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Reductive β-scission of the hydroperoxides of fatty acids and xenobiotics: Role of alcohol-inducible cytochrome P-450

(lipid peroxidation/isozymes of rabbit cytochrome P-450/formation of 13-oxo-9,11-tridecadienoic acid/hydrocarbon formation in microsomal membranes/reductive cleavage of hydroperoxides by P-450 form IIE1)

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ABSTRACT **As shown previously in this laboratory, purified rabbit liver microsomal cytochrome P-450 form 2 (P-450 IIB4) catalyzes the reductive cleavage of hydroperoxides to yield hydrocarbons and either aldehydes or ketones. We have proposed that lipid hydroperoxides are the physiological substrates for the cleavage reaction and have shown that with 13-hydroperoxy-9,11-octadecadienoic acid the formation of pentane is roughly equimolar with respect to the NADPH consumed. In the present study, the other product was isolated and identified as 13-oxo-9,11-tridecadienoic acid. Of particular interest, the alcohol-inducible form of liver microsomal cytochrome P-450 form 3a (P-450 IIE1) is the most active of the isozymes examined in the reductive β-scission of the 13-hydroperoxide derived from linoleic acid and the 15-hydroperoxide derived from arachidonic acid as well as the model compounds cumyl hydroperoxide (α,α-dimethylbenzyl hydroperoxide) and *t*-butyl hydroperoxide. In general, the forms of P-450 with lower activity, as judged by the rate of NADPH oxidation in the reconstituted system, give less of the cleavage products (hydrocarbon and oxo compound) and catalyze direct reduction of the hydroperoxides to the corresponding hydroxy compounds. The occurrence of the reductive cleavage reaction in liver microsomal membranes was demonstrated, and microsomes from animals treated with ethanol or acetone (P-450 IIE1 inducers) or phenobarbital (a P-450 IIB4 inducer) were more active than those from untreated animals. We suggest that the alcohol-inducible P-450, in addition to its known deleterious effects in chemical toxicity and chemical carcinogenesis, may enhance the reductive cleavage of lipid hydroperoxides with a resultant loss in membrane integrity.**

Many reactions are known in which the cytochrome P-450 enzyme system catalyzes the hydroxylation of various naturally occurring as well as foreign compounds in the presence of molecular oxygen and a reduced pyridine nucleotide (1). In addition, peroxy compounds can serve as the oxygen donor in the absence of a reductant, as shown with liver microsomes (2–4) and with purified P-450 (5). With cumyl hydroperoxide (α,α-dimethylbenzyl hydroperoxide), for example, P-450 functions as a peroxygenase by a mechanism apparently involving homolytic oxygen-oxygen bond cleavage, and cumyl alcohol (α,α-dimethylbenzyl alcohol) and the hydroxylated substrate are the products formed (1, 6).

This laboratory found that P-450 catalyzes the formation of hydrocarbons from hydroperoxides (7). In a reconstituted system containing purified rabbit liver microsomal P-450 form 2*, NADPH-cytochrome P-450 reductase (NADPH:ferricytochrome oxidoreductase, EC 1.6.2.4), and NADPH, cumyl hydroperoxide gives methane and acetophenone, but no cumyl alcohol is formed. Since 13-hydroperoxy-9,11-octadecadienoic acid (13-HOO-$C_{18:2}$), derived from linoleic acid, yields pentane under these conditions, we proposed that lipid peroxides may be the physiological substrates for the reductive cleavage reaction we found using xenobiotic hydroperoxides as substrates (7). The report by Hochstein and Ernster (9) that ADP-activated lipid peroxidation is coupled to the NADPH oxidase of microsomes was the first to implicate this enzyme system in the degradative process. As reviewed in our earlier paper (7), others have considered various ways in which microsomal enzymes might initiate or promote lipid peroxidation, but the role of P-450 in hydrocarbon formation may now be attributed to catalysis of the following general reaction (Eq. 1), in which X represents an alkyl or aryl group (or carboxyalkenyl group in the case of the fatty acid hydroperoxides) and R and R′ are either alkyl groups or hydrogen atoms:

$$XRR'C\text{-}OOH + NADPH + H^+ \rightarrow XRCO + R'H + H_2O + NADP^+. \quad [1]$$

In the present paper we present evidence that the alcohol-inducible form of P-450 is the most active in the reductive β-scission of fatty acid and xenobiotic hydroperoxides to give hydrocarbons and aldehydes or ketones. The additional products formed along with pentane from 13-HOO-$C_{18:2}$ have been identified as the 11-*cis* and 11-*trans* forms of 13-oxo-9,11-tridecadienoic acid. Cumyl alcohol is not produced from cumyl hydroperoxide and the hydroxy fatty acid is not produced from the fatty acid hydroperoxide when P-450 form 3a or form 2 is present, but these hydroxy compounds are generated by P-450 forms 3b, 3c, and 4, which are less active as judged by the rate of hydroperoxide-dependent NADPH oxidation. The occurrence of the β-scission reaction in microsomal membranes has also been established.

MATERIALS AND METHODS

Microsomal Preparations and Purified Enzymes. Male New Zealand White rabbits (2.0–2.5 kg in weight) were (*i*) untreated, (*ii*) given 10% (vol/vol) ethanol for 14 days, 1% (vol/vol) acetone for 7 days, or 0.1% (wt/vol) phenobarbital in place of the drinking water, or (*iii*) given isosafrole (150 mg/kg) dissolved in corn oil intraperitoneally for 3 consecutive days. All animals were fasted 12–14 hr before being sacrificed. Pyrophosphate-washed liver microsomes were prepared as described (10) and stored at −70°C in 100 mM Tris acetate (pH 7.4) containing 0.1 mM EDTA and 20%

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Abbreviations: 13-HOO-$C_{18:2}$, 13-hydroperoxy-9,11-octadecadienoic acid; 13-HO-$C_{18:2}$, 13-hydroxy-9,11-octadecadienoic acid; 15-HOO-$C_{20:4}$, 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid.

*P-450 forms 2, 3a, 3b, 3c, 4, and 6 represent individual forms of rabbit liver microsomal cytochrome P-450, which have been designated, on the basis of structural homology, as forms IIB4, IIE1, IIC3, IIIA6, IA2, and IA1, respectively (8).

(vol/vol) glycerol. The microsomes prepared in this way had a specific content of P-450, expressed as nmol per mg of protein for the various treatments, as follows: untreated, 1.6; ethanol, 1.2; acetone, 1.9; isosafrole, 1.9; and phenobarbital, 2.6. The individual P-450 cytochromes were purified from rabbit liver microsomes as summarized (11), except that form 3a is now routinely purified from liver microsomes of acetone-induced (12) rather than alcohol-induced animals (13) because of the improved yield. NADPH-cytochrome P-450 reductase was also purified from rabbit liver microsomes (14).

Product Analysis. Quantitative analysis of the ketone or alcohol product was done on a Waters μBondapack C_{18} reverse-phase analytical column by an automated HPLC system consisting of a Waters model 600 solvent delivery system and a model 490 UV/visible detector (or a Waters model 501 solvent delivery system and a model 484 absorbance detector set at the appropriate wavelength maximum of the product), a Waters WISP model 710 auto-sampler, and a Hewlett–Packard model 3600 integrator. An isocratic solvent system consisting of acetonitrile and water was used for the analysis of the products from cumyl hydroperoxide, whereas acetonitrile and water with 0.1% (vol/vol) trifluoroacetic acid was used for the analysis of the products from 13-HOO-$C_{18:2}$. The concentration of acetonitrile in the solvent system was adjusted so that the alcohol or ketone product did not appear as a shoulder on the NADP/NADPH front. The reactions were quenched with perchloric acid at a final concentration of 3%, and 50–150 μl of each assay mixture was used for analysis. The areas of the peaks were found to be linear with respect to known amounts of the standards in the range of 5–1000 pmol for acetophenone, 2.5–75 nmol for cumyl alcohol, 30–700 pmol for the aldehyde acid, and 150–600 pmol for 13-hydroxy-9,11-octadecadienoic acid (13-HO-$C_{18:2}$).

Isolation and NMR Analysis of 13-Oxo-9,11-Tridecadienoic Acid. A 25-ml reaction mixture containing 50 nmol of P-450 form 2, 50 nmol of NADPH-cytochrome P-450 reductase, and 800 μg of a sonicated aqueous suspension of 1,2-dilauroyl-*sn*-glycero-3-phosphocholine in 50 mM potassium phosphate buffer (pH 7.4) was made anaerobic by repeated purging with oxygen-free, water-saturated nitrogen. Into this mixture was injected 1.0 ml of an anaerobic NADPH solution (50 μmol) followed by 0.2 ml of an ethanolic solution of 13-HOO-$C_{18:2}$ (40 μmol). The mixture was incubated at 25°C for 45 min, after which 5 ml of 6 M aqueous HCl was injected to quench the reaction, and extraction was carried out with two 3-ml aliquots of $CHCl_3$. The combined extract was dried under a stream of dry nitrogen, and the residue was dissolved in 0.4 ml of acetonitrile, diluted to 1.5 ml with distilled water, and examined by HPLC. For this purpose, the solution was applied to a Whatman preparative C_{18} reverse-phase column (10 mm × 30 cm) that was developed isocratically with 55% acetonitrile/45% water containing 0.1% trifluoroacetic acid at a flow rate of 6.0 ml/min. The effluent was monitored at 280 nm, and the peak fractions, which appeared between 12 and 16 min, were pooled, diluted with 30 ml of 1 M KH_2PO_4, and extracted with three 5-ml aliquots of methylene chloride. The combined extract was dried over anhydrous sodium sulfate, and the solvent was evaporated under a stream of nitrogen. The residue was dried under reduced pressure (20

Table 1. Activity of various forms of liver microsomal cytochrome P-450 in catalyzing NADPH oxidation by model hydroperoxides

Form of P-450	Hydroperoxide-dependent rate of NADPH oxidation, nmol/min per nmol of P-450	
	Cumyl hydroperoxide	*t*-Butyl hydroperoxide
2	31 ± 2	60 ± 4
3a	58 ± 2	84 ± 3
3b	16 ± 1	4 ± 1
3c	21 ± 2	5 ± 2
4	17 ± 3	9 ± 1
6	13 ± 3	4 ± 1

The complete anaerobic system used for rate measurements was as described (7), with 0.5 μM NADPH and hydroperoxides at the following concentrations: cumyl, 0.4 mM; *t*-butyl, 1.0 mM. The form of P-450 used and the reductase were at a 1:1 molar ratio, 1,2-dilauroyl-*sn*-glycero-3-phosphocholine was used at a concentration of 30 μg/ml, and the rates were determined at 25°C. The data are an average of the results from at least three experiments. The rate of hydroperoxide-dependent NADPH oxidation was constant for at least 3 min and directly proportional to the concentration of the P-450 isozymes; each cytochrome was tested at concentrations of 0.1, 0.3, and 0.5 μM.

Table 2. Stoichiometry of product formation from model hydroperoxides with various forms of P-450

Form of P-450	Hydroperoxide	Product formed, nmol				Peroxide consumed/ ketone formed/ alcohol formed/ methane formed, molar ratio
		Acetophenone	Cumyl alcohol	Acetone	Methane	
2	Cumyl	91 ± 2	ND		70 ± 4	1.0:0.9:0:0.7
	t-Butyl			174 ± 8	190 ± 7	1.0:0.9:—:1.0
3a	Cumyl	87 ± 1	ND		71 ± 8	1.0:0.9:0:0.7
	t-Butyl			181 ± 7	188 ± 5	1.0:0.9:—:0.9
3b	Cumyl	35 ± 5	60 ± 4		<10*	1.0:0.4:0.6:<0.1
	t-Butyl			80 ± 3	43 ± 17	1.0:0.4:—:0.2
3c	Cumyl	21 ± 3	75 ± 9		<10	1.0:0.2:0.8:<0.1
	t-Butyl			60 ± 8	53 ± 10	1.0:0.3:—:0.3
4	Cumyl	51 ± 4	43 ± 8		60 ± 0	1.0:0.5:0.4:0.6
	t-Butyl			59 ± 2	41 ± 6	1.0:0.3:—:0.2
6	Cumyl	40 ± 4	—		46 ± 6	1.0:0.4:—:0.5
	t-Butyl			71 ± 7	62 ± 4	1.0:0.4:—:0.3

The complete anaerobic reaction mixtures used for product analysis were as described (7), with 2 μM P-450 and reductase, 0.2 mM NADPH, 0.033 mM cumyl hydroperoxide or 0.067 mM *t*-butyl hydroperoxide, and 1,2-dilauroyl-*sn*-glycero-3-phosphocholine at a concentration of 102 μg per ml, in a volume of 3.0 ml at 25°C. When NADPH oxidation had ceased, as determined spectrophotometrically, the reaction mixtures were analyzed. The production of *t*-butanol, the other expected product in the experiments with *t*-butyl hydroperoxide, was not measured. The hydroperoxides were found to be completely consumed. The data are the mean of three experiments. ND, not detected; —, not determined.
*The cause of the unexpectedly low yield of methane, which was reproducible, is not known.

Table 3. Activity of various forms of liver microsomal cytochrome P-450 in catalyzing NADPH oxidation by lipid hydroperoxides

Form of P-450	Hydroperoxide-dependent rate of NADPH oxidation, nmol/min per nmol of P-450	
	13-HOO-$C_{18:2}$	15-HOO-$C_{20:4}$
2	24 ± 4	36 ± 5
3a	33 ± 6	41 ± 6
3b	4 ± 1	
3c	10 ± 1	
4	6 ± 0	

The conditions were as described in Table 1, with 0.05 mM hydroperoxide and 0.15 mM NADPH in a volume of 1.0 ml. The fatty acid hydroperoxides were prepared with soybean lipoxidase as described for 13-HOO-$C_{18:2}$ (7). The rate was constant for at least 4 min. The data are an average of two experiments.

μm of Hg) overnight and then dissolved in 0.5 ml of C^2HCl_3 (99.9%), and the ^1H NMR spectrum was recorded with a General Electric model GN 500 NMR spectrometer.

RESULTS AND DISCUSSION

The relative activities of six purified forms of rabbit liver microsomal cytochrome P-450 in hydroperoxide-dependent NADPH oxidation in the reconstituted enzyme system are shown in Table 1. Alcohol-inducible P-450 form 3a was clearly the most active with both cumyl hydroperoxide and *t*-butyl hydroperoxide, followed by phenobarbital-inducible P-450 form 2, whereas the other four forms of the cytochrome were much less active. It should be noted that P-450 form 2 was the isozyme used in previous experiments in which the β-scission reaction was found (7).

The stoichiometry of the reaction was then examined with all of the P-450 isozymes under conditions where the NADPH was in excess and the reaction was allowed to go to completion. As shown previously with P-450 form 2, under these conditions the hydroperoxide is completely consumed and NADPH is oxidized in an equimolar amount (7). The same was found to be true for all of the other P-450 isozymes used in the experiments summarized in Table 2, in which acetophenone and methane were determined to be the products of cumyl hydroperoxide cleavage and acetone and methane were determined to be the products of *t*-butyl hydroperoxide cleavage. The results show that P-450 isozymes 3a and 2 give the highest yields of these products at completion of the reaction and that the carbonyl compound and the alkane are produced in roughly a 1:1 molar ratio with respect to the peroxide consumed. On the other hand, the four forms of P-450 that were less active in the rate measurements of NADPH oxidation were found to give much lower amounts of the ketone and methane in end-point determinations than expected from the amounts of hydroperoxide and NADPH consumed. In the experiments with cumyl hydroperoxide, the reaction mixtures were therefore examined for cumyl alcohol; none was detected with P-450 forms 2 and 3a, but with forms 3b, 3c, and 4, the amount of the alcohol formed was compatible with the low yield of acetophenone. No attempt was made to look for *t*-butanol, the other expected product in the experiments with *t*-butyl hydroperoxide, but with cumyl hydroperoxide as the substrate, it is evident that the P-450 isozymes that are less active in the reductive cleavage reaction carry out to a significant extent the direct reduction of the hydroperoxide to the corresponding alcohol.

We have previously proposed that lipid hydroperoxides are the naturally occurring substrates for the reductive cleavage reaction and have shown that the expected hydrocarbon, pentane, is indeed produced from 13-HOO-$C_{18:2}$, the hydroperoxide derived from linoleic acid (7). As shown in Table 3, P-450 isozyme 3a, followed by isozyme 2, is the most active as judged by the rate of NADPH oxidation in the reconstituted system when either 13-HOO-$C_{18:2}$ or 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid (15-HOO-$C_{20:4}$), derived from arachidonic acid, serves as the substrate; isozymes 3b, 3c, and 4 are less than one-third as active as isozyme 3a with the C_{18} fatty acid hydroperoxide.

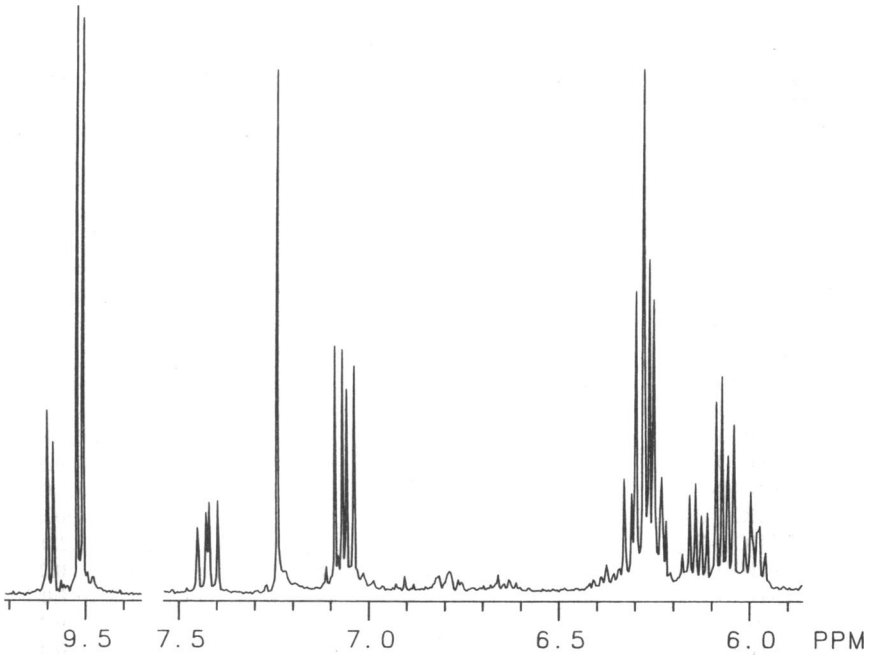

FIG. 1. NMR spectrum of the carbonyl compound derived from reductive cleavage of 13-HOO-$C_{18:2}$. The assignment of the 9, 10, 11, 12, and 13 protons, obtained by proton decoupling experiments, is as follows. For the 11-*cis* isomer: H-13, 9.59 ppm, $J_{13,12}$ = 7.5 Hz; H-12, 6.13 ppm, $J_{12,13}$ = 7.5 Hz and $J_{12,11}$ = 6.0 Hz; H-11, 7.42 ppm, $J_{11,12}$ = 6.0 Hz and $J_{11,10}$ = 12.5 Hz; H-10, 6.25 ppm (multiplet); H-9, 6.23 ppm (multiplet). For the 11-*trans* isomer: H-13, 9.51 ppm, $J_{13,12}$ = 7.5 Hz; H-12, 6.06 ppm, $J_{12,13}$ = 7.5 Hz and $J_{12,11}$ = 16.0 Hz; H-11, 7.07 ppm, $J_{11,12}$ = 16.0 Hz and $J_{11,10}$ = 10.0 Hz; H-10, 6.29 ppm (multiplet); H-9, 5.98 ppm (multiplet).

According to Eq. 1, the reductive rearrangement of the fatty acid hydroperoxides to give pentane would be expected to yield an aldehyde acid as the other product. In the case of 13-HOO-$C_{18:2}$, the reaction was carried out on a large scale, and the product was purified as described in *Materials and Methods*. The UV absorption was that expected for a conjugated carbonyl compound, with a maximum at 280 nm, and the behavior on HPLC under different conditions was identical to that of authentic 13-oxo-9,11-tridecadienoic acid (15). The NMR analysis (Fig. 1) indicated that the product was a 1:3 mixture of the 9-*cis* and 9-*trans* isomers of the unsaturated aldehyde acid, which were not resolved by HPLC under the conditions used.

The stoichiometry of the reaction with fatty acid hydroperoxides, including peroxide consumption, NADPH oxidation, pentane formation, and, in some instances with 13-HOO-$C_{18:2}$, aldehyde acid formation, was then determined with the various P-450 isozymes. As shown in Table 4, the results support the application of Eq. 1 to fatty acid hydroperoxides as substrates in that the consumption of the two reactants and formation of the two products were roughly equimolar when the P-450 isozymes of highest catalytic activity, forms 3a and 2, were used. Thus, the findings are in accord with the stoichiometric determinations made with the model hydroperoxides. A further similarity is that with the fatty acid hydroperoxides the reductive β-scission reaction, as judged by pentane formation, is not the sole pathway with the less active cytochromes, forms 3b, 3c, 4, and 6. With 13-HOO-$C_{18:2}$ and isozymes 3b, 3c, and 4, the aldehyde acid was formed at approximately the same low level as the pentane. By analogy to the increased formation of cumyl alcohol from cumyl hydroperoxide with the P-450 cytochromes of lower catalytic activity, 13-HO-$C_{18:2}$ would be expected to be the product of direct reduction not involving pentane formation. The authentic compound, made by sodium borohydride reduction of 13-HOO-$C_{18:2}$, had the same retention time as the enzymatically generated product, the amount of which is indicated in Table 4 in five of the experiments.

To determine the extent of the β-scission reaction in intact liver microsomal membranes from animals that were (*i*) uninduced, (*ii*) treated with ethanol or acetone as inducers of P-450 form 3a, or (*iii*) treated with phenobarbital as an inducer of P-450 form 2, the formation of methane from cumyl hydroperoxide was determined as shown in Table 5. The procedures known to induce the two isozymes most active in the β-scission reaction increased the rate of the reaction significantly over that in the microsomes from the uninduced animals. Experiments for which the results are not presented were then carried out to determine the effect of these inducers as well as of isosafrole, an inducer of P-450 form 4 (16), on acetophenone and cumyl alcohol formation. The results show that the β-scission reaction occurs with all of the preparations, as judged by formation of the ketone, as well as direct reduction of the hydroperoxide with formation of the alcohol. The largest yield of cumyl alcohol was with microsomes from the isosafrole-treated animals, which is in accord with the known effect of isozyme 4 in favoring peroxide reduction without β-scission, as indicated above. In other experiments that are not presented, microsomal membranes from animals treated in various ways were found to convert added 13-HOO-$C_{18:2}$ to the aldehyde acid and pentane, as well as other products that remain to be identified. In this connection, Ekström and Ingelman-Sundberg (17) have reported that the formation of thiobarbituric acid-reactive substances is correlated with the level of P-450 IIE1 in rat liver microsomes.

Table 5. Methane formation from cumyl hydroperoxide in microsomes

Inducer	Turnover number, nmol of CH_4 per min per nmol of total P-450
None	1.1 ± 0.1
Ethanol	1.7 ± 0.2
Acetone	1.6 ± 0.1
Phenobarbital	1.9 ± 0.1

The reaction mixtures contained liver microsomes from male New Zealand White rabbits that had been treated as indicated (2 μM total P-450), 0.75 mM cumyl hydroperoxide, and 1.0 mM NADPH in potassium phosphate buffer, pH 7.4. The incubation was for 15 min, and methane in the head space was then determined by gas chromatographic analysis (7). The results are the mean of three experiments. Methane formation was linear up to 25 min under the conditions used.

Epidemiological evidence has revealed a link between chronic alcohol intake and cancer and other pathological states (18, 19), and increasing attention has been paid in recent years to the role of cytochrome P-450 in alcohol-related chemical toxicities, mutagenesis, and carcinogenesis. In many instances, the alcohol-inducible form of the cy-

Table 4. Stoichiometry of product formation from fatty acid hydroperoxides with various forms of P-450

Form of P-450	Hydroperoxide	Product formed, nmol: Aldehyde acid	Hydroxy acid	Pentane	Hydroperoxide consumed/ aldehyde acid formed/ hydroxy acid formed/ pentane formed, molar ratio
2	13-HOO-$C_{18:2}$	47 ± 2	ND	43 ± 5	1.0:0.9:0:0.9
	15-HOO-$C_{20:4}$			43 ± 8	1.0:—:—:0.9
3a	13-HOO-$C_{18:2}$	46 ± 2	ND	41 ± 7	1.0:0.9:0:0.8
	15-HOO-$C_{20:4}$			46 ± 8	1.0:—:—:0.9
3b	13-HOO-$C_{18:2}$	21 ± 5	5 ± 0	15 ± 4	1.0:0.4:0.1:0.3
	15-HOO-$C_{20:4}$			18 ± 1	1.0:—:—:0.4
3c	13-HOO-$C_{18:2}$	10 ± 1	7 ± 2	9 ± 3	1.0:0.2:0.2:0.2
	15-HOO-$C_{20:4}$			8 ± 3	1.0:—:—:0.2
4	13-HOO-$C_{18:2}$	15 ± 1	7 ± 2	11 ± 3	1.0:0.3:0.2:0.2
	15-HOO-$C_{20:4}$			11 ± 3	1.0:—:—:0.2
6	13-HOO-$C_{18:2}$			19 ± 4	1.0:—:—:0.4
	15-HOO-$C_{20:4}$			5 ± 3	1.0:—:—:0.1

The reaction mixtures were as described in Table 3, but the reactions were allowed to go to completion as judged by NADPH oxidation. NADPH oxidation was equimolar with respect to hydroperoxide consumption. The data are the average of three experiments. ND, not detected; —, not determined.

tochrome is primarily involved. For example, chronic ethanol ingestion results in increased hepatic microsomal *N*-nitrosodimethylamine demethylase activity and an increase in the microsomal activation of this agent to a mutagen (20). Yang *et al.* (21) examined the dealkylation of a series of nitrosamines by the purified cytochromes and showed that P-450 form 3a is the most active toward *N*-nitrosodimethylamine and is the "low K_m" form of the cytochrome responsible for the activation of this substrate in microsomes. As another example, acetaminophen, a widely used antipyretic and analgesic drug, is normally nontoxic, but in large doses or after chronic exposure of animals and humans to ethanol produces acute hepatic necrosis (22, 23). In this case, P-450 form 3a proved to be more active than isozymes 2, 3b, 3c, and 6 (but not isozyme 4) in converting the drug to the reactive intermediate that is capable of conjugating with glutathione in the reconstituted enzyme system (11). A variety of other chemicals also undergo increased metabolism after ethanol treatment of animals, including procarcinogens such as benzene (24, 25), as reviewed elsewhere (26). The findings in the present paper on the relatively high activity of P-450 form 3a in the reductive cleavage of lipid hydroperoxides raise the interesting question of whether the enhancement of this reaction, with a consequent loss of membrane integrity, may be yet another harmful effect of increased levels of alcohol-inducible cytochrome P-450. The possible importance of P-450 IIE1-dependent lipid peroxidation *in vivo* after ethanol abuse has also been pointed out by Ekström and Ingelman-Sundberg (17).

It should be noted that P-450 is one of a number of autoxidizable, biologically occurring electron carriers that may contribute to the formation of lipid hydroperoxides by generating oxygen radicals (27). On the other hand, P-450 cytochromes, particularly alcohol-inducible form 3a and, secondarily, phenobarbital-inducible form 2, are apparently the only membranous enzymes capable of converting these intermediates to hydrocarbons with shortening of the fatty acyl chains.

A function has yet to be established for lipid peroxidation, which is generally looked on as a destructive event in various membranes of living cells. Several products have been reported to arise, including alkanes, and hydrocarbon exhalation is believed to be a measure of this pathophysiological process *in vivo* (28). Since hydrocarbons are hydroxylated by the P-450 system to form the alcohols (29, 30), eventual oxidation to carbon dioxide, by means of the fatty acids, may occur. In the case of the other product of the β-scission reaction, the aldehyde acid, biological roles may exist. Glasgow *et al.* (31) have isolated a 12-oxododecatrienoic acid formed from arachidonic acid in leukocytes and shown that it has activity in the activation of such cells. An analogous compound called traumatin has long been recognized as a plant wound hormone that induces cell elongation and division (32, 33). Stremler *et al.* (34) have recently reported that purified platelet-activating factor acetylhydrolase catalyzes the hydrolysis of the oxidized fragments of arachidonic acid from the *sn*-2 position of phosphatidylcholine. One of the preferred substrates appeared, by mass spectrometry, to have a 5-oxovaleryl residue at the *sn*-2 position, which is the structure that would be predicted from the P-450-catalyzed β-scission of an arachidonyl residue that had formed the 5-hydroperoxide.

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