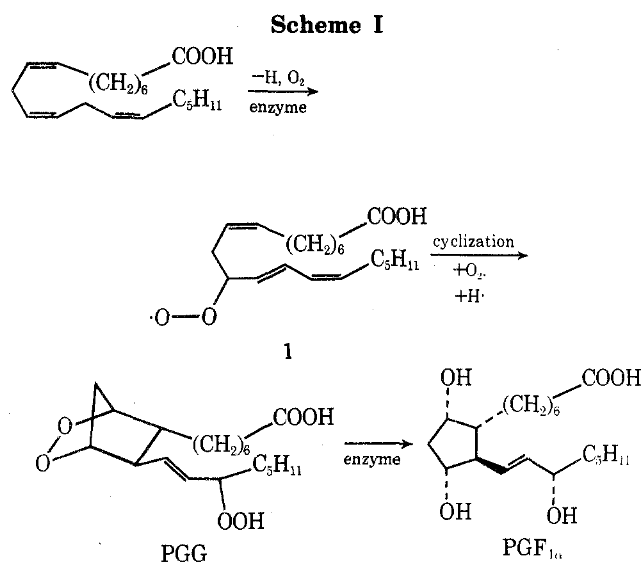
Communications

Peroxy Radical Cyclization as a Model for Prostaglandin Biosynthesis

Summary: Unsaturated lipid hydroperoxides are converted into prostaglandin analogs by free-radical initiators.

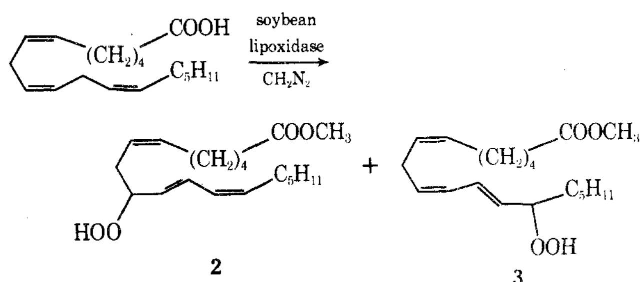
Sir: The biosynthesis of prostaglandins has been the focus of extensive investigation over the past several years.[1] A peroxy radical cyclization mechanism leading to intermediate endoperoxides has been proposed for this biosynthesis (Scheme I). The intermediacy of endoperoxides has, in fact, been concretely established by the isolation of two such compounds, PGG and PGH, from tissue homogenates which were actively engaged in the production of prostaglandins.[2]

Scheme I



Recently, we reported a method for generating specific peroxy radicals from the corresponding hydroperoxide.[3] This method is based on the fact that hydroperoxy hydrogens are readily abstracted by free radicals. Thus, hydroperoxides act as a source of the corresponding peroxy radical when treated with appropriate free radical initiators. We report here the application of this method to a hydroperoxide which should lead to a peroxy radical analogous to 1.

Polyunsaturated fatty acids are converted into hydroperoxides by a class of enzymes known as lipoxygenases (fatty acid: oxygen oxidoreductase). These enzymes have been identified in extracts from a variety of plant sources including soybeans,[4] potato tubers,[5] and wheat flour.[6] With the soybean enzyme,[7] γ-linolenic acid (*all-cis*-6,9,12-octadecatrienoic acid) could be converted into a 1:1 mixture of the ω-10 and ω-6 hydroperoxides 2 and 3. The reaction was carried out on the ammonium salt of the fatty acid at pH 7 for 2 min.[8] The crude hydroperoxide fatty acids were then converted into the hydroperoxide methyl esters 2 and 3 by reaction with diazomethane. 2 and 3 could be separated by high performance liquid chromatography (HPLC) with 6 ft × 1/8 in. of Corasil II and 0.24% isopropyl alcohol in hexane solvent. Thus, pure 2 could be isolated in an overall 15–20% yield based on starting fatty acid. The structures of 2 and 3



were assigned by conversion to the ketostearates followed by mass spectral analysis.[4b]

Incubation of 2 (10.8 mg, .0333 mmol) with di-*tert*-butyl peroxyoxalate (DBPO; 3.22 mg, 0.014 mmol) in O_2-saturated benzene at 25° for 16 hr led to complete consumption of 2. The products of the reaction, all of which were more polar than starting hydroperoxide, were reduced with $NaBH_4$ (25 mg, 0.661 mmol in 10 ml of CH_3OH) and then converted into the trimethylsilyl ethers for analysis by gas chromatography (GLC). Chromatography of the reduced and silylated product mixture on a 1% OV-17 column at 220° revealed at least four volatile products with retention times expected for PGF-type products from a C-18 fatty acid.[9]

Combined GLC–MS analysis of the product mixture indicates that two of these four compounds (retention times 9.5 and 10.5 min) are structurally analogous to authentic $PGF_{1\alpha}$. Prominent fragments of one of these compounds and $PGF_{1\alpha}$ analyzed under identical GLC–MS conditions are presented in Table I.

Table I
Mass Spectral Data (I.P. = 70 eV) of Trimethysilyl Ethers and Methyl Esters of $PFG_{1\alpha}$ and Synthetic C-18 PGF (Retention Time 9.5 min)

$PGF_{1\alpha}$			C-18 PGF		
Fragment[a]	*m/e*	%	Fragment[a]	*m/e*	%
M − 90	496	2	M − 90	468	2
	355	24		355	48
M − 233	353	100	M − 233	325	72
M − 251	335	72	M − 251	307	61
M − 277	309	80	M − 277	281	75
M − 289	297	62	M − 289	269	100
	295	50		295	22

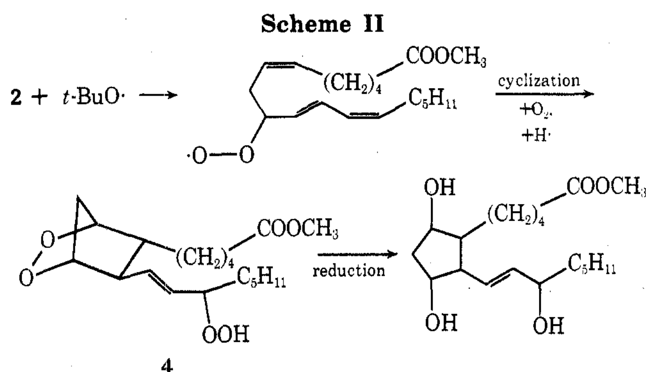
[a] Fragments between 200 and 500 *m/e* are shown and referred to the largest peak in this region as base.

The mass spectra of the synthetic PGF's (which have carboxyl side chains smaller than $PGF_{1\alpha}$ by C_2H_4, 28 *m/e*) are strikingly similar to $PGF_{1\alpha}$.[10] Every fragment from the synthetic PGF's between 200 and 500 *m/e* with a relative intensity greater than 20% of base corresponds either to a fragment of the same mass (loss of the carboxyl side chain) or to a fragment of *m/e* − 28 (retention of carboxyl side chain) from $PGF_{1\alpha}$.[11]

A mechanism consistent with our observations is presented in Scheme II. *tert*-butoxy radicals generated by de-

composition of DBPO abstract the labile hydroperoxide hydrogen[3] from 2 giving the peroxy radical. Cyclization of this radical to the endoperoxide followed by reduction[12] leads to PGF-type products.

Scheme II



The work reported here lends support to the notion that prostaglandin biosynthesis is a controlled free-radical reaction.[1] Other workers have noted the formation of prostaglandins in autoxidizing lipid.[11,13] The method reported here has the advantage of producing specific peroxy radicals for study as compared to the rather random autoxidation format.

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References and Notes

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(11) (a) Our mass spectra were compared to those of compounds obtained by autoxidation of methyl α-linolenate (W. A. Pryor and J. P. Stanley). Fragmentation patterns were generally similar with some exceptions that may be explicable by differences in the proposed structures. We thank Professor Pryor for this information before publication. (b) W. A. Pryor and J. P. Stanley, Abstracts of Papers, American Chemical Society Meeting, Chicago, Ill., Aug 25–29, 1975, paper ORGN-50.
(12) An alternate route to PGF compounds is via isomerization of 4 to PGE's, followed by reduction of PGE to PGF.
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A Suggested Mechanism for the Production of Malonaldehyde during the Autoxidation of Polyunsaturated Fatty Acids. Nonenzymatic Production of Prostaglandin Endoperoxides during Autoxidation

Summary: Autoxidation of methyl linolenate is shown to yield materials which give positive tests for both prostaglandin E and malonaldehyde, and it is suggested that both tests respond to prostaglandin-like endoperoxides which can be formed by autoxidation.

Sir: When polyunsaturated fatty acids (PUFA) or esters containing three or more double bonds undergo autoxidation, a material is produced which develops color in a sensitive test with thiobarbituric acid (TBA).[1–9] This TBA test is the most frequently used index of lipid peroxidation both in vitro and in vivo. Although the TBA-reactive material is frequently referred to as malonaldehyde,[5,6,9,10] it has been known for some time[3,4] that this material is predominantly nonvolatile; therefore, it is not malonaldehyde, but rather is a nonvolatile precursor of malonaldehyde.

In 1962, Holman et al.[4] suggested that a five-membered monocyclic peroxide is the nonvolatile malonaldehyde precursor. However, their mechanism does not appear to accommodate all the known facts of TBA-color production during PUFA autoxidation.[1] A more attractive mechanism, in our view, is one in which the nonvolatile malonaldehyde precursor is a bicyclic endoperoxide analogous to that which is formed in the biosynthesis of prostaglandins.[11–20] Figure 1 shows this mechanism as applied to methyl linolenate (18:3). Abstraction of an "internal" allylic hydrogen followed by reaction with O_2 leads to peroxy radicals 4 and 5. Radical 4 has a structure which allows cyclization to endoperoxide radicals 9, which are allylic, probably via the oxy-bridged radicals 6. Radicals 9, then, can become converted into endoperoxides 10 or 11.[21–23] Radicals 4 and 5 also can lead to the conjugated hydroperoxides 7 and 8 which are known products of autoxidations.

Our first indication that the nonvolatile malonaldehyde precursor is an endoperoxide came from comparisons of the responses of autoxidized solutions of 18:3 to the TBA test and a test developed for prostaglandin E (PGE).[19,24,25] The PGE test, which involves rapid formation of absorption at 278 nm upon addition of alcoholic base,[19,24,25] probably is relatively unspecific, but it is believed to convert PGE compounds into conjugated dienones such as PGB.[24] Since base is known to rapidly decompose secondary dialkyl peroxides to form ketones and alcohols,[26–28] we expected that endoperoxides, if produced in our autoxidations, would be converted by base into PGE-type compounds. The PGE-type compounds would then react further with base to give PGB-type chromophores and a positive PGE test. It appeared reasonable a priori that endoperoxides could be formed nonenzymatically by autoxidation in our system since the suggested mechanism for their biosynthesis involves a radical cyclization;[11–20] furthermore, Nugteren et al.[29a] have shown that autoxidation of 8,11,14-eicosatrienoic acid gives prostaglandins. Thus, we hypothesized that endoperoxides are produced on autoxidation of 18:3 and are the precursors of malonaldehyde under TBA-test conditions and PGB under conditions for the PGE test. (Note that 10 and 11 should give malonaldehyde but only 11 should give a PGE test.)

Indeed, autoxidized 18:3 does give a PGE test. This is true regardless of whether the oxidation is spontaneous (i.e., effected by pure air), or is initiated[1,2] by ozone or NO_2. On the other hand, 18:2, autoxidized under the same condi-