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IRON-PEPLOMYCIN CATALYZED OXYGENATION OF LINOLEIC ACID

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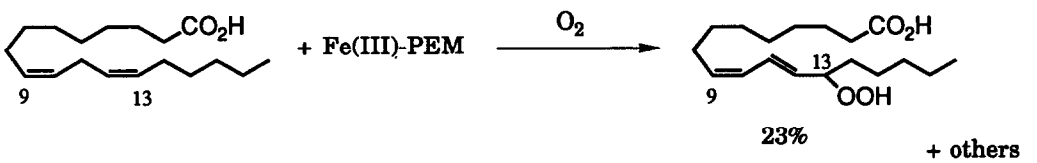
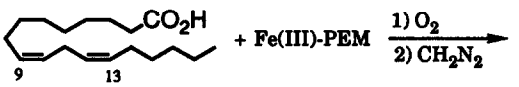
Summary: Incubation of linoleic acid with Fe(III)-peplomycin under aerobic conditions produced a mixture of hydroperoxides ***1a-d*** *as primary products which then gave rise to the formation of the corresponding dienones (2a-d), alcohols (3a-d) and epoxyenones (4a,b).*

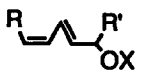
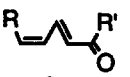
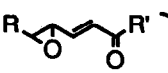
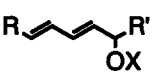
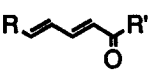
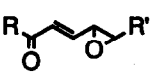
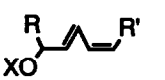
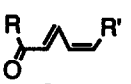
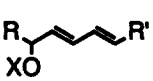
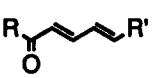
Lipoxygenase, a member of nonheme iron dioxygenases, catalyzes the conversion of 1,4-*cis,cis*-pentadienyl moiety of unsaturated fatty acids to 1-hydroperoxy-2,4-*trans,cis*-pentadiene system in a regio- and stereospecific manner[1] and plays a central role in fatty acid-cascades such as in the leucotrienes and jasmonic acid biosynthesis.[2] Hypothetical mechanisms involving a pentadienyl radical[3] or an organoiron intermediate[4] have been proposed in the enzymatic oxygenation. While the actual mechanism of the enzymatic oxygenation is still a matter of debate,[5] an important approach to the understanding of the lipoxygenase reaction is to discover an appropriate catalytic model system which can mediate hydroperoxidation of polyunsaturated fatty acids with molecular oxygen. We disclose herein an intriguing model system in which iron-complex of peplomycin (PEM), a family of antitumor antibiotic bleomycins, exhibits a lipoxygenase-like activity upon aerobic incubation with linoleic acid to produce hydroperoxides as primary oxidation products.[6]



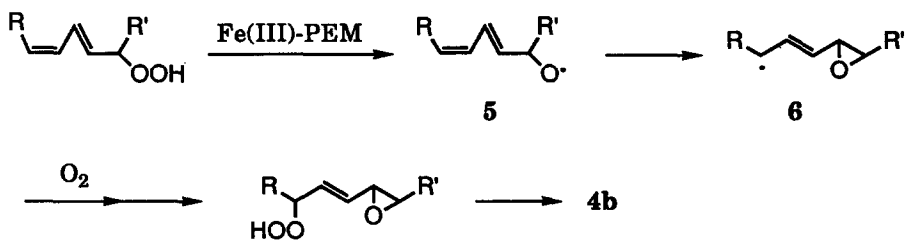
When linoleic acid (500 μM) was incubated with Fe(III)-PEM (20 μM) in 0.1 M phosphate buffer (pH 8) under oxygen atmosphere at room temperature, the substrate was smoothly consumed to give a mixtue of oxygenated products. After 15 min incubation, the mixture was converted to methyl esters and carefully analyzed by means of normal-phase silica gel HPLC (400 : 1 hexane-isopropanol). The major products identified by comparison with synthetic reference samples were four hydroperoxides (total 17.1% yield at 36.2% substrate consumption), including methyl 13-hydroperoxy-9,11-*cis,trans*-octadecadienoate (**1a**), 9,11-*trans,trans*-13-hydroperoxide (**1b**), 10,12-*trans,cis*-9-hydroperoxide (**1c**), and 10,12-*trans,trans*-9-hydroperoxide (**1d**), with the major hydroperoxide being **1a** (23% based on

consumed linoleic acid). In addition to these hydroperoxides, dienones **2a-d** (total 11.9%), alcohols **3a-d** (total 2.8%), and small amounts of epoxyenones **4a,b** (total 1.5% as an inseparable mixture of 9- and 13-keto isomers) were also formed. Interestingly, simple mono-oxygenation products like epoxy octadecaenoic acid could not be detected. The reference samples were prepared as follows. Hydroperoxide **1a** was prepared by reaction of linoleic acid with soybean lipoxygenase followed by treatment with diazomethane. Alcohol **3a** was obtained by reduction of **1a** with trimethyl phosphite. Oxidation of **3a** with lead tetraacetate in pyridine gave ketone **2a**.[7] Dienone **2a** was isomerized to **2b** by treatment with trifluoroacetic acid. Epoxyenone **4a** was prepared by MCPBA oxidation of **2b**. The isomeric hydroperoxides **1b-d** were prepared by singlet oxygenation of methyl linoleate followed by HPLC separation.[8] Alcohols **3b-d** and dienones **2b-d** were prepared similarly from the corresponding hydroperoxides **1b-d**, respectively.[9]



	yield (%)		yield (%)		yield (%)
 **1a** (X = OH)	8.2	 **2a**	3.8	 **4a**	1.5
3a (X = H)	1.3				
 **1b** (X = OH)	2.3	 **2b**	2.1	 **4b**	
3b (X = H)	0.4				
 **1c** (X = OH)	5.3	 **2c**	3.9	R = -(CH2)7CO2CH3	
3c (X = H)	0.8			R' = -(CH2)4CH3	
 **1d** (X = OH)	1.3	 **2d**	2.1		
3d (X = H)	0.3				

At higher conversion, the formation of dienones **2a-d**, alcohols **3a-d**, and epoxyenones **4a,b** became more prominent compared to that of hydroperoxides **1a-d**. For example, when the incubation was continued for 60 min, 76.6% of linoleic acid was consumed and the total yields of **2a-d**, **3a-d**, and **4a-b** increased to 37.7, 5.3, and 5.0%, respectively, whereas the total yield of hydroperoxides **1a-d** was decreased to 12.9%. Thus, it seems likely that dienones **2a-d**, alcohols **3a-d**, and epoxyenones **4a,b** would arise from the initially formed hydroperoxides **1a-d**, respectively, by the reaction with Fe(III)-PEM. Actually, when hydroperoxide **1a** (500 μM) was incubated for 30 min with Fe(III)-PEM (20 μM) in phosphate buffer (pH 8) in the absence of linoleic acid under the aerobic conditions followed by methylation, **2a** (19.2%), **3a** (5.5%), and **4a,b** (7.5%) were produced with 46.1% recovery of **1a**, while **2a** and **3a** were totally unchanged under the same conditons. A similar O-O bond cleavage of hydroperoxides catalyzed by Fe(III)-bleomycins has been precedented,[10] whereas the formation of epoxyenone **4b** (or **4a**) is assumed to arise from the addition of alkoxy radical **5** to the next double bond followed by trapping of the resulting radical **6** with molecular oxygen as illustrated below.



Oxidative DNA cleavage[11] and the oxidation of olefinic substrates[12] by iron-bleomycins have been studied extensively. It has been well documented that either oxygen plus reductant like ascorbate or a mono-oxygen atom donor such as iodosylbenzene is necessary for the production of activated bleomycins.[11] In this context, the present observation is rather surprising, since the oxidation was initiated only by Fe(III)-PEM and oxygen without any additives. Since a considerable induction period was observed when freshly prepared Fe(II)-PEM was used, the Fe(III)-complex should be the active species responsible for the oxygenation, analogous to the case of enzyme lipoxygenase.[1,4] It is also noteworthy that Fe(III)-PEM acts as a catalyst for the oxygenation of linoleic acid as evident from the catalytic turnover number of Fe(III)-PEM (TN = 4.3) in the formation of hydroperoxides **1a-d.**

The present results also suggest that even in the absence of reductant aerobic incubation of Fe(III)-PEM with linoleic acid would produce activated bleomycin which can induce DNA cleavage. In fact, aerobic incubation of Fe(III)-PEM (1 μM) with linoleic acid (10 μM) in the presence of ϕX 174 DNA (60 μM base concentration) in sodium cacodylate buffer (pH 8.0) induced relaxation of supercoiled circular DNA (form I) to form II (relaxed circular)

and form III (linear) DNA.[13] While further work is apparently necessary for the elucidation of the mechanism of the Fe(III)-PEM-mediated oxygenation of linoleic acid, the present results constitute a chemical model for lipoxygenase reaction and suggest that lipid peroxidation may be relevant to the pulmonary toxicity caused by bleomycins.[6,14]

References and Notes

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9. These synthetic samples comigrated with the oxidation products on HPLC and their 400 MHz ^{1}H NMR spectra were identical in each case. Representative HPLC data are as follows: Waters Cosmosil 5SL column (4.6 x 250 mm) detected at 234 nm; elution with hexane-isopropanol (400 : 1) at a flow rate of 3 mL/min; retention time (min), **1a** (21.8), **1b** (26.4), **1c** (31.2), **1d** (32.1), **2a** (7.0), **2b** (8.1), **2c** (7.8), **2d** (9.3), **3a** (24.3), **3b** (28.6), **3c** (36.3), **3d** (40.4), **4a** + **4b** (14.9).
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12. (a) E. C. Long and S. M. Hecht, *Tetrahedron Lett.*, **29**, 6413 (1988). (b) Y. Kaku, M. Otsuka, and M. Ohno, *Chem. Lett.*, 611 (1989), and references therein.
13. DNA cleavage by Fe(III)-bleomycin and 10-hydroperoxy-8,12-octadecadienoic acid has been reported.[10]

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