diameter[2] ($\rho = 0.956$), the solute molar volume[2] ($\rho = 0.988$), and the solute polarizability[2] ($\rho = 0.988$) so that values of $\Delta S_s°$ are also linearly related to these solute properties.[10] If the relationship between $\Delta S_s°$ and $\bar{S}°$ is fundamental, then the observed $\Delta S_s°$ values might be attributed to loss of degrees of freedom of the solute. However, if, for example, the fundamental correlation is that between $\Delta S_s°$ and solute size, the origin of the $\Delta S_s°$ values might then be interpreted in terms of SPT—the larger the solute, the larger the required cavity in the solvent and the more negative the entropy of cavity formation. This illustrates the difficulty (if not the impossibility) of obtaining information on the molecular level from purely macroscopic quantitities (i.e., $\Delta S_s°$ and $\bar{S}°$).

Wertz[7] also suggested that partition coefficients, or $\Delta G_t°$ values, of hydrocarbons between water and 1-octanol could be calculated through eq 4, where $\Delta S_s°$ is the entropy of solution of the hydrocarbon in water. In the deduction of eq 4, Wertz assumed that the corresponding value of $\Delta H_t°$ was zero. Using eq 4, Wertz was able to reproduce the $\Delta G_t°$ values for the alkanes methane

$$\Delta G_t° \text{ (water} \rightarrow \text{1-octanol)} = -0.63T\Delta S_s° \text{ (in water)} \quad (4)$$

to *n*-butane. There is a difficulty over standard states, since $\Delta G_t°$ seems to refer to standard states of unit mole fraction in water and 1-octanol whereas $\Delta S_s°$ in eq 4 corresponds to standard states of 0.0408 mol dm^{-3} in water and the gas phase. But this aside, the fundamental assumption that $\Delta H_t° = 0$ is just not correct. Thus for methane, $\Delta G_t° = 2.8$ kcal mol^{-1} (mole fraction standard states) but $\Delta H_t°$ is -2.4 kcal mol^{-1},[9,11] so the apparent success of eq 4 is somewhat fortuitous.

(10) Correlation constants for plots of $\Delta S_s°$ in water against these solute properties are ($n = 9$ in all cases) 0.938 (hard-sphere diameter), 0.977 (molar volume), and 0.991 (solute polarizability).

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Free Radical Epoxidation of 7,8-Dihydroxy-7,8-Dihydrobenzo[*a*]pyrene by Hematin and Polyunsaturated Fatty Acid Hydroperoxides

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The hydroperoxidase activity of the heme protein prostaglandin endoperoxide synthetase catalyzes the hydroperoxide-dependent oxidation of polycyclic hydrocarbons, aromatic amines, and nitrofurans.[1] The natural hydroperoxide substrate is the hydroperoxy intermediate of prostaglandin biosynthesis, PGG_2, but polyunsaturated fatty acid hydroperoxides will also trigger oxidation.[2] One of the compounds oxidized is 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (BP-7,8-diol) which is a metabolite of the ubiquitous chemical carcinogen, benzo[*a*]pyrene.[3] BP-7,8-diol is oxygenated during prostaglandin biosynthesis to strongly mutagenic derivatives which have been identified as diol epoxides.[4] Since these diol epoxides are generally regarded as the ultimate carcinogenic forms of benzo[*a*]pyrene, it is possible that prostaglandin endoperoxide synthetase plays a role in polycyclic hydrocarbon carcinogenesis.[5] We have found that other heme proteins with peroxidase activity, such as methemoglobin, will catalyze the hydroperoxide-dependent epoxidation of BP-7,8-diol.[6] We now report that hematin, in the absence of protein, will catalyze the epoxidation of BP-7,8-diol by fatty acid hydroperoxides and that peroxy radicals derived from the carbon skeleton of the hydroperoxide appear to be the epoxidizing agents.

Table I. Hydroperoxide Specificity of O_2 Uptake and BP-7,8-diol Oxidation

hydroperoxide	O_2 Uptake, μM[a]	diol,[b] μM/min
13-OOH-18:2[c]	160 ± 10	12.3 ± 0.9
9-OOH-18:2[d]	160 ± 5	12 ± 1
15-OOH-20:4[e]	160 ± 10	12 ± 1
cumene hydroperoxide	0	1.9 ± 0.1
n-butyl hydroperoxide	0	0
hydrogen peroxide	0	0

[a] [Hydroperoxide] = 500 μM, [hematin] = 5 μM, [Tween 20] = 200 μM, 0.1 M $NaPO_4$ (pH 7.8), 25 °C. [b] [Hydroperoxide] = 50 μM, [hematin] = 0.5 μM, [BP-7,8-diol] = 18 μM, [Tween 20] = 200 μM, 0.1 M $NaPO_4$(pH 7.8), 25 °C. [c] 13-hydroperoxy-9,11-octadecadienoic acid. [d] 9-hydroperoxy-10,12-octadecadienoic acid. [e] 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid.

The initial velocity of BP-7,8-diol oxidation was determined by monitoring the decrease in absorbance at 370 nm. Saturation kinetics were observed for hematin and 13-hydroperoxy-9,11-octadecadienoic acid with half-maximal velocities at 0.25 and 20 μM, respectively. Maximal velocities were observed at 0.5 μM hematin and 50 μM hydroperoxide. A linear concentration dependence was observed for BP-7,8-diol in the range 0.1–50 μM; higher concentrations could not be experimentally attained.

No oxidation of BP-7,8-diol is observed at Tween 20 concentrations below 50 μM, its critical micellar concentration.[7] A sharp increase to maximal initial velocities occurs between 70 and 100 μM Tween. There is an increase in solubility accompanied by a shift in the absorbance maximum of BP-7,8-diol to longer wavelengths as the Tween 20 concentration is increased from 50 to 100 μM. This indicates that the detergent solubilizes the hydrophobic substrate. In addition, it appears that detergents stimulate the peroxidase activity of the hematin. Oxidation of the water-soluble aromatic hydrocarbon, 9,10-bis(carboxyethyl)anthracene,[8] is increased sixfold by raising the Tween 20 concentration from 25 to 100 μM. Thus, it appears that the basis for the detergent requirement is more complex than simple substrate solubilization.

The major products of BP-7,8-diol oxidation are the isomeric tetraols **3**–**6** which are the hydrolysis products of epoxides **1** and **2** (Scheme I).[9] In a typical experiment 13-hydroperoxy-9,11-octadecadienoic acid (5 μmol) is added to a solution of [7-^{14}C]-BP-7,8-diol (1.8 μmol) and hematin (0.05 μmol) in 100 mL 0.1 M sodium phosphate (pH 7.8) containing Tween 20 (20 μmol). After 5 min, 2-*tert*-butyl-4-methoxyphenol is added to terminate the reaction, and the solution is extracted with ethyl acetate. The extract is concentrated and analyzed by HPLC on a reversed-phase column (Radial Pak B) eluted with water–methanol gradients. Tetraols **3**–**6** account for 33% of the total radioactivity eluting

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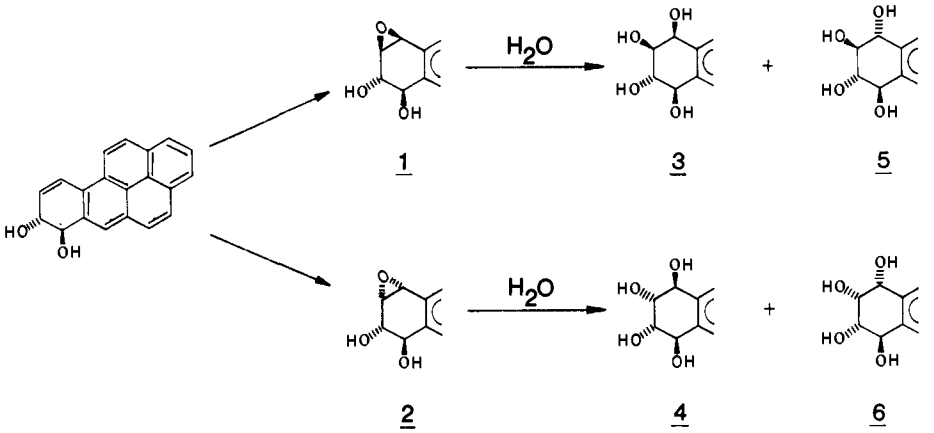
Scheme I



from the column; a compound which cochromatographs with the 7,8-quinone of benzo[*a*]pyrene constitutes 15% of the radioactivity.[10] No other product peaks are seen consistently in the HPLC profiles.[11] The total conversion of BP-7,8-diol in 5 min is 48%.

It is found that 80% of the tetraols are derived from **2** and 20% from **1**, indicating that a degree of stereoselectivity exists in the epoxidation. Inclusion of a chiral nucleophile, polyguanylic acid, in reaction mixtures containing (±)-BP-7,8-diol leads to the formation of diastereomeric adducts derived from enantiomeric epoxides. Digestion of the nucleic acid and HPLC separation of the nucleoside adducts produces a chromatographic profile identical with that observed when polyguanylic acid is reacted with (±)-**1** and (±)-**2**.[12] This suggests that both enantiomers of **1** and **2** are produced in equal amounts from (±)-BP-7,8-diol and that the epoxidations are not stereospecific.[13]

The principal source of the oxygen incorporated into BP-7,8-diol is molecular oxygen. Reactions performed under an atmosphere of $^{18}O_2$ (94 atom % excess) lead to the incorporation of ^{18}O into the epoxide-derived products (87 atom % excess). Argon deoxygenation reduces the initial velocity of BP-7,8-diol oxidation from 12.3 to 1.2 μM/min. *In the absence of BP-7,8-diol*, solutions of polyunsaturated fatty acid hydroperoxides and hematin consume O_2 at a rapid rate. Table I lists the extent of O_2 uptake observed with a series of hydroperoxides. It is clear that hydroperoxides containing multiple sites of unsaturation trigger the most vigorous O_2 consumption. Furthermore, Table I demonstrates that the ability to stimulate O_2 uptake correlates directly with the efficacy of individual hydroperoxides to trigger BP-7,8-diol epoxidation, suggesting a causal relationship between the two reactions. Finally, it should be noted that 1 μM 2-*tert*-butyl-4-methoxyphenol or 10 μM 2,6-di-*tert*-butyl-*p*-cresol completely inhibit epoxidation.

These experiments indicate that hematin-catalyzed epoxidation of BP-7,8-diol by polyunsaturated fatty acid hydroperoxides requires O_2 and sites of unsaturation in the hydroperoxide and is potently inhibited by antioxidants. A mechanistic hypothesis which is consistent with these observations is outlined in Scheme II. The key step is the rearrangement of the initial alkoxy radical to a carbon-centered radical derived from the unsaturated portion of the hydroperoxide.[14] The carbon-centered radical is scavenged by O_2 to generate a peroxy radical which acts as the epoxidizing agent. Hamberg has reported that 61% of the products isolated following the reaction of 13-hydroperoxy-9-11-octadecadienoic acid with methemoglobin are epoxy alcohols which are presumably derived from reduction of the initially formed epoxy hydroperoxides.[15] Gardner et al. have isolated these epoxy hydroperoxides in low yield following the reaction of 13-hydroperoxy-9,11-octadecadienoic acid with ferrous ion and have shown that they are reduced to epoxy alcohols under the conditions of the reaction.[16] In addition, Gardner et al. have trapped the carbon-centered radical which is the precursor of the peroxy radical by carrying out the reactions in the presence of tocopherol.[17] Thus there is ample literature precedent for the rearrangement of unsaturated fatty acid alkoxy radicals to carbon-centered radicals which react with dioxygen to form peroxy radicals. Epoxidation of aliphatic double bonds by peroxy radicals is well precedented, although the reason for the observed stereoselectivity is obscure.[18,19] Scheme II, therefore, is consistent with the key experimental results detailed above as well as with the work of other investigators.

The present report demonstrates that hematin can catalyze the epoxidation of BP-7,8-diol by unsaturated fatty acid hydroperoxides. The epoxidation appears to be effected by peroxy radicals derived from the carbon skeleton of the hydroperoxide. Peroxy radicals derived from the unsaturated acyl moieties of phospholipids have been implicated as chain-propagating agents in the peroxidation of membrane lipids, which eventually leads to cell dysfunction and death.[21] Our observation that these peroxy radicals epoxidize BP-7,8-diol indicates that they also may play

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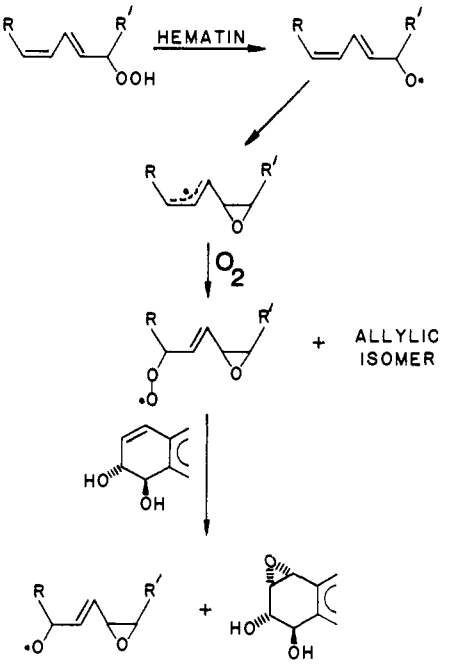
Scheme II



a role in cell transformation by the oxidative activation of xenobiotics to carcinogenic derivatives. This may contribute to the association of dietary fat and carcinogenesis.[22]

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^{109}Ag and ^{103}Rh NMR Spectroscopy with Proton Polarization Transfer

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Since the paper of Morris and Freeman[1] first describing a polarization-transfer sequence between two J coupled spins I and S (INEPT sequence), several communications have appeared applying this method for NMR measurements of ^{13}C,[2a] ^{15}N,[2b] and ^{14}N,[2c] (S, ^{1}H; I, observed nucleus). The gain in signal over noise obtained using the INEPT sequence can reach, under optimal

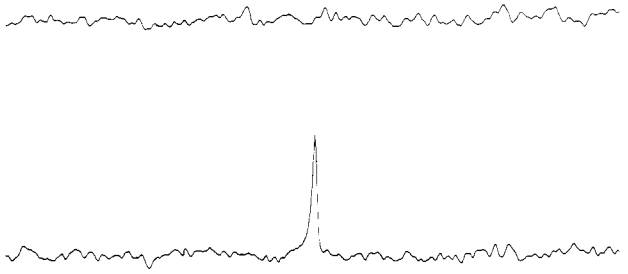
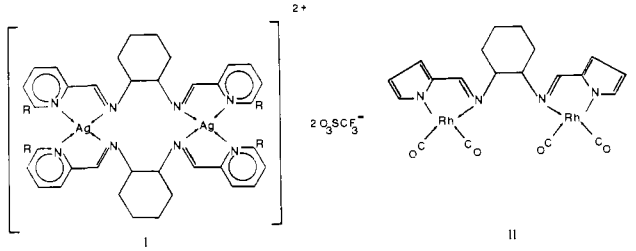


Figure 1. ^{109}Ag spectrum of a 4M solution of $AgNO_3$ in H_2O (1 scan, 90° pulse). Top, with solvent proton irradiation; bottom, normal acquisition.

experimental conditions, a factor equal to $\gamma S/\gamma I$ for an AX spin system. This enhancement is particularly welcome for NMR observations of isotopes with small γ values. We now report results which show the possibilities offered by the INEPT sequence for direct observation of ^{109}Ag and ^{103}Rh in complexes where these nuclei have a ^{1}H resolved scalar coupling [^3J(M,^{1}H)].[3]

From a practical point of view NMR experiments on transition-metal nuclei with small γ values suffer from several drawbacks. Firstly, these nuclei often possess a negative γ which implies a negative η (nuclear Overhauser enhancement) when measuring the sample using broadband proton decoupling irradiation; in general the metal center is not directly bonded to hydrogen and the effect of a remote ^{1}H environment will reduce the theoretical enhancement of the metal resonance to a few percent. As the maximum theoretical η value can be quite large ($\eta = -10.7$ for ^{109}Ag), this small operative mechanism often leads to observed η values of ca. -1 and gives a zero nuclear Overhauser effect (the "null signal" problem). Such a phenomenon has been found during measurements of transition-metal salts in protio solvents,[4] and Figure 1 clearly shows this effect on the ^{109}Ag resonance of an $AgNO_3$ solution (4 M in 90:10 (v/v) H_2O/D_2O) when observed with and without broadband ^{1}H decoupling. Secondly, ^{1}H decoupling inescapably produces a slight increase of the sample temperature. Because of the large $\Delta\delta/K$ for many transition-metal nuclei,[5] the comparison of chemical-shift data can be unreliable. Finally, since low-γ spin $^1/_2$ nuclei sometimes have very large T_1 values (900–1000 s for ^{109}Ag in aqueous solutions of $AgNO_3$[6]) very long relaxation delays and small pulse angles are required.

During the course of a detailed NMR study of complexes of the type $[Ag^I_2[\mu$-(R,S)-1,2-$[(6$-R-2-$C_5H_3N)C(H)=N]_2$cyclohexane$]_2](O_3SCF_3)_2$ (I, R = H)[7] and $[\{Rh^I(CO)_2\}(\mu$-(R,S)-1,2-$[(2$-$C_4H_3N)C(H)=N]_2$cyclohexane)] (II) we considered the use of ^{109}Ag and ^{103}Rh NMR spectroscopy to gain more insight into the electronic environment and the structural and dynamic behavior of these complexes in solution. Fortunately they all show



I II

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(3) The γ values for ^{109}Ag, ^{103}Rh (I spins), and ^{1}H (S spin) are -1.2449, -0.8420 and 26.7510×10^7 rad T^{-1} s^{-1}, respectively. INEPT sensitivity gain is then equal to 21.48 for ^{109}Ag and 31.77 for ^{103}Rh with a corresponding theoretical drop in accumulation time by a factor of 461 for ^{109}Ag and 1000 for ^{103}Rh.

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