazole, a potent inhibitor of ADH but not of aldehyde dehydrogenase (22, 23), on the stimulation of NAD reduction by ethanol, 12-OH-L-Me, and 20-OH-PGE_1 (Table III). Surprisingly, pyrazole markedly inhibited the stimulation of NAD reduction by the compounds examined, indicating that the guinea pig liver enzyme has certain ADH characteristics.

To determine the nature of the products formed from incubations of 20-OH-PGB_1-Me and 20-OH-PGE_1, the products were isolated and characterized by a combination of their solubility characteristics and chromatographic polarities in hplc and by GC/MS fragmentation patterns of the derivatized products. Figure 4 demonstrates the GC/MS fragmentation of the esterified and silylated metabolites from incubations of 20-OH-PGB_1-Me and 20-OH-PGE_1. The spectra exhibited a molecular ion at *m/e* 508 and a peak at *m/e* 477 ($M^+ - CH_3O$). Other fragments were at *m/e* 451 (M^+ – [57

TABLE III

EFFECT OF PYRAZOLE ON THE OXIDATION OF ETHANOL, 12-HYDROXYLAURATE METHYL ESTER (12-OH-L-Me), AND 20-OH-PGE_1 BY GUINEA PIG LIVER CYTOSOL[a]

Substrate (mM)	Pyrazole (mM)	ΔA_{340}/min	Percentage control
Ethanol (1.0)	—	0.046	100
Ethanol (1.0)	1	0.006	13
Ethanol (2.0)	—	0.062	100
Ethanol (2.0)	1	0.001	2
Ethanol (170)	—	0.051	100
Ethanol (170)	1	0.030	59
Ethanol (170)	2	0.020	39
20-OH-PGE_1 (0.5)	—	0.013	100
20-OH-PGE_1 (0.5)	2	0.002	15
12-OH-L-Me (0.2)	—	0.047	100
12-OH-L-Me (0.2)	1	0.015	32
12-OH-L-Me (0.2)	2	0.010	21

[a] Conditions as in Table I; phosphate buffer, pH 8.4, NAD (4 mM), and 0.1 ml of cytosol was used.

which brought the pH to ≥10.0, yielding transient dark yellow color with λ_{max} at about 500 nm which faded after a few minutes (this suggestive evidence for 15 keto-PGE_1 was pointed out to us by Dr. N. H. Andersen).

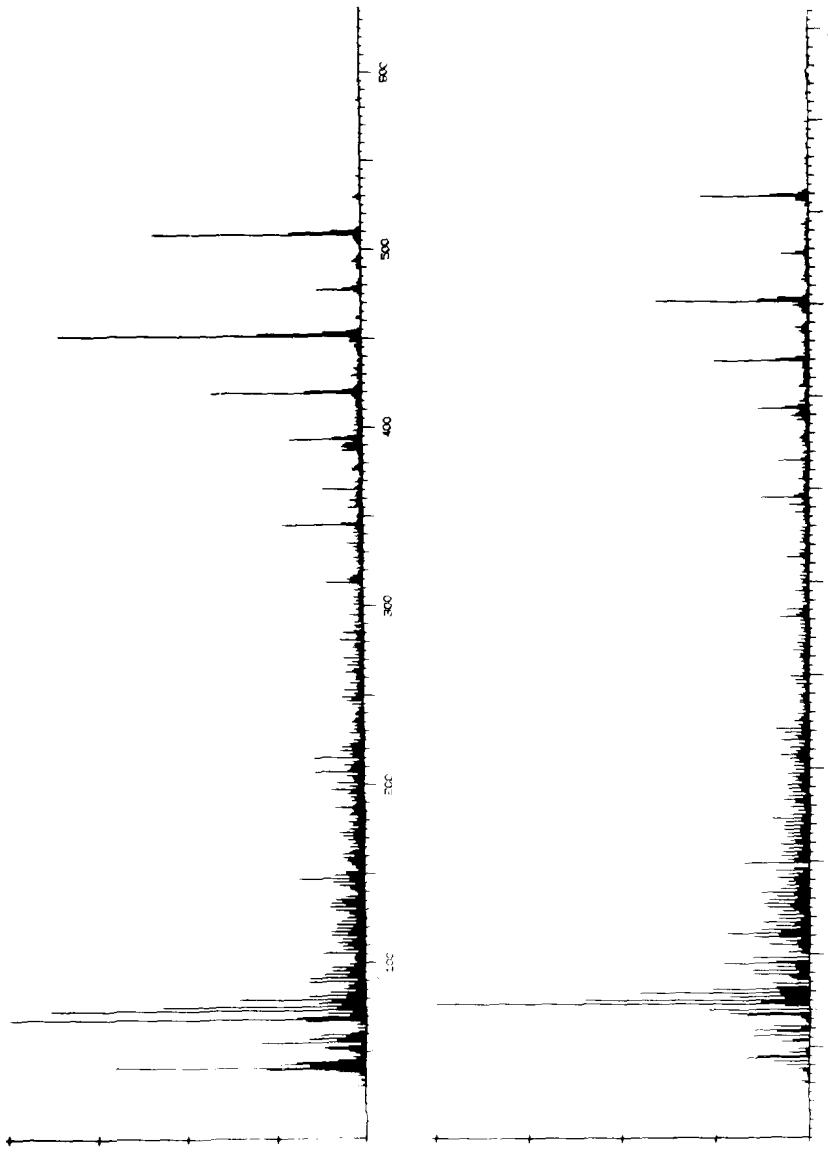

FIG. 4. GC/MS fragmentation patterns of the t-butyldimethylsilyl methyl esters of metabolites isolated from incubations of 20-OH-PGB$_1$-Me (upper figure) and 20-OH-PGE$_1$ (lower figure). The isolation, preparation, and purification of derivatives are described under Materials and Methods; the conditions of the GC/MS were essentially as previously described (9); however, the GC column (1% SE30 on Gaschrom Q) was operated isothermally at 250°C; injection port 250°C; manifold 250°C. The esterified metabolites from 20-OH-PGB$_1$-Me and of 20-OH-PGE$_1$ after base treatment had similar GC retention times each exhibiting two peaks: a minor peak (10 min) and a major peak (10.7 min); both peaks showed similar mass fragmentations, suggesting that they represent different stereoisomers or enantiomers of the same compound.

+ CH_3OH]), 393, 365 (M^+ − 143[side chain]), 345, 313. Though a similarly derivatized authentic 9-keto-15-hydroxyprosta-8(12),13-diene-1,20-dioic acid was not available to us, the mass fragmentations of the dimethyl ester silylated metabolites from incubations of 20-OH-PGB_1-Me and 20-OH-PGE_1 are consistent with that structure.

In addition, the evidence derived from the differential extraction from bicarbonate of the parent compound versus the metabolite from incubation of 20-OH-PGB_1-Me and the relative retention times in hplc of the metabolites as free acids, monomethyl esters, and dimethyl esters supports our conclusion of the oxidation of the ω-hydroxyl to the corresponding dicarboxylic acid. Finally the oxidation product of 12-OH-L-Me was isolated by a procedure similar to that of the metabolite from incubation of 20-OH-PGB_1-Me. The product was esterified with CH_2N_2 and characterized by GC/MS as the dimethyldodecanedioate by comparing with an authentic dimethyldodecanedioate (purchased from Analabs, Inc., New Haven, Conn.).

In conclusion, we have established that mammalian liver cytosol from several species contains an NAD-dependent ω-OH-FA dehydrogenase which catalyzes effectively the oxidation of ω-OH-PGs to the corresponding dicarboxylic acids. Whether the oxidation of ω-OH-PGs involves two discrete steps catalyzed by different enzymes is currently not known. Also, whether the formation of the dicarboxylic PG derivatives *in vivo* is catalyzed by the enzyme in liver or in other tissues has not been yet established. Nevertheless, these findings support the speculation that urinary ω-carboxy-PG metabolites are formed by ω-hydroxylation catalyzed by an NADPH-dependent microsomal *P*-450 monooxygenase with the subsequent oxidation of the ω-hydroxyl by an NAD-dependent cytosolic ω-OH-"fatty acid" dehydrogenase.

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