Biochimica et Biophysica Acta, 713 (1982) 173–176
Elsevier Biomedical Press

BBA Report

BBA 50022

STRUCTURAL ANALYSIS OF DIASTEREOMERIC METHYL-9-HYDROXY *trans*-12,13-EPOXY-10-*trans*-OCTADECENOATES

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(Received June 17th, 1982)

Key words: Polyunsaturated fatty acid; Oxygenation; Chirality; Diastereomer; GLC

Reaction of 13-L$_S$-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid * with cysteine and $FeCl_3$ results in formation of a number of products, among which are *trans*-12,13-epoxy-9-oxo-*trans*-10-octadecenoic acid, *trans*-12,13-epoxy-9-hydroxy-*trans*-10-octadecenoic acid and *trans*-12,13-epoxy-9-hydroperoxy-*trans*-10-octadecenoic acid. It appears that formation of the latter compound by oxygenation at C_9 is a key step in this reaction. For the assessment of the enantioselectivity of this oxygenation, a method for the determination of the chirality at C_9 is necessary. For this purpose, *trans*-12,13-epoxy-9-oxo-*trans*-10-octadecenoic acid has been reduced, yielding a mixture of two compounds which can be separated by chromatography. Confirmation of the diastereomeric relation of these compounds has been obtained by 360 MHz ^{1}H-NMR. Subsequently, the absolute configurations at C_9 have been determined by a capillary GLC method, involving ozonolysis of the epoxyhydroxyenes. The results enable us to deduce the configuration of these compounds from their chromatographic behaviour.**

Oxygenation of polyunsaturated fatty acids having a 1,4-*cis, cis*-pentadiene system is catalysed by lipoxygenase (EC 1.13.11.12). The resulting *cis-trans* conjugated hydroperoxides can be converted into a variety of secondary products by different catalysts containing iron, such as a $FeCl_3$/cysteine couple [1], hemoglobin [2] and lipoxygenase from various plant sources.

Purified soybean lipoxygenase-1 can exhibit such activity under aerobic as well as under anaerobic conditions [3–6]. The latter reactions require a relatively high enzyme concentration.

Starting from 13-L$_S$-hydroperoxy-*cis*-9, *trans*-11-octadecadienoic acid as substrate, the major product appears to be *trans*-12,13-epoxy-*threo*-11-hydroxy-*cis*-9-octadecenoic acid [3]. The formation of the *threo* form only points to a stereochemically controlled reaction which is in contrast to experiments in which hemoglobin is used as a catalyst [2]. The stereochemical control of the lipoxygenase-catalysed reaction was confirmed by experiments with ^{18}O-labelled hydroperoxide, which resulted in a high retention (approx 90%) of both hydroperoxy oxygen atoms in the end product. The conversion rate of hydroperoxides can be stimulated greatly by addition of suitable hydrogen donors, e.g., guaiacol [7] under aerobic conditions, or by linoleic acid [8,9] under anaerobic conditions. A similar stimulation by linoleic acid has been reported in the reaction of hydroper-

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*** L and D refer to the nomenclature according to the Fischer convention; *S* and *R* refer to the nomenclature according to Cahn, Ingold and Prelog.

oxides with a pea lipoxygenase [10].

Gardner and Kleiman [11] have reported that reaction products resulting from decomposition of 13-L$_S$-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid by a soy homogenate are very similar to those obtained from a $FeCl_3$/cysteine-catalysed reaction. Both systems give rise to the formation of 13-oxo-*cis*-9, *trans*-11-octadecadienoic acid, 13-hydroxy-*cis*-9, *trans*-11-octadecadienoic acid, *trans*-12,13-epoxy-9-oxo-*trans*-10-octadecenoic acid, 12,13-epoxy-11-hydroxy-*cis*-9-octadecenoic acid and *trans*-12,13-epoxy-9-oxo-*trans*-10-octadecenoic acid [11,12].

Reduction of methyl *trans*-12,13-epoxy-9-oxo-*trans*-10-octadecenoate with $NaBH_4$ yields a 1:1 mixture of two products which can be separated by TLC on silica gel using multiple development with hexane/diethyl ether (60:40, v/v) as an eluent. A similar mixture was obtained by reduction of *trans*-12,13-epoxy-9-hydroperoxy-*trans*-10-octadecenoic acid derived from 13-L$_S$-hydroperoxy-*cis*-9, *trans*-11-octadecadienoic acid by reaction with either $FeCl_3$/cysteine or with a soy-extract. These epoxyhydroxyenes have recently been reported as products of the $FeCl_3$/cysteine-catalysed conversion of 13-L$_S$-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid [13]. It was proposed that these compounds are diastereomers differing in configuration at C_9. Mass spectra of trimethylsilyl derivatives of both compounds are virtually identical and a list of prominent fragment ions has been published [13]. However, the mass spectra can hardly be interpreted in terms of the presence of positional isomers. For example, a mass spectrum of the trimethylsilyl derivative of *trans*-12,13-epoxy-9-hydroxy-*trans*-10-octadecenoate differs from a spectrum of the corresponding *trans*-9,10-epoxy-13-hydroxy-*trans*-11-octadecenoate only in ion intensities [13].

In the present study we elaborated the stereochemistry of these compounds obtained by $FeCl_3$/cysteine-catalysed reaction of enzymically prepared hydroperoxides of linoleic acid. The epoxyhydroxyenes were synthesized by $NaBH_4$ reduction of methyl *trans*-12,13-epoxy-9-oxo-*trans*-10-octadecenoate, which was obtained and purified as described before [1,14] except for the use of a SilicAR CC-4-packed column (Mallinckrodt) in the purification of 13-L-ROOH and the introduction of pure O_2 in a solvent mixture of methanol/H_2O (4:1, v/v) in the $FeCl_3$/cysteine reaction. The epoxyhydroxyenes were obtained in a pure state by high-performance liquid chromatography on a preparative scale using a microporasil column (Waters; particle size, 10 μm; column dimensions, 7.8 mm × 30 cm) eluted with 4% acetone in hexane at 2 ml/min. The compound with the longest retention time is designated A, the other B. Both compounds show a plain negative ORD curve. The optical rotation data are summarized in Table I.

The 360 MHz ^{1}H-NMR spectra of compounds A and B in C^2HCl_3 were recorded on a Bruker HX-360 operating in the FT mode. In this way, subtle chemical shift differences between the spectra of compound A and B were detected. These differences are so small as not to be detected by 90 MHz spectroscopy (cf. Ref. 11). The spectrum of compound A is shown in Fig. 1. The spectral features are characteristic of a *trans* epoxy group, $J_{12,13} = 2.1$ Hz, and of a *trans* double bond, $J_{10,11} = 15.6$ Hz, in both compounds A and B. Close inspection of the H_9–H_{13} resonances in the 360 MHz ^{1}H-NMR spectra of compound A, compound B and of a 1:1 mixture of A and B reveals subtle chemical shift differences for protons 9,11 and 13 (Fig. 2). The chemical shifts of the latter resonances are summarized in Table II together with the shift increments.

To deduce the configuration of the chiral centers it is essential to realize that the carbon-oxygen bond of the hydroperoxy group is not broken in the epoxide formation. Therefore, the absolute

TABLE I

OPTICAL ROTATIONS OF COMPOUNDS A AND B

Concentrations: A 0.596 g/100 ml MeOH; B 0.553 g/100 ml MeOH.

λ (nm)	$[\alpha]^{24}$	
	Compound A	Compound B
589	−37.2°	−14.8°
578	−38.8°	−15.6°
546	−43.8°	−17.2°
436	−74.8°	−25.3°
365	−118.1°	−31.3°

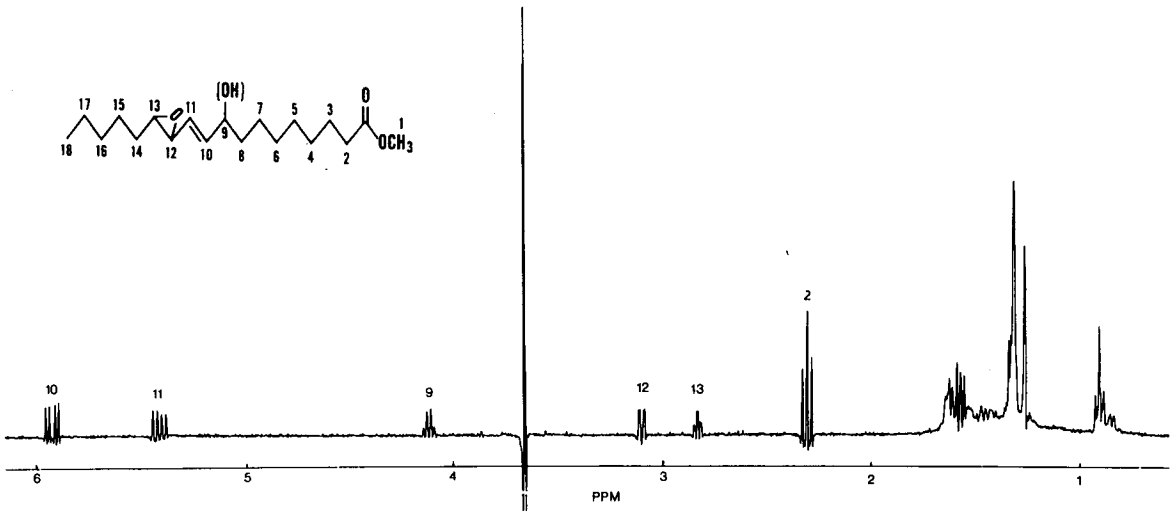

Fig. 1. 360 MHz ^{1}H-NMR spectrum of compound A. Resolution enhancement was achieved by Lorentzian to Gaussian transformation according to the method of Ernst [16].

TABLE II

RELEVANT CHEMICAL SHIFTS OF COMPOUNDS A AND B

Proton no.	Compound A, δ(ppm)	Compound B, δ(ppm)	Increment, δ(ppm)
9	4.118	4.131	0.013
10	5.917	5.915	
11	5.410	5.416	0.006
12	3.097	3.097	
13	2.826	2.823	−0.003

configuration at C_{13} is fixed by the L_S configuration of the starting hydroperoxide. Compounds A and B possess identical *trans* epoxy groups and *trans* double bonds. In consequence, they have the structural element C_{10}–C_{13} in common and differ only in configuration at C_9.

This is in line with the synthetic route leading to these products. To determine the absolute configurations at C_9, compounds A and B were analysed according to a previously described

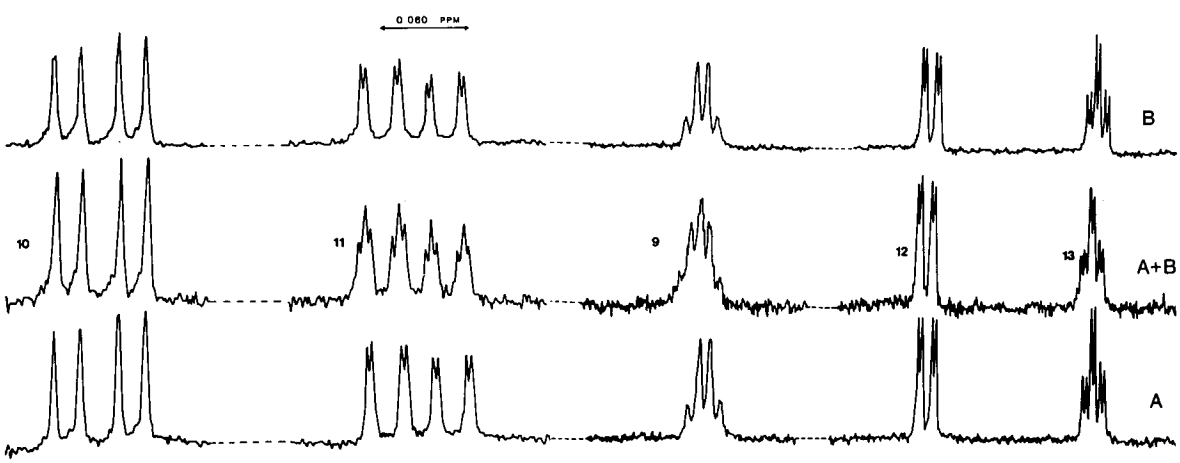

Fig. 2. Expanded parts of the 360 MHz ^{1}H-NMR spectra of compound A, compound B and of a 1 : 1 mixture of A and B showing the resonances of H_9–H_{13}.

method [15], comprising oxidative ozonolysis of the acetylated epoxyhydroxyenes. This degradation led to hydroxy sebacic acid derivatives which were treated with *R*-(−)-2-butanol and analysed by capillary gas-liquid chromatography. From the resulting chromatograms (Fig. 3) it can be seen that compound A yields the acetylated di-*R*-(−)-2 butyl ester of D_R-2-hydroxysebacic acid, whereas compound B yields the L_S analogue. The formation of these products gives independent evidence for the positions of the hydroxyl group and of the double bond in the epoxyhydroxyenes. From these data we conclude that compound A is identical with methyl (12*S*, 13*S*, 9*R*)-12,13-epoxy-9-hydroxy-*trans*-10-octadecenoate and compound B represents the corresponding 9*S* diastereomer *.

It should be noted that Hamberg [2] obtained a similar compound from the decomposition of 13-L-ROOH by hemoglobin. He investigated the configuration at C_9 and found 52% 9-D and 48% 9-L. However, he apparently did not detect the presence of diastereomers.

The identification of the compounds as described in this study makes it possible to correlate the absolute configuration of methyl (12*S*, 13*S*)-12,13-epoxy-9-hydroxy-*trans*-10-octadecenoate diastereomers with their chromatographic behaviour. These reference data can be applied to compare the diastereomeric compositions of (12*S*, 13*S*)-12,13-epoxy-9-hydroperoxy-*trans*-10-octadecenoic acids formed from 13-L_S-hydroperoxy-*cis*-9, *trans*-11-octadecadienoic acid by various catalysts.

Thanks are due to Dr. H. Van Halbeek for NMR spectra and to Mrs. G.P.M. Rijke-Schilder for technical assistance. This investigation was supported in part by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

* Assignments of the stereochemistry of C_9 for compounds A and B by the Fischer convention are L and D, respectively. Because the Fischer configurations of C_9 in A and B are the opposite of their 2-hydroxysebacic acid derivatives, this point could be confused easily. It follows that the upwards orientation of the acetylated 2-hydroxy group of the sebacate ester used in the Fischer convention is in the opposite orientation when compared to the carboxyl group of the parent compounds (A and B).

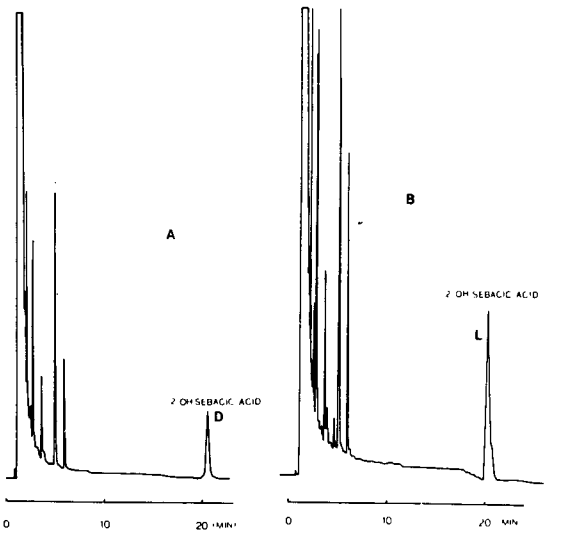

Fig. 3. Capillary gas-liquid chromatograms of the acetylated *R*-(−)-2-butyl esters of 2-hydroxysebacic acids obtained from compounds A and B after oxidative ozonolysis.

References

1 Gardner, H.W., Kleiman, R. and Weisleder, D. (1974) Lipids 9, 696–705
2 Hamberg, M. (1975) Lipids 10, 87–92
3 Garssen, G.J., Veldink, G.A., Vliegenthart, J.F.G. and Boldingh, J. (1976) Eur. J. Biochem. 62, 33–36
4 Verhagen, J., Bouman, A.A., Vliegenthart, J.F.G. and Boldingh, J. (1977) Biochim. Biophys. Acta 486, 114–120
5 Verhagen, J., Veldink, G.A., Vliegenthart, J.F.G. and Boldingh, J. (1979) in Advances in the Biochemistry and Physiology of Plant Lipids (Appelquist, L.Å. and Liljenberg, C., eds.), pp. 231–236, Elsevier/North-Holland, Amsterdam
6 Verhagen, J. (1978) Thesis, University of Utrecht
7 Streckert, G. and Stan, H.J. (1975) Lipids 10, 847–854
8 Garssen, G.J., Vliegenthart, J.F.G. and Boldingh, J. (1971) Biochem. J. 122, 327–332
9 Garssen, G.J., Vliegenthart, J.F.G. and Boldingh, J. (1972) Biochem. J. 130, 435–442
10 Arens, D. and Grosch, W. (1974) Z. Lebensm. Unters. Forsch. 156, 292–299
11 Gardner, H.W. and Kleiman, R. (1977) Lipids 12, 941–944
12 Gardner, H.W., Weisleder, D. and Kleiman, R. (1978) Lipids 13, 246–252
13 Gardner, H.W. and Kleiman, R. (1981) Biochim. Biophys. Acta 665, 113–125
14 Gardner, H.W. (1975) Lipids 10, 248–252
15 Van Os, C.P.A., Rijke-Schilder, G.P.M., Kamerling, J.P., Gerwig G.J., and Vliegenthart, J.F.G. (1980) Biochim. Biophys. Acta 620, 326–331
16 Ernst, R.R. (1966) in Advances in Magnetic Resonance (Waugh, J.S., ed.) Vol. 2, pp. 1–135, Academic Press, New York