hexamethylbenzene-d_{18} were the same to within the experimental uncertainties.

Electron Spin Resonance Studies of Arene Cation Radicals as Intermediates. The direct observation of arene cation radicals as intermediates in the oxidative substitutions with iron(III) was carried out as follows. Separate trifluoroacetic acid solutions of 6.3 mM hexamethylbenzene and $Fe(phen)_3(PF_6)_3$ (prepared from 11 mg of complex and 2.0 mL of solvent by heating over a steam bath until complete dissolution) were added to an ESR tube with a side arm affixed. The tube was sealed in vacuo, and the contents were mixed by rapid shaking immediately prior to insertion in the ESR cavity (Varian E-112). The concentration of $HMB^{+\cdot}$ was followed periodically by monitoring the intensity of the $M_1 = 0$ line. The dashed line in Figure 1 indicates that 15–20 s were required to obtain the first data point. Since the disappearance of the cation radical was temperature dependent, the ESR spectrum shown in the inset was obtained from a similar reaction which was cooled to −30 °C prior to mixing.

Evaluation of the Free Energy Terms. The free energy change $\Delta G_0'$ was determined from the standard oxidation potentials in Table I according to eq 20. The work term w_p was considered to be only of electrostatic origin, i.e., $w_p = 2e^2/Dd^*$ as given in ref 45. The activation free energy ΔG^* was evaluated from the rate constant k^* listed in Table III and eq 21, where $w_r \cong 0$ and the collision frequency was taken to be 10^{11} M^{-1} s^{-1}. The value of k^* was deduced from k_1 by taking into account the diffusional contributions according to eq 17.[36] For rapid oxidative substitutions, the effect of diffusion on k_1 is small, but increases in importance for the slowest rates, as shown in Table VII.

The intrinsic barriers were computed from the values of $\Delta G_0'$ and ΔG^* for each system, with the aid of the free energy relationship in eq 22–24. For each methylarene, the three values of ΔG_0^* (obtained with the three iron(III) oxidants) were averaged for construction of Figure 6.

Derivation of the Kinetic Rate Expressions. The kinetics were based on the stoichiometry in eq 6 and the mechanism presented in Scheme II for the general case in which back electron transfer (k_{-1}) competes with the followup step (k_3). All derivatives assumed a zero order dependence on arene (Ar) in excess, and a negligible consumption of the pyridine base. The rate of iron(III) disappearance (taking into account the steady state concentration of $ArCH_2\cdot$) is

$$-d[Fe(III)]/2\,dt = k_1[Fe(III)][Ar] - k_{-1}[Fe(II)][Ar^{+\cdot}] \quad (30)$$

Since at steady state, $[Ar^{+\cdot}] = k_1[Fe(III)][Ar]/(k_{-1}[Fe(II)] + k_3[py])$, eq 30 reduces to

$$-d[Fe(III)]/dt = 2k_1[Fe(III)][Ar]\{(1 - k_{-1}[Fe(II)]/k_{-1}[Fe(II)] + k_3[py])\} \quad (31)$$

For $[Fe(II)] = [Fe(III)]_0 - [Fe(III)]$ and $x = [Fe(III)]/[Fe(III)]_0$, eq 31 is simplified to

$$(-dx/x)(1 - x + k_2[py]/(k_{-1}[Fe(III)]_0)) = (2k_1[Ar]k_3[py]/(k_{-1}[Fe(III)]_0))\,dt \quad (32)$$

Integration of eq 32 between the limits of $t = 0$ ($x = 1$) and $t(x)$ yields

$$(1 + p)\ln x + p(1 - x) = -2k_1[Ar]t \quad (33)$$

where $p = k_{-1}[Fe(III)]_0/(k_3[py])$.

Two limiting situations derive from eq 33, that is, $p \cong 0$ and $p >> 1$, which describe rate-limiting electron transfer (eq 14) and pre-equilibrium electron transfer (eq 12), respectively. The same results are obtained in the latter case, if $[Ar^{+\cdot}]$ is assumed to attain equilibrium rapidly, i.e., $k_1/k_{-1} = [Ar^{+\cdot}][Fe(II)]/([Fe(III)][Ar])$.

Note that the previously measured rate constants[12e] did not take into account the reversibility in the electron transfer. Correction of this factor, however, does not fundamentally affect the conclusions presented therein.

Acknowledgment. We thank the National Science Foundation for financial support of this research and for a fellowship to C.A. under the auspices of the United States–France (NSF-CNRS) cooperative program, Dr. W. Lau for help with the ESR experiments, and J. Goncalves for the computer interface of the spectral data.

Registry No. $(phen)_3Fe^{2+}$, 14708-99-7; $(5\text{-}Cl(phen))_3Fe^{2+}$, 15053-59-5; $(5\text{-}NO_2(phen))_3Fe^{2+}$, 15245-50-8; $(phen)_3Fe^{3+}\cdot(PF_6)_3^-$, 28277-57-8; $(5\text{-}Cl(phen))_3Fe^{3+}\cdot(PF_6)_3^-$, 89556-59-2; $(5\text{-}NO_2(phen))_3Fe^{3+}\cdot(PF_6)_3^-$, 89578-85-8; $C_6H_4(CH_3)(OCH_3)$-1,2, 578-58-5; HMB, 87-85-4; HMB-d_{18}, 4342-40-9; PMB, 700-12-9; DUR, 95-93-2; TMB, 488-23-3; *p*-methoxytoluene, 104-93-8; pyridine, 110-86-1; deuterium, 7782-39-0.

Stereochemical Course of the Autoxidative Cyclization of Lipid Hydroperoxides to Prostaglandin-like Bicyclo Endoperoxides

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Contribution from The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio 45247. *Received September 26, 1983*

Abstract: The stereochemical course of the autoxidative cyclization of the two polyunsaturated fatty acid hydroperoxides **1a** and **1b** to form prostaglandin-like bicyclo endoperoxides has been found to be highly selective. The reaction generates four independent chiral centers, giving rise to the possibility of eight different stereoisomers having the basic prostaglandin structure. Seven of these eight diastereoisomers were observed: four, representing three different ring stereochemistries, were isolated and characterized as relatively pure single isomers; three others were characterized as minor components of the major isomers. The major bicyclo endoperoxide products of the reaction were the two C-15 epimers of the endo,endo bicyclo endoperoxides **9** and **10**. While the two C-15 epimers of the exo,exo bicyclo endoperoxides **7** and **8** were also formed in significant yield, the products arising from trans ring substitutents (including those having the natural prostaglandin ring stereochemistry) were only minor components. Interestingly, the isomers that could arise from oxygenation of intermediate **6** at the carbon adjacent to the bicyclic ring were not observed. Thus, the reaction shows high selectivity disfavoring the formation of the natural prostaglandin stereochemistry.

Since their discovery by von Euler, Goldblatt, and Kurzrok,[1] the prostaglandins have been noted for their remarkably potent and varied biological effects. It was not until the early 1960s that the complete structures were elucidated for "primary" prostaglandins (PGF's, PGE's), and in the ensuing two decades the astounding complexity of the arachidonic acid cascade has slowly become apparent.[2] The common intermediate to all the prostaglandins, thromboxanes, and prostacyclin is the bicyclo endoperoxide PGG, which results from the action of the prostaglandin synthetase (cyclooxygenase) enzyme on 20-carbon polyunsaturated

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(1) A concise historical perspective is given by: von Euler, U. S. In "Progress in Lipid Research"; Holman, R. T., Ed.; Pergamon Press: Elmsford, NY, 1982; Vol. 20, pp xxxi–xxxv.

(2) Nelson, N. A.; Kelly, R. C.; Johnson, R. A. *Chem. Eng. News* **1982**, *60* (*33*), 30–44.

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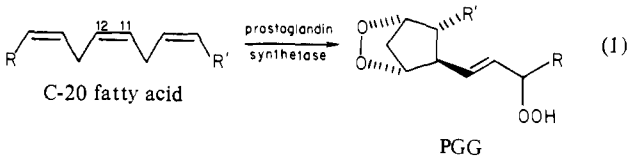
fatty acids having three or more double bonds (eq 1). The



C-20 fatty acid PGG (1)

mechanisms employed by the enzyme in this remarkable conversion are not known with certainty; however, it is commonly accepted that the available data support a controlled free-radical pathway proceeding through either an 11-hydroperoxide or an 11-peroxy radical.[3,4] Bicyclization of this intermediate, which has never been isolated, generates the PGG structure with its carefully manipulated and crucial stereochemistry.

Surprisingly little work has been done on the nonenzymic conversion of polyunsaturated fatty acids to prostaglandins. Unilever workers[5] were the first to document the formation of PGE_1 from autoxidized eicosatrienoic acid and warned that this propensity for oxidation could lead to artificial results when measurements of very low levels of prostaglandins are performed. Some years later Pryor et al.[6] autoxidized methyl α-linolenate and obtained mass spectra evidence for the formation of PGF-type products after reduction of the oxidation mixture. In more detailed work, Porter and co-workers[7] prepared a single hydroperoxy fatty ester from γ-linolenic acid and allowed it to react in the presence of oxygen and a free-radical source. After reduction, GC/MS indicated the presence of a number of PGF-like materials, one of which was identical with an authentic sample of dinor-$PGF_{1\alpha}$ obtained from the UpJohn Co.

These model studies supported the feasibility of a peroxy radical cyclization route from polyunsaturated fatty acids to the prostaglandins; however, they did not answer the important question of stereoselectivity in the various bond-forming steps, nor were attempts made to isolate the PGG-like products which were the necessary intermediates in these preparations. Following our demonstration that the initial peroxy radical cyclization occurs with the necessary cis selectivity required for further conversion to PGG-like materials,[8] we developed a program to define the stereochemical preferences of bicyclo endoperoxide formation in these in vitro autoxidations. Our initial results[9] demonstrated quite high selectivities for stereoisomers different from those obtained by the enzyme-mediated pathway. We now report in detail our completed stereochemical profile of bicyclo endoperoxide formation from two different substrate hydroperoxides, one of which leads to the dinor-PG_1 series.

Results

The two hydroperoxides used in this study were methyl 13-hydroperoxy-*cis*-9,*trans*-11,*cis*-15-octadecatrienoate (**1a**) and methyl 9-hydroperoxy-*cis*-6,*trans*-10,*cis*-12-octadecatrienoate (**1b**), both of which were prepared following procedures of Porter et al.[10] Hydroperoxide **1a** was obtained in 60–70% yield by the soybean lipoxygenase-catalyzed oxidation of α-linolenic acid followed by esterification of the product with diazomethane and chromatography. Hamberg and Samuelsson[11] have shown that this enzyme oxidation gives the 13-hydroperoxy derivative; our own analysis by ^1H and ^{13}C NMR was consistent with the assigned structure, and indicated it was not contaminated by other isomers, within the limits of detection. The dinor-PG_1 precursor **1b** was prepared in 20–25% yield along with approximately equal amounts of methyl 13-hydroperoxy-*cis*-6,*cis*-9,*trans*-11-octadecatrienoate (**2**) by the soybean lipoxygenase-catalyzed oxidation of γ-linolenic acid followed by esterification of the product with diazomethane. The **1b** and **2** were each obtained as pure compounds by medium-pressure chromatography on silica gel using 1.5% isopropyl alcohol in hexane as the eluent.

The absolute configuration and the optical purity of the hydroperoxides **1a** and **1b** prepared by soybean lipoxygenase oxidation have not been reported. It would be a reasonable presumption that both have predominantly the *S* configuration at the hydroperoxy-bearing carbon, based on the known configurations of similarly oxidized polyunsaturated fatty acids.[11] Experiments were carried out which did, in fact, verify that this presumption is correct. The configurations of both **1a** and **1b** were determined to be at least enriched in *S*, on the basis of a positive CD absorption at 236 nm[12] and a positive and increasing rotation with decreasing wavelength,[13] both of which were determined on the derived alcohols in methanol solvent. The enantiomeric purity was not determined for either **1a** or **1b**. However, we did determine that **1b** substantially racemizes during autoxidation. The **1b** starting material gave $[\alpha]^{25}_D$ −9.73° (*c* 1.15, CCl_4) while the hydroperoxide recovered from the autoxidation, confirmed to be **1b** by ^{13}C NMR, had a rotation of less than half that value ($[\alpha]^{25}_D$ −3.91° (*c* 2.95, CCl_4)). This observation is consistent with previous studies by Chan et al. which showed oxygen exchange during autoxidative cyclization of **1a**.[14]

Bicyclo endoperoxides derived from the (*S*)-hydroperoxides will have absolute stereochemistries analogous to the enantiomers of the natural prostaglandins and likely are formed in greater abundance than those derived from the (*R*)-hydroperoxides. Nevertheless, the structures throughout this paper are drawn as if they were derived from (*R*)-hydroperoxides to emphasize their relationship to the prostaglandins. All references to absolute stereochemistry are based on the structure as drawn.

The autoxidations of **1a** and **1b** were carried out under atmospheric pressure of oxygen or air in carbon tetrachloride (0.15 M solution). These oxidations lead to complex mixtures of more highly oxidized materials which include significant quantities of monocyclic and bicyclic endoperoxides. The accepted pathway by which these materials are formed is shown in Scheme I. The autoxidation of **1a** gave the best yields of bicyclo endoperoxides at room temperature; lower temperatures favored the formation of monocyclic peroxides at the expense of the bicyclic peroxides. The autoxidation of **1b** gave the best results at 5 °C; in this case higher temperatures gave rapid autoxidation, but led to the formation of substantial amounts of highly polar oxidation products at the expense of the derived bicyclo endoperoxides. Various other solvents (benzene, acetonitrile, methanol) were tried, but none gave results as good as those obtained in carbon tetrachloride. Interestingly, benzene, with or without initiators, led to lower levels of cyclic peroxides and large amounts of more polar byproducts that were not completely characterized.

The separation of products derived from either **1a** or **1b** could be carried out on a medium-pressure liquid chromatography unit[15]

(3) (a) For a timely review covering the formation and conversions of endoperoxides see: Porter, N. A. In "Free Radicals in Biology"; Pryor, W. A., Ed.; Academic Press: New York, 1980; Vol. IV, Chapter 8. (b) For the first isolation of an endoperoxide intermediate in prostaglandin biosynthesis see: Hamberg, M.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 899–903.

(4) Porter, N. A.; Wolf, R. A.; Pagels, W. R.; Marnett, L. J. *Biochem. Biophys. Res. Commun.* **1980**, *92*, 349–55.

(5) Nugteren, D. H.; Vonkeman, H.; Van Dorp, D. A. *Recl. Trav. Chim. Pays-Bas* **1967**, *86*, 1237–45.

(6) Pryor, W. A.; Stanley, J. P.; Blair, E. *Lipids* **1976**, *11*, 370–9.

(7) (a) Porter, N. A.; Funk, M. O. *J. Org. Chem.* **1975**, *40*, 3614–15. (b) Porter, N. A.; Funk, M. O.; Gilmore, D. W.; Isaac, S. R.; Menzel, D. B.; Nixon, J. R.; Roycroft, J. H. In "Biochemical Aspects of Prostaglandins and Thromboxanes"; Kharasch, N., Fried, J., Eds.; Academic Press: New York, 1977; pp 39–53.

(8) Mihelich, E. D. *J. Am. Chem. Soc.* **1980**, *102*, 7141–43. See also: Beckwith, A. L. J.; Wagner, R. D. *Ibid.* **1979**, *101*, 7099–7100; *J. Chem. Soc., Chem. Commun.* **1980**, 485–6.

(9) O'Connor, D. E.; Mihelich, E. D.; Coleman, M. C. *J. Am. Soc.* **1981**, *103*, 223–4.

(10) Funk, M. O.; Isaac, R.; Porter, N. A. *Lipids* **1976**, *11*, 113–7.

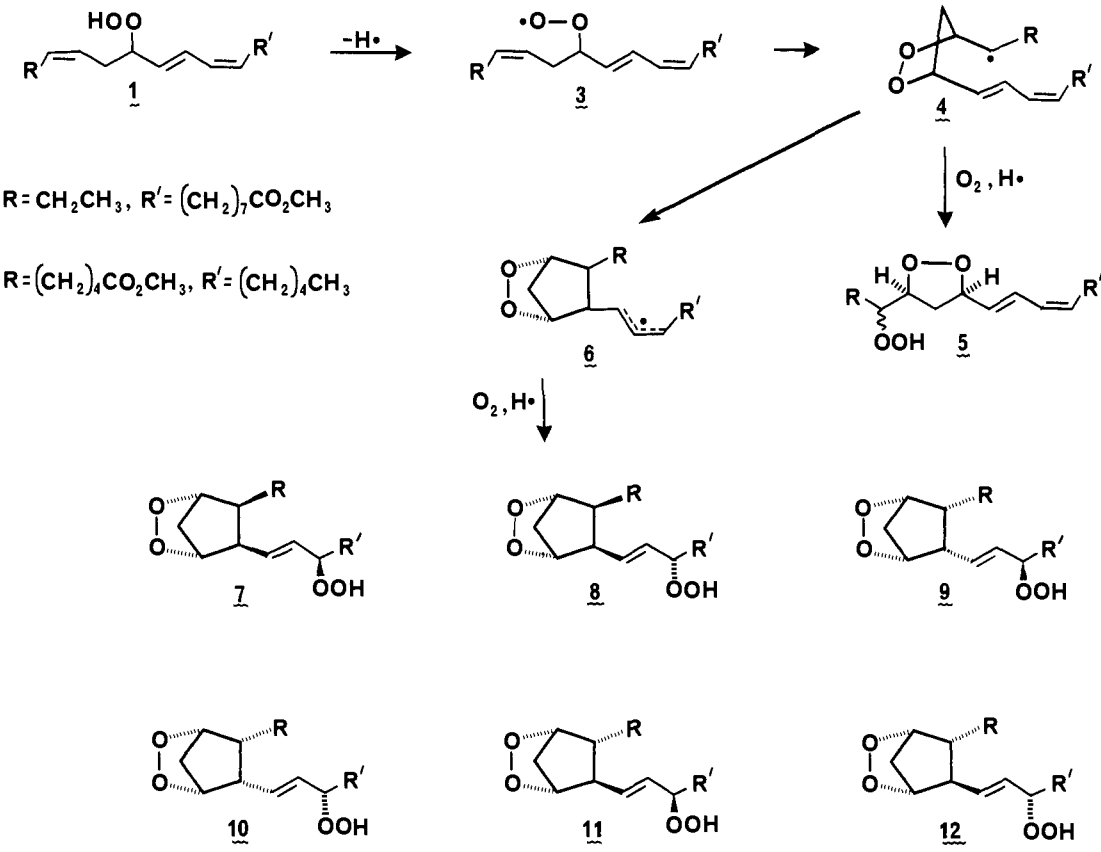
(11) Hamberg, M.; Samuelsson, B. *J. Biol. Chem.* **1967**, *242*, 5329–35.

(12) Van Os, C. P. A.; Rijke-Schilder, G. P. M.; Vliegenthart, J. F. G. *Biochim. Biophys. Acta* **1979**, *575*, 479–84.

(13) Corey, E. J.; Albright, J. O.; Barton, A. E.; Hashimoto, S. *J. Am. Chem. Soc.* **1980**, *102*, 1435–36 and references cited therein.

(14) Chan, H. W.-S.; Matthew, J. A.; Coxon, D. T. *J. Chem. Soc., Chem. Commun.* **1980**, 235–6. Chan, H. W.-S.; Levett, G.; Matthew, J. A. *Chem. Phys. Lipids* **1979**, *24*, 245–56.

Scheme I



over silica gel using hexane–ethyl acetate (75–25) as eluting solvent. In each case the first significant oxidation products to elute were two diastereomeric monocyclic peroxides (**5**), which constituted ~25% of the oxidized material. These were followed by a series of fractions of bicyclic peroxides which, combined, accounted for 15–20% of the total. The balance of the material was more polar components which we believe were decomposition products of the mono- and bicyclic peroxides. Total recovery of material from the column ranged from 70 to 85%.

The bicyclic endoperoxide components derived from **1a** were seen as four major peaks by high-pressure liquid chromatography (HPLC) (75:25 hexane–ethyl acetate on μ-Porasil) having capacity factors (cf) of 1.1, 1.4, 1.7, and 2.4. The 1.1-, 1.4-, and 2.4-cf components were shown to be **7a**, **9a**, and **10a**, respectively, while the 1.7-cf material was shown to be a mixture containing the two isomers **8a** and **12a**. The bicyclo endoperoxide component derived from **1b** showed significant peaks having capacity factors of 1.2, 1.5, 1.7, and 3.0. These components were each found to be primarily a single isomer and were subsequently shown to be **7b**, **11b**, **9b**, and **10b**, respectively.

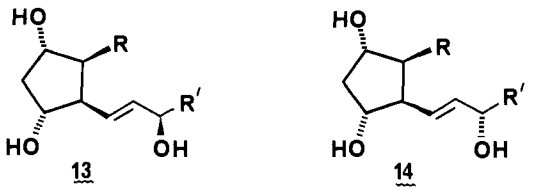
Quantitative yield data were difficult to obtain because of the complexity of the reaction mixture and the limited stability of the peroxides. Analysis of the crude oxidation mixtures by both HPLC and ^{13}C NMR suggests the true yield of monocyclic peroxides is 40–50%, while the combined yield of all bicyclo endoperoxides is probably higher than the 15–20% isolated yield. For example, HPLC analysis of a typical **b** series sample indicated relative percentages based on all peaks detected were 49% **5b**, 5% **7b**, 3% **8b**, 21% **9b**, 11% **10b**, and 3% **11b**. Similar HPLC analysis of an **a** series sample showed 46% **5a**, 5% **7a**, 5% **8a** containing some **12a**, 10% **9a** and 4% **10a**. The total yield of bicyclo endoperoxides and the relative yields of the various isomers were similar in both the **a** and **b** series. The endo,endo-substituted isomers **9** and **10** were the major products; the exo,exo-substituted isomers **7** and **8** were also significant products, while the isomers with trans ring substituents were minor products. The isomers having an *R* configuration at the allylic hydroperoxide group predominated over those having the *S* configuration.

Reduction of the bicyclo endoperoxides to the triols was carried out with stannous chloride.[16] The reductions went better with this reagent than with sodium borohydride, triphenylphosphine, or hydrogen over Lindlar's catalyst. Analysis of the crude reduction products by TLC indicated that the triols were formed in very good yield; however, chromatography (medium-pressure, high-pressure, or flash) of the reduction products invariably gave poor yields of the triols, especially in the case of the all-cis products (those derived from **9** and **10**). In our earlier work with the triols derived from **1a**, we performed the chromatographies and obtained quite pure triols. In most cases, the triols from **1b** were not chromatographed; they were analyzed as crude reduction products. Their ^{13}C NMR spectra were not affected by the impurities in the spectral regions of interest, e.g., below 35 ppm, and their mass spectra were obtained on pure gas chromatographic peaks of their trimethylsilyl derivatives.

The structural assignments of these isomers are based on various spectroscopic and chromatographic observations, all of which are internally consistent and consistent with literature. The process of identification can be summarized as follows: The isomers **7a**, **9a**, and **10a** were isolated and characterized in relatively pure form. Each was reduced to its corresponding triol, **13a**, **15a**, and **16a**, respectively, by known methods. Comparison with the model compounds as described previously[9] by both ^{1}H and ^{13}C NMR spectroscopy established their ring stereochemistry and their double bond regio- and stereochemistry. The γ-linolenate-derived products **7b**, **9b**, **10b**, and **11b** were subsequently isolated and characterized. The very close correspondence in chromatographic behavior and ^{13}C NMR spectra of **7a** to **7b**, **9a** to **9b**, and **10a** to **10b** provided strong evidence for the structures of these **b** series compounds. Characterization of the reduction products **13b**, **15b**, **16b**, and **17b** by ^{13}C NMR and comparisons with model com-

(15) Meyers, A. J.; Slade, J.; Smith, R. K.; Mihelich, E. D.; Hershenson, F. M.; Liang, C. D. *J. Org. Chem.* **1979**, *44*, 2247.

(16) Hamberg, M.; Svensson, J.; Wakabayashi, T.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *171*, 345–9.



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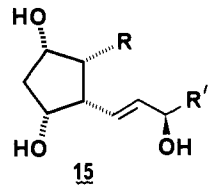

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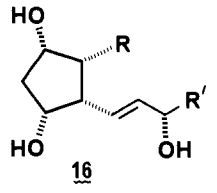

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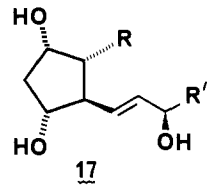

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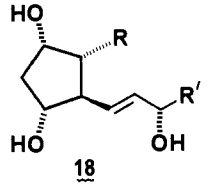


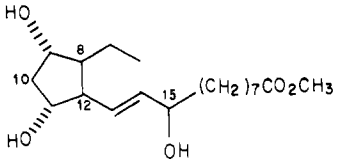
18

a, $R = C_2H_5$, $R' = (CH_2)_7CO_2CH_3$

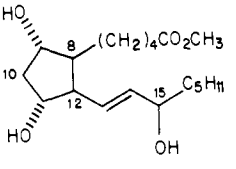
b, $R = (CH_2)_4CO_2CH_3$, $R' = C_5H_{11}$

pounds provided further evidence for their structure. The assignments of the structures of isomers that have the same ring stereochemistry but that are epimeric at their hydroperoxy-substituted carbons were based on differences in the chemical shifts of their olefinic protons and olefinic carbons and their TLC mobilities. Exact mass spectra of the triol isomers established their basic structures.

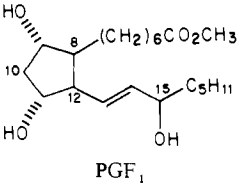
In the discussion of results and in the tables it is necessary to refer to specific carbon atoms by number. In order to simplify comparisons among the **a** and **b** series and the natural prostaglandins, both the **a** and **b** series are numbered in the same way as the natural prostaglandins.



a series



b series



PGF_1

NMR Analysis of Bicyclo Endoperoxides. To our knowledge, there are no previous reports of NMR spectroscopy on PGG_1 or PGH_1 and only a few on the 2-series bicyclo endoperoxides.[17] Consequently, our isolation work allowed the first extensive spectroscopic investigation of prostanoid endoperoxides of varying structure and stereochemistry. The ^1H NMR data are given in Table I. For the series of bicyclo endoperoxides derived from methyl α-linolenate four isomers with cis side chains, **7a**, **8a**, **9a**, and **10a**, were isolated in quite pure form; in addition, two isomers with trans substituents, **11a** and **12a**, were characterized as minor components of **9a** and **8a**, respectively. For each ring stereochemistry there are two C-15 epimers which are well-resolved from one another chromatographically. However, the ^1H NMR spectra of these epimeric pairs (e.g., **7a**, **8a**, and **9a**, **10a**) were nearly identical; only the H-11 proton displayed an upfield shift for the 15(*R*) epimer in the **7–10** compounds.

Major differences were observed when ring stereochemistries were compared. The best single diagnostic chemical shift is that of H-12, which is at 2.92 ppm for the exo,exo isomer, 2.66 ppm for the endo,endo, and 2.3 ppm for the natural trans stereochemistry. The combined values for H-9 and H-11 were also unique for the three different ring stereochemistries *within each series* (either **a** or **b**). Thus, it is very easy to distinguish the substituent pattern on the bicyclo endoperoxide nucleus based on ^1H NMR data. Although complete structure assignments were not made at this point, an interesting feature in the bicyclo endoperoxide spectra is the downfield shift of H-13 to ~6 ppm when the alkenyl substitutent is endo. The absence of such a resonance in the samples containing **11a** and **12a** strongly suggested these isomers had the natural trans stereochemistry. This observation is supported by other PG modeling studies done in the laboratories of Professor R. Marshall Wilson.[18]

The proton assignments shown in Table I were made on the basis of extensive homonuclear decoupling experiments performed on all samples. The most informative irradiation was that of H-12, which collapsed H-13 and H-8 and also sharpened H-11. Decoupling of H-15 simplified H-14 to a doublet and had no effect on H-12. This confirmed the assigned regiochemistry of oxygenation of allylic radical **6**. We found no evidence of oxygenation at C-13 in any of our chromatographic samples, although very low levels of such compounds could have escaped detection.

The ^1H NMR spectra of our **b** series bicyclic peroxides were nearly identical with those of the corresponding **a** series materials. The H-8 and H-9 resonances were the only ones to exhibit small differences for isomers **7** to **10**. We were also able to obtain in pure form the natural trans isomer, **11b**, by HPLC separation. It is interesting to note that this gave the least informative spectrum of all. The olefin protons overlapped one another, and H-12 was hidden under the methylene adjacent to the carboxyl group. Homonuclear decoupling at this position collapsed the low-field half of the olefin multiplet, sharpened H-11, and had no effect on H-15. Only the bridgehead singlets and H-15 were well resolved. Similar patterns were previously reported for PGH_2.[17a] Recently, very high-field ^1H NMR (500 MHz) has been used to completely assign the spectrum of PGH_2.[19] The chemical shift of H-12 in that system is 2.2 ppm—very close to the 2.3 ppm value we have assigned to H-12 in **11b**.

The ^{13}C NMR data for all bicyclo endoperoxides are given in Table II. Compounds having different ring stereochemistries, e.g., **7a** vs. **9a**, showed very significant differences in their ^{13}C NMR spectra, most notably at C-7, C-8, C-10, and C-12, which are consistent with the assigned structures. (As noted earlier, definitive structural determinations were made on the corresponding triols.) Each of the two isomers of the C-15 epimeric pairs, **7a**, **8a**, and **9a**, **10a**, had ^{13}C NMR spectra that were essentially identical with one another.

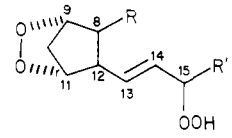
In the series of bicyclo endoperoxides derived from methyl γ-linolenate four isomers, **7b**, **9b**, **10b**, and **11b**, representing three different ring stereochemistries, were isolated in relatively pure form in sufficient quantity for ^{13}C NMR analysis. Structure **8b** was seen only as a minor component in **9b**; however, the chemical shifts of its distinctive ^{13}C NMR absorbances were essentially

(17) (a) Gorman, R. R.; Sun, F. F.; Miller, O. V.; Johnson, R. A. *Prostaglandins* **1977**, *13*, 1043–53. (b) Porter, N. A.; Byers, J. D.; Holden, K. M.; Menzel, D. B. *J. Am. Chem. Soc.* **1979**, *101*, 4319–22. (c) Porter, N. A.; Byers, J. D.; Ali, A. E.; Eling, T. E. *Ibid.* **1980**, *102*, 1183–4.

(18) Wilson, R. M., et al. University of Cincinnati, unpublished results. We thank Professor Wilson for sharing his data with us prior to publication.

(19) Andersen, N. H.; Wilson, C. H. "Abstracts of Papers", 185th National Meeting of the American Chemical Society, Seattle, WA, March 20–25, American Chemical Society: Washington, DC, 1983; ORGN 267. We thank Professor Anderson for providing us with this information.

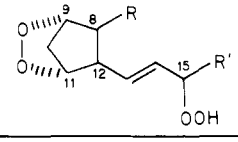
Table I. ^{1}H NMR Data of Bicyclo Endoperoxides[a]



	chemical shift, δ								coupling constants, Hz			
compd	H-6	H-8	H-9	H-11	H-12	H-13	H-14	H-15	$J_{8,12}$	$J_{12,13}$	$J_{13,14}$	$J_{14,15}$
7a	0.95	2.16	4.53	4.42	2.92	5.44	5.52	4.26	9.5	9.5	15.5	7.2
7b		2.18	4.46	4.40	2.92	5.41	5.52	4.25	10.0	9.6	15.5	7.6
8a[b]	0.94	2.16	4.53	4.46	2.92	5.44	5.52	4.27	9.5	9.7	15.5	7.6
9a[b]	0.88	1.90	4.64	4.47	2.66	6.02	5.47	4.33	10.2	9.9	15.5	7.5
9b		1.97	4.58	4.46	2.65	6.01	5.47	4.34	10.0	9.9	15.2	8.2
10a	0.88	1.90	4.66	4.53	2.67	6.03	5.48	4.35	10.0	9.9	15.5	8
10b		1.98	4.60	4.51	2.66	6.01	5.47	4.34	10.0	10.2	15.7	7.7
11b			4.54	4.40	~2.32	~5.47	~5.47	4.24				

[a] Measured in $CDCl_3$ solution at either 270-MHz or 300-MHz field strength. [b] Spectrum exhibited small bridgehead singlets at δ 4.59 and 4.45 indicative of isomers **11a** (in **9a**) and **12a** (in **8a**).

Table II. ^{13}C NMR Data of Bicyclo Endoperoxides[a]



	chemical shift, ppm							
compd	C-6	C-7	C-8	C-9, C-11[b]	C-10	C-12	C-13, C-14[b]	C-15
7a	13.2	21.9	48.1	81.7, 80.5	39.3	48.7	132.5, 131.2	86.3
7b			46.1	81.8, 81.2	39.4	48.7	133.2, 131.1	86.5
8a	13.2	21.9	48.1	81.7, 80.5	39.3	48.7	132.4, 131.3	86.4
8b[c]			46.1		39.0	48.6		
9a	13.0	18.8	45.3	83.0, 80.1	44.1	46.1	132.7, 132.2	86.5
9b			43.3	83.1, 81.0	44.2	45.8	132.8, 132.4	86.6
10a	12.9	18.8	45.3	82.7, 80.0	44.1	45.9	132.5, 132.4	86.6
10b			43.3	82.7, 80.3	44.2	45.9	132.6, 132.5	86.7
11a	12.6	22.6	50.0	82.3, 79.8	42.5	50.8	135.0, 129.8	86.3
11b			47.7	82.5, 80.4	42.6	51.0	134.9, 130.7	86.4

[a] Measured in $CDCl_3$ with tetramethylsilane as an internal reference; the **b** series spectra were obtained by using an INEPT program. [b] The olefin carbons and the bridgehead carbons were not assigned. [c] This isomer was observed as a minor component of **9b**.

identical with those of **7b** and left no doubt that the two had the same ring stereochemistry and must, therefore, be epimeric at their hydroperoxy carbons. The portions of the ^{13}C spectra below 35 ppm of **7b**, **9b**, and **10b** were similar to those of the correponding structures **7a**, **9a**, and **10a**, the only significant difference being an expected 2 ppm shift to higher field for C-8.[20] This very close correpondence provided one basis for their structural assignments. The chemical shifts of the ring carbons of **11b** correponded closely with those of PGH_2, reported by Porter et al.[17b]

The distinctive part of these bicyclo endoperoxide spectra is in the 39–51 ppm range. Each of the different ring stereochemical isomers shows a unique set of three peaks in this region due to C-8, C-10, and C-12. The chemical shift of C-10 is particularly distinctive: Its chemical shifts in the **a** and **b** series are identical for a given substitution pattern, and it shows the expected variation in chemical shift with changes in ring stereochemistry.

NMR Analysis of Triols. Definitive stereochemical assignments for the bicyclo endoperoxides were accomplished by reducing them to the correponding triols (structures 13–18) and correlating their ^{1}H and ^{13}C NMR spectra to established literature precedent[21,22] in the prostaglandin field. Our analysis of the **a** series compounds has been reported earlier[9] and is equally applicable to the **b** series. The ^{13}C NMR data on these triols are shown in Table III. In addition, ^{1}H NMR data on the **a** series compounds (Tables IV and V), as well as detailed analyses of the ^{1}H and ^{13}C NMR spectra, are offered as supplementary material.

Mass Spectrometric Analysis of Triols. The chemical compositions of the various triols were established by gas chromatography/mass spectrometry (GC/MS) of their trimethylsilyl derivatives. The observed fragmentation patterns were very similar to those of various prostaglandins and prostaglandin analogues reported by earlier workers.[6,7b,23] In fact, the fragmentation patterns of individual GC components served as a fingerprint to differentiate between those that had the basic prostaglandin structure and those that did not. These fragmentation patterns did not vary with stereochemistry, although some peak intensities did show some consistent variations. The structures of all the significant fragments above 190 mass units were assigned, on the basis of the excellent agreement between observed and calculated masses (generally, less than 5 mmu difference). The data are tabulated (Tables VI and VII) and discussed in the supplementary material.

Chromatographic Analysis. Chromatography (TLC, HPLC, and GC) was used extensively in this work. A tabulation of the chromatographic data (Table VIII) is presented in the supplementary material.

Other Isomers. Two additional isomers were observed as minor components (<10% of each) in one large sample of the major

(20) This upfield shift induced by the γ-effect [Stothers, J. B. "Carbon-13 NMR Spectroscopy"; Academic Press: New York, 1972; Chapter 3] present in the **b** series but absent in the **a** series, in fact, allows us to assign C-8 and, by default, C-12. The INEPT programming sequence enabled routine assignment of the third important peak in this region of the spectrum, C-10.

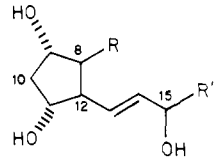
(21) DeClerq, P.; Samson, M. *Org. Magn. Reson.* **1977**, *9*, 385–8.

(22) Mizsak, S. A.; Slomp, G. *Prostaglandins* **1975**, *10*, 807–12.

(23) Granstrom E.; Lands, W. E. M.; Samuelsson, B. *J. Biol. Chem.* **1968**, *243*, 4104–8.

(24) "Methods in Enzymology"; Academic Press: New York, 1982; Vol. 86, p 384.

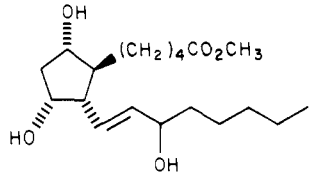
Table III. ^{13}C NMR Data of Triols[a]



compd	chemical shift, ppm									
	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15
13a	12.8	22.1	52.4	76.4[b]	42.6	76.5[b]	53.5	128.7	135.7	72.7
13b			50.1	76.9[b]	42.6	77.0[b]	53.7	128.4	136.3	72.6
14a	12.8	22.1	52.5		42.7		53.7	128.8	135.8	72.9
14b[c]					42.5		53.7	129.3	136.1	
15a	12.9	19.3	49.0	73.8	43.0	76.3	50.1	128.0	137.7	72.6
15b			46.8	73.9	42.9	75.6	50.0	127.1	137.8	72.1
16a	12.8	19.2	49.0	73.8	42.9		49.9	129.4	137.2	73.1
16b			46.8	74.0	42.7	75.7	50.0	128.8	137.6	73.0
17b			50.4	73.8	43.0	78.8	56.6	132.3	134.6	72.7
18b[d]				73.2		78.0	56.1	133.1	135.2	
15-epi-$PGF_{1\alpha}$[e]			50.6	73.5	43.0	78.6	56.2	132.2	134.5	72.5
$PGF_{1\alpha}$[e]			50.2	73.2	43.1	78.1	56.2	133.2	135.0	73.0

[a] Spectra were measured in $CDCl_3$ with tetramethylsilane as internal reference; the **b** series spectra were obtained by using an INEPT program. [b] These values were obtained in CD_2Cl_2; the C-9 and C-11 assignment could be reversed. [c] This isomer was consistently observed as a minor component of **15b**. [d] This isomer was observed as a minor component in one large sample of **15b**. [e] These data are taken from: Cooper, G. F.; Fried, J. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 1579–84.

isomer, **15b**. One of these isomers was identified as **18b** on the following basis: It (Me_3Si derivative) had a GC retention time very similar to that of **17b** but a different R_f on TLC; it gave a classical prostaglandin-like MS fragmentation pattern and exhibited distinguishable ^{13}C NMR absorbances at 135.2, 133.1, 78.0, 73.2, and 56.1 ppm, closely matching those of $PGF_{1\alpha}$ (see Table III). A second minor isomer seen in the same sample was assigned the structure **19b** on the basis of the following data: It



19b

(Me_3Si derivative) had a GC retention time of 15.16 min, which is different from those of the other isomers, showed a typical prostaglandin-like fragmentation pattern, and had distinguishable ^{13}C NMR absorbances at 135.6, 129.6, 54.6, and 51.1 ppm, which match reasonably well the corresponding absorbances of 11-epi-$PGF_{2\beta}$ (135.6, 130.2, 53.4, 51.8). The bicyclo endoperoxide isomers from which these components were derived were not distinguishable in the precursor sample (**9b**).

Discussion

The primary goal of our studies was the isolation and complete characterization of the PGG-type endoperoxides that had been implicated in published work[5-7] on the autoxidation of polyunsaturated fatty acids to prostanoids. The successful outcome of our endeavors was a direct result of several favorable circumstances.

The availability of the pure isomer **1a** in multigram quantities offered the advantages of reducing regioisomeric difficulties encountered by others[5,6] but still allowing work on a scale that might allow the isolation of low yields of individual stereoisomers. Furthermore, the first radical cyclization occurs stereospecifically in the desired sense to give **4**, which is suitably disposed for further cyclization to **6**. Lower partial pressures of oxygen decreased the conversion of **4** to monocyclic peroxides **5**. Pryor's work[6] on methyl linolenate autoxidation had indicated the endoperoxide intermediates had quite good stabilities (half-life estimated to be ca. 3.3 h at 80 °C in organic solvent), yet he chose not to attempt their isolation since it seemed likely that they would be minor components of a complex mixture of peroxidic materials. Instead, we have found that relatively large quantities of the stereoisomers **7–10** are formed and are sufficiently stable to survive chromatographic separation on silica gel. Both of these attributes were unexpected and contributed significantly to the experimental success of the work.

Autoxidation of hydroperoxide **1a** provided, as major isomers, bicyclo endoperoxides **7a–10a** that were easily separated by MPLC. The spectral properties of these materials provided hints as to their stereochemistry, but complete structural elucidation was accomplished on the corresponding triols **13a–16a** (and their derivatives). In this series, minor bicyclo endoperoxides **11a** and **12a** could not be separated from the major bicyclic peroxides. It was clear that they had a trans relationship of the side chains, but unequivocal assignment was difficult.

These findings were revealing for several reasons. The preferred formation of **9a** and **10a** (>50% of all bicyclic peroxides) was unprecedented in radical cyclization chemistry[25] and indicated kinetic control in [2.2.1] ring formation must be operative. Furthermore, we only found products of oxygenation of intermediate **6** at C-15 (none at C-13) which led to the correct functional relationships of C-8 through C-15 (prostaglandin numbering) when comparing our compounds to the natural prostanoids. Since our model hydroperoxide, **1a**, affords bicyclic peroxides with side chains that are very different from the natural products, we felt it prudent to confirm our findings in another system. This was facilitated by the elegant studies of Porter's group[7,10] which provide ready access to hydroperoxide **1b**. This material had been autoxidized and reduced to triols, one of which gave a GC–MS trace that was identical with that of authentic dinor-$PGF_{1\alpha}$.[7b] It was noted that other PGF-type compounds were also formed.

We have extended Porter's observations by autoxidizing **1b** and isolating the bicyclo endoperoxides in a fashion analogous to our work with **1a**. We were gratified to find that the isomer ratios were essentially the same as in the **a** series. Additionally, the chromatographic mobility of **11b** was sufficiently different from the other bicyclic peroxides that we were able to isolate it by HPLC uncontaminated with other isomers. There is no doubt that it has the natural prostaglandin ring stereochemistry and confirms both the identification work of Porter and our own suppositions regarding **11a** and **12a**. Working with the γ-linolenate-derived species allowed easier spectral comparisons of our

(25) Beckwith, A. L. J. *Tetrahedron* **1981**, *37*, 3073–3100.

triols to the PGF_1 and PGF_2 literature, which is extensive. In total, we have evidence that seven of the eight possible dinor-$PGF_{1\alpha}$ stereoisomers have been formed; however, the isomers with side chains trans are highly disfavored. The cyclization chemistry of peroxy radicals such as **3** (Scheme I) to PGG-type structures deviates from the enzyme-controlled reaction in a dramatic way only at the carbon–carbon forming step (**4** → **6**). The implication is that a major function of the cyclooxygenase enzyme is to maintain a proper orientation of the side chains for ring closure in a trans fashion, a mode that we now know to be unfavorable in solution. Excellent evidence for a similar enzymic function in the biosynthesis of steroids has recently culminated very extensive studies in that field.[26]

The fact that autoxidative cyclizations of lipid hydroperoxides lead to prostanoids that are stereochemically distinct from cyclooxygenase products should prove generally useful in a variety of lipid oxidation studies. One system that should be reinvestigated in light of our findings is the soybean lipoxygenase-2 conversion of arachidonic acid to prostanoids as reported by Axelrod and co-workers.[27] Since this enzyme has been shown to display predominantly (ω − 10)-oxygenation specificity,[12] it may be that the PGF-type products arose from oxidative cyclization of enzymatically produced 11-hydroperoxy-5(*Z*),(8*Z*),12(*E*),14(*Z*)-eicosatetraenoic acid.

Our studies with the C-18 compounds indicate that TLC comparisons of authentic PGF's to oxidation products of unusual origin may be misleading. In a commonly used solvent system, we found that triols with the natural prostaglandin ring stereochemistry (**17**, **18**) had R_f values that were nearly identical with those of the major triol isomers from autoxidation (**15**, **16**) for a given configuration at C-15. If this correlation holds for the C-20 series, mistaking PGF's formed by simple autoxidation with the natural material is a very real possibility. Thus, the cautions of Nugteren et al.[5] were well founded. Future studies should always include serious attempts to isolate and characterize PGG/H intermediates. If this proves impractical, we suggest increased use of capillary GC analysis of triol reduction products (as Me_3Si ether methyl esters) as a key tool in stereochemical determinations. Of course, if sample quantities permit, ^{13}C NMR is the most effective means of distinguishing among ring stereoisomers.

Experimental Section

General. α-Linolenic acid (99%) was obtained from Sigma Chemical Co. γ-Linolenic acid (99%) was obtained from Nu-Chek Preparations, Inc. Lipoxygenase-1 (50 000 units/mg and 250 000 unit/mg) was obtained from P-L Biochemicals. Solvents were either reagent grade or chromatography grade and were used without further purification.

Exact mass spectra were obtained on an AEI/Kratos MS-30 dual beam, double focusing mass spectrometer or on a ZAB-2F manufactured by Vacuum Generators. NMR spectra were obtained on JEOL FX-270 or FX-90, Varian EM360 or CFT-20, and Bruker CXP-300 instruments in dilute deuteriochloroform solutions using tetramethylsilane as an internal reference, except where noted otherwise. Infrared spectra were recorded on a Perkin-Elmer 298 spectrometer. Optical rotations were obtained by using a Rudolph Autopol III instrument. Circular dichroism (CD) measurements were done by using a 1-cm path length cell in a Jasco J-500C instrument with repetitive scanning capabilities (DP-500N data processor). The number of scans used for each CD determination is indicated with the molecular ellipticity (units are (deg·cm_2)/dmol). Analytical high-pressure liquid chromatography (HPLC) was done on a Waters Analytical HPLC (ALC/GPC 201) equipped with an M-6000 pumping system, M-U6K injector, M-R401 differential refractometer, and M-440 UV spectrophotometer, using μ-Porasil as the stationary phase and chromatography grade solvents as noted. Gas chromatography was performed on a Hewlett-Packard 5880A using a DB-1 capillary column (30 m × 0.25 mm with a 0.25-μm film thickness). Thin layer chromatography (TLC) was performed by using Analtech Silica Gel G plates of 0.25-mm thickness containing a fluorescer (W-F-254) (designated SG-GF). "Flash chromatography" was run according to the method of Still.[28] Medium-pressure liquid chromatography (MPLC) was performed by the method of Meyers.[15]

Preparation of Model Hydroperoxides. A 3-g sample of α-linolenic acid was enzymatically oxidized to its 13-hydroperoxide following a procedure of Porter et al.[10] to yield, after esterification and flash chromatography, 2.05 g (71%) of pure methyl 13-hydroperoxy-*cis*-9,*trans*-11,*cis*-15-octadecatrienoate. By a similar procedure γ-linolenic acid was converted to a mixture of methyl 9-hydroperoxy-*cis*-6,*trans*-10,*cis*-12-octadecatrienoate (ω − 10 isomer) (27% yield) and methyl 13-hydroperoxy-*cis*-6,*cis*-9,*trans*-11-octadecatrieonate (ω-6-isomer) (31% yield). Details of these syntheses along with various spectroscopic data are shown in the supplementary material.

Autoxidation of Methyl 13-Hydroperoxy-*cis*-9,*trans*-11,*cis*-15-octadecatrieonate (1a). A 0.15 M solution of the hydroperoxide in carbon tetrachloride was bubbled with oxygen for 15 min. The solution was stored at room temperature open to the atmosphere; it was bubbled with oxygen daily over a 10-day period. At this point TLC indicated most of the hydroperoxide had been consumed. Analytical HPLC on μ-Porasil using hexane–ethyl acetate (75:25) indicated 57% of the mixture was monocyclic peroxides, 18% was bicyclic peroxides (in four peaks having capacity factors of 1.1, 1.4, 1.7, and 2.4), and 22% was more polar material. A ^{13}C NMR spectrum of the crude mixture showed a multitude of peaks in the 35–55 ppm region.

The separation of these oxidation products was carried out on a medium-pressure liquid chromatography apparatus at room temperature over Silica Gel G (230–400 mesh) using hexane–ethyl acetate (70:30 or 75:25) as the mobile phase. Usually 200 25-mL fractions were collected, after which the remaining material was eluted from the column with ethyl acetate. The individual fractions were analyzed by TLC using two different systems—hexane–ethyl acetate (70:30) and pentane–ether–chloroform (55:35:10). Both TLC systems were used as a guide in combining the individual fractions.

The separation of 1.5 g using 70:30 hexane–ethyl acetate gave the following results: frations 11–17, 55 mg of recovered monohydroperoxide; fractions 18–30, 220 mg of **5a** (both diastereomers); fractions 35–42, 47 mg of **7a**; fractions 43–48, 78 mg of **9a**; fractions 52–60, 48 mg of a mixture of **8a** and **12a**; fractions 67–77, 45 mg of **10a**. A more polar fraction (eluting primarily in the neat ethyl acetate fraction) constituted ~0.5 g. The total weight recovery, including intermediate fractions which were mixtures, was ~70%.

Autoxidation of Methyl 9-Hydroperoxy-*cis*-6,*trans*-10,*cis*-12-octadecatrienoate (1b). In a manner similar to that described above, this autoxidation was carried out by using a 0.15 M solution of substrate in carbon tetrachloride at 5 °C. After 11 days TLC analysis indicated only a small amount of the starting hydroperoxide remained. Analysis of the crude sample by HPLC using hexane–ethyl acetate (75:25) as solvent indicated the mixture contained 49% **5b**, 5% **7b**, 3% **8b**, 21% **9b**, 11% **10b**, and 3% **11b**. An attempt to analyze the crude reaction mixture by quantitative ^{13}C NMR (10-s delay between pulses) analysis was not completely successful. Integration of various absorbances in the 35–50 ppm region relative to the methyl ester carbon indicated the relative percentages were about right, but suggested the total mono- and bicyclic peroxide content of the sample was somewhat lower.

The separation of these oxidation products was carried out on a medium-pressure liquid chromatography apparatus at 0 °C over Silica Gel G (230–400 mesh) using hexane–ethyl acetate (3:1) as the mobile phase at a flow rate of 21 mL/min. The individual fractions, each containing ~25 mL, were analyzed by TLC using two systems—hexane–ethyl acetate (70:30) and pentane–ether–chloroform (55:35:10). Both systems were used as a guide in compositing fractions.

The separation of 346 mg gave the following results: fractions 10–14, 45 mg of recovered monohydroperoxide; fractions 22–34, 75 mg of **5b** (two monocyclic diastereomers); fractions 35–40, 10 mg of **7b**; fractions 42–46, 6 mg of **11b**; fractions 54–67, 29 mg of **9b**; fractions 91–105, 15 mg of **10b**. The intermediate fractions, which were mixtures, and the more polar fractions (later than 105) accounted for most of the remaining material. Spectral data of the isolated fractions are shown in the tables.

Reduction of Bicyclo Endoperoxides to Triols. The reductions of the bicyclo endoperoxides were carried out at 0–5 °C in 0.1 M pH 7 phosphate buffer using 6 equiv of stannous chloride.[16] As an example, 4 mg (12 μmol) of **11b** in 3 mL of 95% ethanol was added to 20 mL of 0.1 M phosphate buffer at 0–5 °C. Then, 23 mg of $SnCl_2 \cdot 2H_2O$ in 3 mL of 95% ethanol was added, and the mixture was stirred 3.5 h. The reduced product was recovered by extracting the aqueous mixture several times with ether. After drying, the ether was stripped off to give 3 mg of an oil. A TLC of this product using aqueous ethyl acetate as solvent showed a major spot at R_f 0.35 subsequently identified as **17b** and a minor spot at 0.30 due to some **13b**. There was also a minor streak running near

(26) (a) van Tamelen, E. E.; Leopold, E. J.; Marson, S. A.; Waespe, H. R. *J. Am. Chem. Soc.* **1982**, *104*, 6479. van Tamelen, E. E. *Ibid.* **1982**, *104*, 6480. (b) See also: *Science* **1982**, *218*, 1297.

(27) Bild, G. S.; Bhat, S. G.; Ramadoss, C. S.; Axelrod, B. *J. Biol. Chem.* **1978**, *253*, 21–3.

(28) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–5.

the solvent front. In a similar manner, 7 mg of **7b** was reduced to give 4.6 mg of **13b**; 27 mg of **9b** was reduced to give 19 mg of **15b**; and 13 mg of **10b** was reduced to give 8.7 mg of **16b**. These samples were all analyzed without purification. The **a** series triols prepared in the same way were purified by flash chromatography (silica gel, neat ethyl acetate), but this treatment resulted in very poor yields.

Preparation of Trimethylsilyl Ether Derivatives of Triols. A mixture of 1 mg of triol, 0.1 mL of (trimethylsilyl)imidazole, and 0.1 mL of dry pyridine was heated at 80 °C for 30 min. The resulting reaction mixture was then analyzed by GC/MS. The results were shown in Tables VI and VII.

Preparation of the Triacetate Derivatives of Triols. The triacetate derivatives were prepared by stirring the triols with an excess of acetic anhydride in methylene chloride in the presence of 2 equiv of 4-(dimethylamino)pyridine. For example, 6 mg (18 μmol) of **13a**, 50 μL of acetic anhydride, and 5 mg (41 μmol) of (dimethylamino)pyridine in 3 mL of methylene chloride were stirred 15 min at room temperature. The reaction product was then flash chromatographed using 4:1 ether-pentane.

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Supplementary Material Available: Discussion of NMR and MS data on triols, spectroscopic data and experimental details on preparation of hydroperoxides, and tables of NMR, MS, and chromatographic data (15 pages).

Rearrangement and Catalysis in the Seyferth Reaction

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Abstract: The Seyferth reagent $PhHgCBr_3$ reacts with *trans*-1,2-dichloroethene to give two major products, *trans*-1,1-dibromo-2,3-dichlorocyclopropane (C) and 1,1-dibromo-3,3-dichloropropene (P). The stereospecifically formed cyclopropane is consonant with a singlet carbene mechanism, but the rearranged propene requires a second intermediate. Observation that the concentration ratio [P]/[C] is inversely proportional to the concentration of the alkene demonstrates that there are two intermediates, that the cyclopropane comes from the first-formed intermediate, and that the propene comes from the second-formed intermediate. The further observation that [P]/[C] is directly proportional to the concentration of starting material (the Seyferth reagent) requires that an additional mole of $PhHgCBr_3$ react with the first intermediate to form the second intermediate. Thus, the second intermediate must be a complex between the Seyferth reagent and the singlet carbene. Measurement of [P]/[C] as a function of aryl substituent in $ArHgCBr_3$ demonstrates that the Seyferth reagent serves as a Lewis base in the catalytic step. The near absence of rearranged material when phenyl is replaced by cyclohexyl in the Seyferth reagent suggests that the phenyl ring or the phenyl-mercury bond is the basic site. The ratio [P]/[C] is linearly proportional to either σ^+ or arene ionization potential in the series $ArHgCBr_3$, so that the second intermediate probably is a σ or π complex between the carbene and the aryl group. Electron donation from the aryl group increases the nucleophilicity of the carbene and heightens its reactivity with the electron-deficient alkene.

Cyclopropanation by the Seyferth reagent $C_6H_5HgCBr_3$ occurs via a free, singlet carbene, $:CBr_2$, in a reaction that has been well studied.[2] When the alkene is electron deficient, however, the singlet reaction is slow. We found that with dichloroethene a rearrangement pathway occurs in comparable yield to that of cyclopropanation.[3] Furthermore, the rearrangement pathway appeared to be catalyzed by the Seyferth reagent itself.[4] Consequently, we have carried out a thorough examination of the Seyferth reaction with dichloroethene in order to understand the mechanism of catalysis. We report here that the Seyferth reagent serves as a Lewis base in the rearrangement pathway, that the aryl ring is the catalytic site, and that mercury may serve as a template for gathering together carbene and alkene.

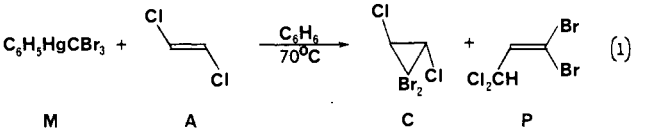
Results

trans-Dichloroethene (A) reacts with phenyl(tribromomethyl)mercury (M) in benzene at 70 °C to give two major products, the expected stereospecifically formed cyclopropane (C) and a rearranged propene (P) (eq 1).[5] The cyclopropane is stable to the reaction conditions, so that the propene must be a primary product. *cis*-Dichloroethene undergoes the same reaction but

Table I. Reaction of 2.5 mol % of $PhHgCBr_3$ with 25 mol % *trans*-CHCl=CHCl as a Function of Temperature[a]

product	60 °C	70 °C	80 °C
propene (P)	0.76	0.75	0.82
cyclopropane (C)	2.69	2.37	2.59
C_6H_5Br[b]	0.41	0.39	0.47
$CHBr_3$[b]	0.17	0.08	0.09
$CBr_2=CBr_2$[b]	0.35	0.32	0.34
$Br(CH_2)_6Br$[c]	1.00	1.00	1.00
[P]/[C]	0.28	0.32	0.32

[a] In benzene for 24 h. [b] Observed also in the absence of alkene. [c] Internal standard, not a reaction product.



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(2) Seyferth, D.; Mui, J. Y.-P.; Burlitch, J. M. *J. Am. Chem. Soc.* **1967**, *89*, 4953-4959.

(3) Lambert, J. B.; Kobayashi, K.; Mueller, P. H. *Tetrahedron Lett.* **1978**, 4253-4256.

(4) Lambert, J. B.; Mueller, P. H.; Gaspar, P. P. *J. Am. Chem. Soc.* **1980**, *102*, 6615-6616.

(5) Freidlina, R. K. *Adv. Free-Radical Chem.* **1965**, *1*, 231f.

0002-7863/84/1506-3584$01.50/0