[Chem. Pharm. Bull. 32(1) 332—335 (1984)]

Isomerization of Linoleic Acid Hydroperoxides under Argon and under Degassed Conditions[1)]

TAKAO KANEKO and MITSUYOSHI MATSUO*

Tokyo Metropolitan Institute of Gerontology, 35–2 Sakae-cho, Itabashi-ku, Tokyo 173, Japan

(Received May 16, 1983)

The rearrangement of linoleic acid hydroperoxides, (9*Z*, 11*E*)-13-hydroperoxy-9,11- and (9*E*, 11*E*)-13-hydroperoxy-9,11-octadecadienoic acids (13-*Z*,*E*-LOOH and 13-*E*,*E*-LOOH, respectively), occurred under argon and under degassed conditions, as well as under aerobic conditions. In argon-saturated benzene, 13-*Z*,*E*-LOOH isomerized to 9-*E*,*E*- and 13-*E*,*E*-LOOHs, and 13-*E*,*E*-LOOH to 9-*E*,*E*-LOOH; the *E*,*E*-isomers hardly isomerized to *E*,*Z*-isomers. The hydroperoxides isomerized rapidly in benzene and chloroform, but slowly in *n*-propyl ether and methanol. However, they decomposed extensively in chloroform, and moderately in benzene and methanol. Decomposition was very slight in *n*-propyl ether. The isomerization rate of the 9*Z*, 11*E*-isomer was much higher than that of the 9*E*, 11*E*-isomer. Since the rate was found to be slow under degassed conditions, traces of dissolved molecular oxygen may be responsible for the isomerization.

Keywords——linoleic acid hydroperoxide; rearrangement; isomerization; solvent effect; molecular oxygen; HPLC

Recently lipid peroxides have been implicated as harmful agents in liver necrosis,[2)] atherosclerosis,[3)] cerebral ischemia,[4)] carcinogenesis[5)] and aging.[6)] Initial products of lipid peroxidation are thought to be hydroperoxides of polyunsaturated fatty acids. However, the chemical properties of the hydroperoxides are little known because of their instability and the difficulty of their purification. Fortunately, recent progress in high performance liquid chromatography (HPLC) means that it is now possible to purify several isomers of fatty acid hydroperoxides.[7)]

Chan and his co-workers observed that methyl linoleate hydroperoxides rearranged under aerobic conditions.[8)] We report here that linoleic acid hydroperoxides undergo rearrangement leading to their isomerization under argon and under degassed conditions to give the *E*,*E*-isomers, and the isomerization rate depends on the solvent used.

Experimental

Analytical HPLC was performed on a Varian 8520 liquid chromatograph equipped with a Varian Vari-Chrom liquid chromatography detector and a MicroPak Si-5 column (4 mm i.d. × 30 cm). Preparative HPLC was carried out on a Varian 5020 liquid chromatograph with a MicroPak Si-10 column (8 mm i.d. × 50 cm). Degassing was conducted with a mercury diffusion pump. Linoleic acid (99% purity) and soybean lipoxygenase-1 were obtained from Sigma Chemical Co. (St. Louis, U.S.A.) and argon (research grade) from Takachiho Shoji Co. (Tokyo, Japan).

Preparation and Purification of Linoleic Acid Hydroperoxides——(9*Z*,11*E*)-13-Hydroperoxy-9,11-octadecadienoic acid (13-*Z*,*E*-LOOH) was prepared by the method of Hamberg[9)]; linoleic acid (2.80 g, 10 mmol) and soybean lipoxygenase-1 (10.5 mg, 1.53×10^5 units/mg) were incubated in 0.1 M NH_4Cl–NH_4OH buffer (pH 9.2, 400 ml) at 5 °C for 6 h under oxygen. After being acidified to pH 3 with diluted HCl, the reaction mixture was extracted with ether, washed with water, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was chromatographed on SilicAR CC-7 (Mallinckrodt Inc., Paris, U.S.A.) by means of stepwise elution with 10, 20 and 30% ether in hexane to give crude 13-*Z*,*E*-LOOH. This 13-*Z*,*E*-LOOH was purified by preparative HPLC eluted with hexane–ethanol–acetic acid (98.5 : 1.45 : 0.05, v/v) at a flow rate of 4.0 ml/min at room temperature, with monitoring

through optical density measurement at 234 nm (diene conjugation).

(9*E*, 11*E*)-13-Hydroperoxy-9,11-octadecadienoic acid (13-*E*,*E*-LOOH) was prepared by the autoxidation of linoleic acid and purified similarly.

Rearrangement of Linoleic Acid Hydroperoxides——Under argon, 0.5 ml of a 50 mM solution of 13-*Z*,*E*- or 13-*E*,*E*-LOOH (7.8 mg, 25 μmol) was incubated at 50 °C for an appropriate period in a 1-ml minivial equipped with a Mininert valve SC-13 (Pierce Chemical Co., Rockford, U.S.A.). Under reduced pressure (0.0005 mmHg), the 13-*Z*,*E*-LOOH solution was degassed by the freeze–thaw procedure (repeated five times) and then incubated at 50 °C in sealed ampoules. The time courses of isomerization and decomposition of the hydroperoxides were followed by the analytical HPLC method.

Estimation of Isomerization and Decomposition——The percentages of isomerization (I) and decomposition (D) were estimated in the following manner:

$$I=(100-A)\times\frac{[\text{areas for LOOH}]-[\text{area of substrate}]}{[\text{total area}]}$$

$$D=A+(100-A)\times\frac{[\text{areas of products other than LOOH}]}{[\text{total area}]}$$

where A is the percentage decrease in the optical density of each sample at 234 nm, and areas represent peak areas on the HPLC chromatogram.

Results

When incubated in benzene under argon at 50 °C, 13-*Z*,*E*-LOOH isomerized to (10*E*, 12*E*)-9-hydroperoxy-10,12-octadecadienoic acid (9-*E*,*E*-LOOH) and 13-*E*,*E*-LOOH. After separation by HPLC, 9-*E*,*E*- and 13-*E*,*E*-LOOHs were identified by means of proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. 9-*E*,*E*-, 13-*E*,*E*- and 13-*Z*,*E*-LOOHs can be separated chromatographically, while the *Z*,*E*- and *E*,*E*-isomers can be distinguished from each other on the basis of the chemical shifts and coupling constants of protons attached to sp^2 carbon atoms in the LOOHs.[10] The 9-*E*,*E*-LOOH fraction in HPLC did not contain (10*E*, 12*Z*)-9-hydroperoxy-10,12-octadecadienoic acid (9-*E*,*Z*-LOOH). This was confirmed by the finding that the 9-*E*,*E*-LOOH fraction was reduced with triphenylphosphine to afford only (10*E*, 12*E*)-9-hydroxy-10,12-octadecadienoic acid. The isomerization proceeded rapidly in the initial incubation period, and decomposition proceeded relatively slowly with advancing reaction time (Fig. 1A). Under the degassed conditions, the isomerization was suppressed considerably (Fig. 1A). Although 13-*E*,*E*-LOOH isomerized to 9-*E*,*E*-LOOH in benzene, the

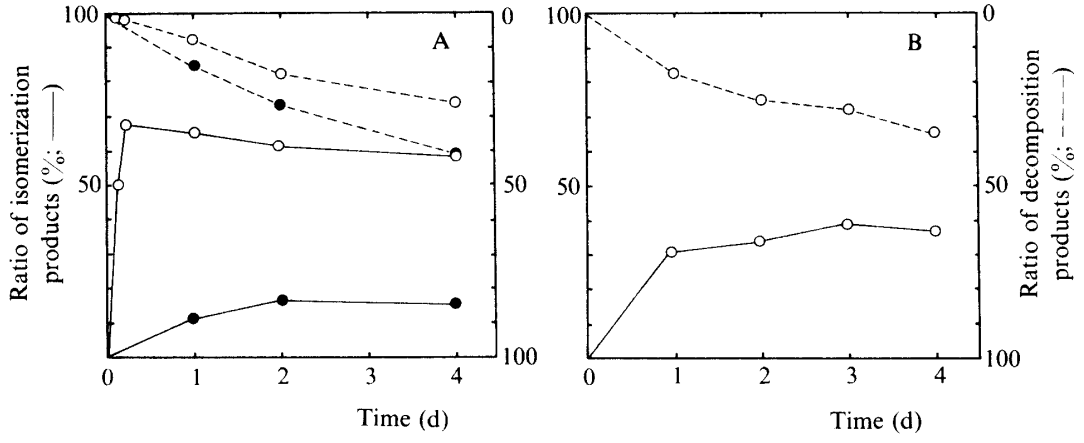

Fig. 1. Time Courses of the Isomerization and Decomposition of Linoleic Acid Hydroperoxides under Argon or under Degassed Conditions

13-*Z*,*E*-LOOH (A) or 13-*E*,*E*-LOOH (B) (50 mM) was incubated in benzene at 50 °C under argon (○) or under degassed conditions (●).
——, isomerization; - - - -, decomposition.

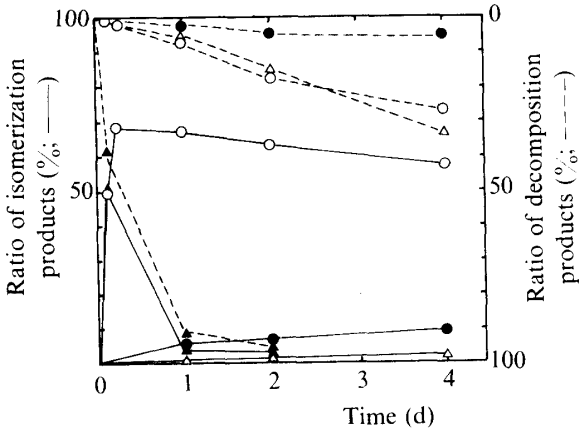

Fig. 2. Effect of Solvents on the Isomerization and Decomposition of 13-*Z*,*E*-LOOH

13-*Z*,*E*-LOOH (50 mM) was incubated at 50 °C under argon.
○, benzene; ●, *n*-propyl ether; △, methanol; ▲, chloroform.

isomerization rate was much slower than that of 13-*Z*,*E*-LOOH (Fig. 1B).

The time courses of the isomerization and decomposition of 13-*Z*,*E*-LOOH in different solvents under argon are illustrated in Fig. 2. The isomerization occurred rapidly in benzene, slowly in *n*-propyl ether and scarcely at all in methanol. The simultaneous decomposition was slow in benzene and methanol, and very slight in *n*-propyl ether. Both the isomerization and decomposition were very fast in chloroform.

Discussion

Chan and his co-workers reported that methyl linoleate hydroperoxides rearranged under aerobic conditions, but not under anaerobic conditions, and they proposed a molecular oxygen-based free radical mechanism for the rearrangement.[8] However, we found that linoleic acid hydroperoxides rearranged in benzene under argon as well as under air, and also rearranged slightly under degassed conditions (0.0005 mmHg). Thus, the rearrangement may occur in the presence of trace amounts of molecular oxygen contained in the solutions.

In argon-saturated benzene, 13-*Z*,*E*-LOOH isomerizes to 9-*E*,*E*- and 13-*E*,*E*-LOOHs, and 13-*E*,*E*-LOOH to 9-*E*,*E*-LOOH; the *E*,*E*-isomers hardly isomerized to the *E*,*Z*-isomers. This may be due to the difference in thermodynamic stability between the *E*,*E*- and *E*,*Z*-radicals, which are presumed to be necessary intermediates in the course of the isomerization,[8] because an *E*,*Z*-pentadienyl radical is converted into an *E*,*E*-pentadienyl radical above *ca.* 170 °K, but the latter does not isomerize to the former even at a temperautre as high as 420 °K.[11]

The isomerization was found to occur rapidly in benzene and chloroform, and very slowly in methanol and *n*-propyl ether. The finding is not consistent with the previous suggestion that solvent polarity is responsible for the solvent effect on the rearrangement.[8]

Since both the isomerization and decomposition rates in *n*-propyl ether are very slow, *n*-propyl ether can be recommended as a solvent for the storage of the hydroperoxides. Particular attention should be given to the susceptibility of polyunsaturated fatty acid hydroperoxides to very small amounts of molecular oxygen in solutions.

References

1) Contribution TMIG-I No. 53.
2) T. F. Slater, "Free Radical Mechanism in Tissue Injury," Pion Limited, London, 1972, pp. 118—170.
3) J. Glavind, S. Hartmann, J. Clemmensen, K. E. Jessen and H. Dam, *Acta Pathol. Microbiol. Scand.*, **30**, 1 (1952).
4) H. B. Demopoulos, E. S. Flamm, D. D. Pietronigro and M. L. Seligman, *Acta Physiol. Scand. Suppl.*, **492**, 91 (1980).
5) R. Shamberger, *J. Natl. Cancer Inst.*, **48**, 1491 (1972).

6) D. R. Sanadi, "Handbook of the Biology of Aging," ed. by C. E. Finch and L. Hayflick, Van Nostrand Reinhold Co., New York, 1977, pp. 90—93.
7) H. W.-S. Chan, C. T. Costaras, F. A. A. Prescott and P. A. T. Swoboda, *Biochim. Biophys. Acta*, **398**, 347 (1975); J. A. Singleton, H. E. Pattee and M. S. Nelson, *J. Am. Oil Chem. Soc.*, **55**, 387 (1978).
8) H. W.-S. Chan, G. Levett and J. A. Matthew, *Chem. Phys. Lipids*, **24**, 245 (1979).
9) M. Hamberg, *Anal. Biochem.*, **43**, 515 (1971).
10) H. W. Gardner and D. Weisleder, *Lipids*, **7**, 191 (1972); D. T. Coxon, K. R. Price and H. W.-S. Chan, *Chem. Phys. Lipids*, **28**, 365 (1981).
11) A. G. Davies, D. Griller, K. U. Ingold, D. A. Lindsay and J. C. Walton, *J. Chem. Soc., Perkin Trans. 2*, **1981**, 633.