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The Radical-Scavenging Reactions of a Vitamin E Model Compound, 2,2,5,7,8-Pentamethylchroman-6-ol, with Radicals from the Fe(II)-Induced Decomposition of a Linoleic Acid Hydroperoxide, (9*Z*,11*E*)-13-Hydroperoxy-9,11-octadecadienoic Acid[1)]

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The radical scavenging reactions of a vitamin E model compound, 2,2,5,7,8-pentamethylchroman-6-ol, with radicals from the Fe(II)-induced decomposition of a linoleic acid hydroperoxide, (9*Z*,11*E*)-13-hydroperoxy-9,11-octadecadienoic acid, were examined extensively. When Fe(II) was added to a mixture of the vitamin E model compound and the linoleic acid hydroperoxide in methanol, (9*E*)-*trans*-12,13-epoxy-*erythro*-11-, (9*E*)-*trans*-12,13-epoxy-*threo*-11-, (9*Z*)-*trans*-12,13-epoxy-*erythro*-11- and (9*Z*)-*trans*-12,13-epoxy-*threo*-11-(2,2,5,7,8-pentamethylchromanoxy)-9-octadecenoic acids, and (9*E*)-13-hydroxy-12-methoxy-11-(2,2,5,7,8-pentamethylchromanoxy)-9-, (10*E*)-13-hydroxy-12-methoxy-9-(2,2,5,7,8-pentamethylchromanoxy)-10- and (11*E*)-13-hydroxy-10-methoxy-9-(2,2,5,7,8-pentamethylchromanoxy)-11-octadecenoic acids were obtained as main products. The hydroxymethoxy acids are presumed to be derived from the epoxy acids. A possible reaction pathway for the formation of the products is discussed.

Keywords——lipid peroxide; radical scavenger; linoleic acid hydroperoxide; vitamin E model compound; ferrous ion; adduct; solvolysis

Lipid peroxides are suspected to be injurious to cells and tissues,[2)] since they decompose to form a variety of radicals and carbonyl compounds that react readily with biological substances.[3)] On the other hand, vitamin E is thought to scavenge such radicals, and to act as a biological antioxidant against lipid peroxidation *in vivo*.[4)] Therefore, considerable attention has been directed to the reaction mechanisms by which vitamin E scavenges radicals.

For the elucidation of the radical-scavenging mechanisms, attempts have been made to analyze the reaction products formed in some lipid peroxide-decomposing systems containing vitamin E or a model compound.[5)] However, isolation and structure determination have been achieved only in part because of the complexity and instability of the products.

In order to identify the reaction products, we examined the radical-scavenging reactions of a vitamin E model compound, 2,2,5,7,8-pentamethylchroman-6-ol, under reaction conditions such that alkoxyl radical species were expected to arise predominantly; by the action of Fe(II), the radical was generated from highly purified (9*Z*,11*E*)-13-hydroperoxy-9,11-octadecadienoic acid (*Z*,*E*-13-LOOH) in methanol. We identified eleven products, including four isomeric epoxy(pentamethylchromanoxy)octadecenoic acids and three isomeric hydroxymethoxy(pentamethylchromanoxy)octadecenoic acids.

Experimental

Materials——A linoleic acid hydroperoxide, *Z*,*E*-13-LOOH, was prepared by oxidation of linoleic acid (99% purity; Sigma Chemical Co., St. Louis, MO, U.S.A.) with soybean lipoxygenase-1 (Sigma Chemical Co., 1.53×10^5

units/mg).[6] The hydroperoxide was purified by high-performance liquid chromatography (HPLC) as described previously.[7] A vitamin E model compound, 2,2,5,7,8-pentamethylchroman-6-ol, was synthesized by the method of Nilsson.[8] Iron(II) perchlorate $Fe(ClO_4)_2 \cdot 6H_2O$ was purchased from Alfa Products (Dambers, MA, U.S.A.).

Reaction Conditions——*Z,E*-13-LOOH (1.12 g, 3.6 mmol) and 2,2,5,7,8-pentamethylchroman-6-ol (0.80 g, 3.7 mmol) were dissolved in methanol (65 ml), argon was bubbled through, and an argon-saturated methanol solution (20 ml) of $Fe(ClO_4)_2 \cdot 6H_2O$ (1.34 g, 3.7 mmol) was added. When the reaction was carried out under aerobic conditions, no argon saturation or bubbling was done. After the addition was completed, argon bubbling was continued for 20 min. The reaction mixture was concentrated to one-tenth of its original volume under reduced pressure. Diethyl ether (40 ml) and water (20 ml) were added to the residue. The mixture was shaken, and the ethereal layer was separated and dried over Na_2SO_4. The ether was evaporated off under reduced pressure. The residue was applied to a silica gel column and eluted stepwise with diethyl ether–hexane mixtures (1 : 5, 1 : 3, 1 : 2, 1 : 1 and 2 : 1, v/v). Furthermore, each fraction was purified by semipreparative HPLC as described previously.[7]

Analytical Methods——Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian XL-200 spectrometer with $CDCl_3$ as a solvent and tetramethylsilane as an internal standard. Infrared (IR) spectra were obtained with a Jasco A-202 spectrometer equipped with a 0.1 mM NaCl cell using CCl_4 as a solvent. Ultraviolet (UV) spectra were taken with a Cary 118C spectrophotometer. Mass spectra (MS) were obtained with a Shimadzu-LKB 9000B gas chromatograph-mass spectrometer. HPLC was performed on a Waters PrepLC/System 500A with a PrePAK-500/Silica column and a Varian 5020 liquid chromatograph with a MicroPak Si-5 or -10 column.

Derivatization——Fatty acids were converted to their methyl esters with diazomethane in diethyl ether. Trimethylsilyloxy derivatives were prepared with a mixture of hexamethyldisilazane, trimethylchlorosilane and pyridine (2 : 2 : 1, v/v).

Results

Under anaerobic conditions, Fe(II) was added to a mixture of highly purified *Z,E*-13-LOOH and a vitamin E model compound, 2,2,5,7,8-pentamethylchroman-6-ol, in methanol. The reaction mixture was analyzed by HPLC (Fig. 1). For the isolation of the products, the reaction mixture was fractionated by silica gel column chromatography and subsequently each fraction was purified by semipreparative HPLC. Although no *Z,E*-13-LOOH remained in the reaction mixture, 30.8% of 2,2,5,7,8-pentamethylchroman-6-ol was recovered. The products isolated were epoxy(pentamethylchromanoxy)octadecenoic acids (I), hydroxy-methoxy(pentamethylchromanoxy)octadecenoic acids (II—IV), 13-hydroxyoctadecadienoic acid (V), 13-oxooctadecadienoic acid (VI), 2-(3-hydroxy-3-methylbut-1-yl)-3,5,6-trimethyl-1,4-benzoquinone (VII) and another compound (VIII). Compounds V, VI and VII were identified by comparison with authentic samples. Spectroscopically, VIII was suggested to be 5-methoxymethyl-2,2,7,8-tetramethylchroman-6-ol.[9] The structures of I—IV were determined as will be described later.

In order to examine the effect of oxygen on the radical scavenging reaction of the vitamin E model compound with the radicals derived from *Z,E*-13-LOOH, we studied the reaction under aerobic conditions. The products obtained were very similar to those from the reaction under anaerobic conditions, *i.e.*, mainly I, II and III. Oxygen has essentially no effect on the radical scavenging reaction of the vitamin E model compound.

Epoxy(2,2,5,7,8-pentamethylchromanoxy)octadecenoic Acids

Compound I was methylated with diazomethane. The products were fractionated by HPLC. As shown in Fig. 2, three compounds, Ia, Ib and Ic, were isolated. Their spectral data indicated that they are all epoxy(pentamethylchromanoxy)octadecenoic acids (Table I). Their mass spectra show a molecular ion peak at *m/e* 528, which is consistent with the molecular weight of the isomers of methyl epoxy(pentamethylchromanoxy)octadecenoate. Their UV spectra exhibit absorptions at 283 and 289 nm due to a 2,2,5,7,8-pentamethylchromanoxy group. Their ^{1}H-NMR spectra have signals at 3.00—2.69 ppm due to methine protons on epoxy rings[10] and signals at 5.53—5.28 ppm due to olefinic protons. The coupling constants (J=2.2—2.3 Hz) between the methine protons and the IR absorptions at 900—895 cm^{-1} show that the configuration of the epoxy groups is *trans*.

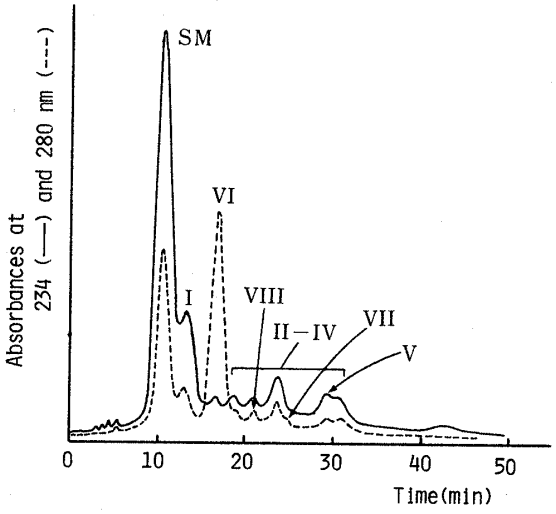


Fig. 1. High-Performance Liquid Chromatograms of the Reaction Mixture Resulting from the Addition of Fe(II) to a Mixture of *Z*,*E*-13-LOOH and 2,2,5,7,8-Pentamethylchroman-6-ol in Methanol under Argon

Solvent, hexane–ethanol–acetic acid (98.5:1.45:0.05, v/v); column, MicroPak Si-5 (4 mm i.d. × 300 mm); detection, UV at 234 (——) or 280 nm (----). Peaks I—VIII, see the text; SM, unreacted 2,2,5,7,8-pentamethylchroman-6-ol.

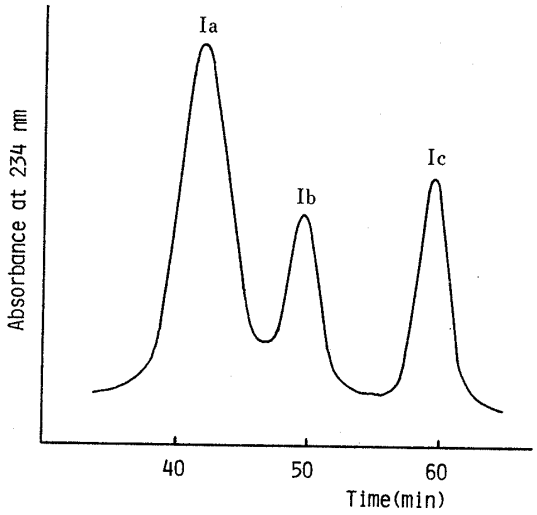


Fig. 2. Separation of Methyl Epoxy(pentamethylchromanoxy)octadecenates by HPLC

Solvent, hexane–diethyl ether (97.5:2.5, v/v); column, MicroPak Si-10 (8 mm i.d. × 500 mm). Peaks Ia—Ic, see the text.

The positions of the epoxy ring, double bond and 2,2,5,7,8-pentamethylchromanoxy group in each molecule were determined from the fragmentation patterns in the mass spectra and by ^{1}H-NMR signal assignment, confirmed by decoupling experiments (Table I).

The *E*–*Z* isomerism of Ia, Ib and Ic was distinguished on the basis of the IR absorption at 970 cm^{-1} due to an *E*-double bond. Since the IR spectrum of Ia shows a relatively weak band at 970 cm^{-1} and the ^{1}H-NMR signal of the methine proton at C-11 attached to the 2,2,5,7,8-pentamethylchromanoxy group is split unequally, Ia is a mixture of an *E*-olefin isomer and a small amount of a *Z*-isomer. The *Z*:*E* ratio was estimated as 3:1 based on the intensity of the split signals.

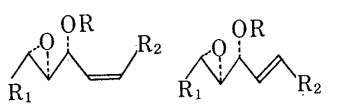
According to the report of Mercier and Agoh on the *erythro–threo* isomerism of *vic*-hydroxyepoxy compounds, coupling constants of 3.25 and 5.00 Hz are expected for the *erythro* and *threo* configurations, respectively.[11] Furthermore, for *erythro*- and *threo*-*E*-alkenyl silanes the coupling constants of the *erythro* isomers are smaller than those of the *threo* isomers.[12] From a consideration of the coupling constants between the protons at C-11 and C-12 in Ia (4.9 Hz), Ib (6.3 Hz) and Ic (6.2 Hz), Ia, Ib and Ic appear to be the *erythro*-, *threo*- and *threo*-(2,2,5,7,8-pentamethylchromanoxy)epoxides, respectively. This is supported by the greater mobility of the *erythro*-isomer on a thin layer plate or a silica gel column, because methyl *erythro*-hydroxyepoxyoctadecenoate migrates faster than the *threo* isomer.[13]

The above results show that Ia is a 3:1 mixture of the (9*Z*)- and (9*E*)-isomers of methyl *trans*-12,13-epoxy-*erythro*-11-(2,2,5,7,8-pentamethylchromanoxy)-9-octadecenoate, and that Ib and Ic are methyl (9*Z*)- and (9*E*)-*trans*-12,13-epoxy-*threo*-11-(2,2,5,7,8-pentamethylchromanoxy)-9-octadecenoates, respectively.

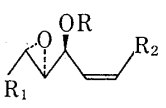
Hydroxymethoxy(2,2,5,7,8-pentamethylchromanoxy)octadecenoic Acids

Seven reaction products, IIa—c, IIIa—c and IV, which were eluted during 18—33 min from a 5 μm silica gel column, were isolated. Comparison of the spectral data between I and

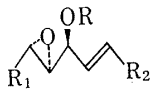
TABLE I. Yields and Spectral Data of Methyl Epoxy(pentamethylchromanoxy)octadecenoates



Ia



Ib



Ic

	Ia	Ib	Ic
Yield (%)	4.6	1.2	2.1
UV λ_{max}^{EtOH} nm (ε)	283sh (2460)	283sh (2270)	283sh (2420)
	289 (2770)	289 (2640)	289 (2700)
IR (CCl_4) cm^{-1}	1745 (C=O)	1745	1745
	970 (*E*-olefin)		970
	900 (*trans*-epoxide)	895	895
MS *m/e*	528 (M^+)	528	528
	428 ($M^+ - CH_3(CH_2)_4CHO$)	428	428
	415 ($M^+ - CH_3(CH_2)_4CH(O)CH$)	415	415
^{1}H-NMR ($CDCl_3$) ppm			
H-9 and 10	5.28 (m, 2H)	5.53 (m, 2H)	5.52 (m, 2H)
H-11	4.40 (dd, 0.75H, J=8.3, 4.9 Hz)[a]	4.25 (dd, 1H, J=8.7,	3.81 (dd, 1H, J=6.4,
	3.87 (dd, 0.25H, J=5.8, 5.6 Hz)[a]	6.3 Hz)	6.2 Hz)
H-12	2.93 (dd, 1H, J=4.9, 2.2 Hz)	3.05 (dd, 1H, J=6.3,	3.04 (dd, 1H, J=6.2,
		2.2 Hz)	2.3 Hz)
H-13	2.69 (m, 1H)	2.80 (dt, 1H, J=5.6,	2.79 (dt, 1H, J=5.7,
		2.2 Hz)	2.3 Hz)

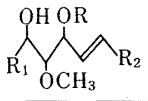
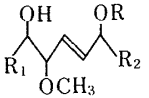
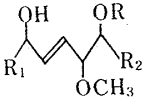
$R_1 = CH_3(CH_2)_4-$; $R_2 = -(CH_2)_7COOCH_3$; RO = 2,2,5,7,8-pentamethylchromanoxy. *a*) *Z*-/*E*-olefin = 3 : 1.

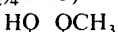
each of these products indicated that the products are all fatty acids with hydroxy (IR), methoxy (^{1}H-NMR) and chromanoxy (UV) groups and a double bond (^{1}H-NMR) (Table II). The mass spectra of their methyl esters show the same molecular ion peak at *m/e* 560, indicating that they are isomeric with each other. The mass spectra of their trimethylsilyl (TMS)-derivatives have the same fragment ion peak at *m/e* 173, which was assigned to a fragment of $CH_3(CH_2)_4CHOTMS$. In addition, the configuration of the double bonds was assigned as *E*-type on the basis of the coupling constants of the olefinic protons (15.3—15.6 Hz) and the IR absorptions at 970—980 cm^{-1}. Therefore, it is evident that II, III and IV are (*E*)-(13-hydroxy)methoxy(2,2,5,7,8-pentamethylchromanoxy)octadecenoic acids.

^{1}H-NMR decoupling experiments and coupling pattern analyses of IIa, IIIa and IV enabled us to assign the ^{1}H-signals due to the protons at C-9—C-13 in the molecules as listed in Table II. The signal assignments show that IIa is a 9-ene, IIIa a 10-ene and IV an 11-ene, and that C-11 and C-12 of IIa, C-9 and C-12 of IIIa and C-9 and C-10 of IV are oxygen-bearing carbon atoms. The fragment ion peak at *m/e* 415 of the methyl esters of IIa and IIIa indicates methoxy substitution at C-12 in IIa and IIIa, and that at *m/e* 243 of the TMS-derivatives of IV indicates methoxy substitution at C-10 in IV. These results indicate that IIa, IIIa and IV are (9*E*)-13-hydroxy-12-methoxy-11-(2,2,5,7,8-pentamethylchromanoxy)-9-octadecenoic acid, (10*E*)-13-hydroxy-12-methoxy-9-(2,2,5,7,8-pentamethylchromanoxy)-10-octadecenoic acid and (11*E*)-13-hydroxy-10-methoxy-9-(2,2,5,7,8-pentamethylchromanoxy)-11-octadecenoic acid, respectively.

Since IIa is spectroscopically identical with IIb and IIc, and IIIa with IIIb and IIIc except for the chemical shifts and coupling constants of the methine protons, it seems reasonable to conclude that IIb and IIc are stereoisomers of IIa, and that IIIb and IIIc are stereoisomers of IIIa. However, the stereochemistry of II, III and IV remains to be assigned.

TABLE II. Yields and Spectral Data of Hydroxymethoxy(pentamethylchromanoxy)octadecenoic Acids

		IIa	IIb	IIc	IIIa	IIIb	IIIc	IV
Yield (%)		4.5	5.2	2.3	4.2	3.8	1.5	1.1
UV λ_{max}^{EtOH} nm (ε)		282.5 (2630) 288.5 (3020)	283sh (2340) 289.5 (2770)	283sh (2480) 289 (2790)	283sh (2340) 289 (2670)	283sh (2310) 289 (2610)	283sh (2350) 289 (2700)	283sh (2300) 289 (2590)
IR (CCl_4) cm^{-1}		3590 (OH) 1715 (C=O) 970 (*E*-olefin)	3550 1715 975	3590 1715 970	3570 1715 980	3600 1715 980	3600 1715 980	3590 1715 980
MS *m/e*	Me ester	560 (M^+)	560	560	560	560	560	560
		460 ($M^+ - CH_3(CH_2)_4CHO$)	460	460	460	460	460	
		415 ($M^+ - CH_3(CH_2)_4$ )	415	415	415	415	415	
		340 (M^+ – chromanol)	340	340	340	340	340	340
	TMS deriv.	632 (M^+)	632	632	632	632	632	632
		415	415	415	415	415	415	
								243($CH_3(CH_2)_4$- OTMS $CHCH=CHOCH_3$)
		173($CH_3(CH_2)_4CHOTMS$)	173	173	173	173	173	173
^{1}H-NMR ($CDCl_3$) ppm	H-9	5.23 (dt, 1H, J=15.3, 6.4 Hz)	5.22 (dt, 1H, J=15.5, 5.9 Hz)	5.16 (dt, 1H, J=15.5, 3.2 Hz)	4.17 (dt, 1H, J=8.3, 5.5 Hz)	4.19 (dt, 1H, J=8.8, 5.8 Hz)	4.18 (dt, 1H, J=8.7, 7.7 Hz)	3.67 (m, 1H)
	H-10	5.45 (dd, 1H, J=15.3, 9.2 Hz)	5.61 (dd, 1H, J=15.5, 9.4 Hz)	5.60 (dd, 1H, J=15.5, 9.5 Hz)	5.68 (dd, 1H, J=15.5, 8.3 Hz)	5.65 (dd, 1H, J=15.5, 8.8 Hz)	5.70 (dd, 1H, J=15.4, 8.7 Hz)	3.98 (dd, 1H, J=6.4, 5.8 Hz)
	H-11	4.32 (dd, 1H, J=9.2, 6.7 Hz)	4.31 (dd, 1H, J=9.4, 3.9 Hz)	4.19 (dd, 1H, J=9.5, 3.2 Hz)	5.26 (dd, 1H, J=15.5, 8.0 Hz)	5.26 (dd, 1H, J=15.5, 8.4 Hz)	5.17 (dd, 1H, J=15.4, 7.7 Hz)	5.27 (dd, 1H J=15.6, 6.4 Hz)
	H-12	3.30 (dd, 1H, J=6.7, 1.7 Hz)	3.45 (dd, 1H, J=6.9, 3.9 Hz)	3.45 (dd, 1H, J=5.5, 3.2 Hz)	3.36 (dd, 1H, J=8.0, 4.2 Hz)	3.39 (dd, 1H, J=8.4, 3.6 Hz)	3.28 (dd, 1H, J=7.7, 5.4 Hz)	5.82 (dd, 1H, J=15.6, 9.4 Hz)
	H-13	3.74 (m, 1H)	3.70 (m, 1H)	3.68 (overlapped with OCH_3 signal, 1H)	3.42 (m, 1H)	3.60 (m, 1H)	3.32 (m, 1H)	4.07 (m, 1H)
	CH_3O	3.66 (s, 3H)	3.59 (s, 3H)	3.68 (s, 3H)	3.22 (s, 3H)	2.92 (s, 3H)	3.22 (s, 3H)	3.58 (s, 3H)

See the footnote to Table I.

Discussion

By the addition of Fe(II) to a mixture of *Z,E*-13-LOOH and the vitamin E model compound, we obtained epoxy(pentamethylchromanoxy)- and hydroxymethoxy(pentamethylchromanoxy)octadecenoic acids and other products. A possible reaction pathway for the formation of the products is shown in Chart 1. Initially *Z,E*-13-LOOH is reduced by Fe(II) to give a fatty acid alkoxyl radical. The alkoxyl radical, in turn, cyclizes to a *trans*-epoxy-*Z*-allylic radical, and this *Z*-allylic radical comes to equilibrium with the corresponding *trans*-epoxy-*E*-allylic radical. The 2,2,5,7,8-pentamethylchroman-6-oxy radical generated by hydrogen abstraction from the vitamin E model compound adds to C-11 of the *E*- and *Z*-allylic radicals to afford the (9*E*)- and (9*Z*)-11-(2,2,5,7,8-pentamethylchromanoxy)-12,13-epoxides I, respectively, each of which is composed of a pair of *erythro*- and *threo*-isomers (Ia, Ib and Ic). The 2,2,5,7,8-pentamethylchromanoxy radical adds to C-9 of the *trans*-epoxy-*E*- and *trans*-epoxy-*Z*-allylic radicals to afford the (10*E*)-9-(2,2,5,7,8-pentamethylchromanoxy)-12,13-epoxide. Methanolysis of the (9*E*)-11-(2,2,5,7,8-pentamethylchromanoxy)-12,13-epoxides yields II, and that of the (10*E*)-9-(2,2,5,7,8-pentamethylchromanoxy)-12,13-epoxide yields III and IV.

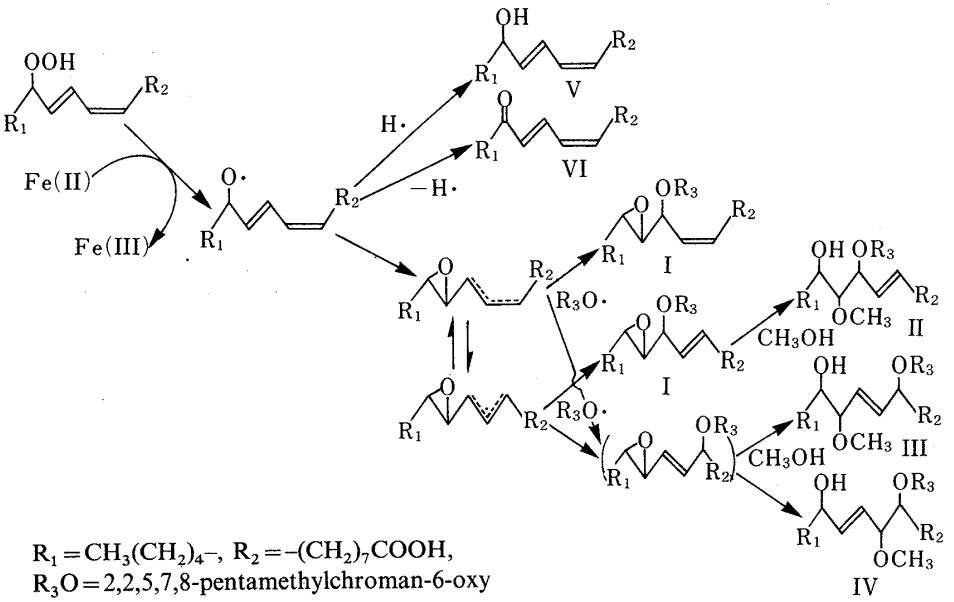


Chart 1. A Possible Reaction Pathway for the Formation of the Reaction Products

On the other hand, Gardner *et al.* reported that a mixture of 9- and 13-hydroperoxyoctadecadienoic acids and the vitamin E model compound in the presence of Fe(III) gave (9*E*)-*cis*-12,13-epoxy-11-(2,2,5,7,8-pentamethylchromanoxy)-9-octadecenoic, (9*E*)-*trans*-12,13-epoxy-11-(2,2,5,7,8-pentamethychromanoxy)-9-octadecenoic and (12*E*)-*cis*-9,10-epoxy-11-(2,2,5,7,8-pentamethylchromanoxy)-12-octadecenoic acids.[5a] In contrast to the results of Gardner *et al.*, we obtained only the *trans*-epoxides. The reason for the discrepancy between their results and ours is unclear. However, it seems reasonable that the *trans*-epoxides are predominant, because the *trans*-epoxides are considered to be formed from the more stable conformer of the fatty acid alkoxyl radical.

As has already been mentioned, the main products obtained by the addition of Fe(II) to a mixture of *Z,E*-13-LOOH and the vitamin E model compound under anaerobic conditions are the same as those obtained under aerobic conditions. This finding shows that molecular oxygen has no effect on the radical scavenging reaction of the vitamin E model compound.

This is supported by the facts that the vitamin E radical and its model radical, which are the key species in radical scavenging (see Chart 1), do not react with molecular oxygen.[14)]

Cysteine is also a biological radical scavenger. However, molecular oxygen appears to affect its radical scavenging ability. When a linoleic acid hydroperoxide was added to a cysteine solution in the presence of Fe(III), cysteine–fatty acid adducts were mainly obtained under anaerobic conditions, but only oxidation products of the fatty acid, such as oxooctadecadienoic, epoxyhydroxyoctadecenoic and epoxyoxooctadecenoic acids, appeared under aerobic conditions.[15)] There seems to be a clear difference in radical scavenging mechanisms between vitamin E and cysteine; vitamin E may be an efficient radical scavenger under both aerobic and anaerobic conditions, while cysteine may be less efficient under aerobic conditions.

References and Notes

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9) VIII: UV λ_{max}^{EtOH}: 298 nm (ε; 4200). IR (CCl_4): 3630 (OH), 1270 (aryl ether) and 1170 cm^{-1} (alkyl ether). MS *m/e*: 250 (M^+), 218 ($M^+ - 32$; loss of CH_3OH), 203, 179, 163, 149 and 135 (characteristic fragment ions of 2,2,5,7,8-pentamethylchroman-6-ol). ^{1}H-NMR ($CDCl_3$) ppm: 4.54 (s, 2H, $C\underline{H}_2OCH_3$), 3.32 (s, 3H, $OC\underline{H}_3$), 2.62 (t, 2H, J=6.8 Hz), 2.24 (s, 3H, 8-CH_3), 2.12 (s, 3H, 7-CH_3), 1.79 (t, 2H, J=6.8 Hz) and 1.29 (s, 6H).
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