Preparation of Triglyceride Hydroperoxides

Kaneyuki SOHDE,* Sadao IZUTANI
and Setsuro MATSUSHITA

Research Institute for Food Science,
Kyoto University, Kyoto

Received February 26, 1973

The toxicity of oxidized lipids has been attributed to both hydroperoxides which are the primary products of lipid oxidation and their secondary degradation products.^{1,2)} However, there are some contradictory discussions on the toxicity levels of hydroperoxides and their secondary degradation products.^{3,4)} Therefore, experiments using pure compounds seem to be necessary for the study of the toxicity of oxidized lipids. Separation of fatty acid hydroperoxides has been achieved by partition chromatography.⁵⁾ However, the isolation and purification of TG hydroperoxides have not been reported. This paper reports the separation and purification of TG hydroperoxides from oxidized safflower oil by silica gel column chromatography and TLC. No complete chemical characterization of TG hydroperoxides has been attempted, but the method is considered to be useful for the studies on the toxicity of oxidized lipid.

Preparation of TG hydroperoxides. Safflower oil was kindly supplied from Nihonkoyu Co., Ltd. Twenty ml of safflower oil in a 11.5 cm dish was placed

FIG. 2. Polarograms of TG and TG Hydroperoxide. Polarograph, Yanaco Model P₈ was used. Electrolyte solution consisted of 0.3 M LiCl in benzene: methanol (50: 50, v/v).⁹⁾ Two μ l of TG hydroperoxide was added to 2 ml of electrolyte solution, and polarographed at 20°C after bubbling argon gas through the solution for 20 min to eliminate dissolved oxygen.

FIG. 1. TLC of the Compounds Fractionated by Column Chromatography. Merck TLC plate, silica gel 0.25 mm, was used. The solvent system was petroleum ether: ethyl ether: 28% ammonia (55: 45: 2). The spots were detected by exposing the plates to iodine vapour.

* Permanent address: Katata Research Laboratory, Toyobo Co., Ltd., Ohtsu, Japan.

Abbreviations: TG, triglyceride; POV, peroxide value; TLC, thin-layer chromatography.

in a glass room for 5 days at about 25°C. Silica gel, 60~200 mesh, was washed with acetone and was activated by heating at 100°C for 1 hr before use. One hundred g of silica gel was thoroughly mixed with 100 ml of immobile solvent, benzene: methanol (9:1, v/v), and was suspended in 200 ml of benzene. The uniform suspension was packed into a 2.5×45 cm column (Pharmacia column for solvent use). One ml of oxidized safflower oil (POV, 960, determined by the method of Sully⁶⁾) was charged on to the top of the column and eluted with 500 ml of benzene (mobile solvent) at a flow rate of 1 ml/min followed by 200 ml of ethylether. The fractions were subjected to TLC identification as shown in Fig. 1. The hydroperoxide fraction (tube No. 40~90) was pooled and concentrated by vacuum evaporation at 37°C. The concentrated fraction was subjected to preparative TLC using the same solvent system as used in the analytical TLC (Fig. 1). The hydroperoxide band detected by UV light was scraped off and extracted with ethyl ether. By evaporating ether, 65 mg of hydroperoxides were obtained. Preparative TLC plates (1.25 mm) were prepared with Merck silica gel PF₂₅₄ containing CaSO₄.

Identification of TG hydroperoxides. The purity of the prepared TG hydroperoxides was confirmed by analytical TLC using different solvent systems.⁷⁾ With every solvent system, a single spot was obtained with an *R_f* value very similar to TG monohydroperoxide. This sample preparation showed clear distinction from diglyceride monohydroperoxide and monoglyceride monohydroperoxide as described by Oette⁷⁾ on TLC analysis. Further this preparation could be clearly separated from unoxidized TG by TLC. The POV of the preparation was 2,100. Safflower oil contains *ca.* 75% of linoleic acid and 15% of oleic acid. By assuming that the TG consists of only linoleic acid, the molecular weight of the TG monohydroperoxide to be 911. Thus the theoretical POV of the TG monohydroperoxide sums up to 2195, in contrast to the POV of TG dihydroperoxide and TG trihydroperoxide which becomes 4,242 and 6,154, respectively. By considering the molecular weight and POV, the contamination of oleic acid can be neglected. Thus the experimental POV of 2,100 determined for the purified TG monohydroperoxide testifies that this preparation is pure TG monohydroperoxide. The TG hydroperoxide preparation showed a polarographic reducing wave having a half wave potential at -0.64 V which are specific for hydroperoxides as described by Willits *et al.*⁸⁾ (Fig. 2).

Infrared absorption spectrum of the TG hydroperoxide preparation is shown in Fig. 3. The absorption at 3,450 cm⁻¹ corresponding to -OOH group¹⁰⁾ and 989 cm⁻¹ and 948 cm⁻¹ due to conjugated diene structure^{11~13)} are found in the purified TG hydro-

FIG. 3. Infrared Absorption Spectra of TG and TG Hydroperoxide.

Infrared absorption spectra were taken in a sodium chloride cell with a Hitachi Model FPI-S₂ spectrophotometer.

peroxide preparation and are not found in the control. The absorptions at 1,750 cm⁻¹ based on C=O and 1,166 cm⁻¹ due to C-O¹⁴⁾ are both found in TG and TG hydroperoxide.

UV absorption at 234 nm specific for *cis,trans*-conjugated diene structure¹⁵⁾ based on linoleic acid hydroperoxides was observed with the preparation.

From these observations, the TG hydroperoxide preparation can be considered as TG monohydroperoxides. However, this TG monohydroperoxides may contain some isomers of linoleic acid hydroperoxides and may contain oleic acid hydroperoxides as a contaminant.

REFERENCES

- 1) H. Kaunitz, in "Lipids and Their Oxidation," ed. by H. W. Schultz *et al.*, AVI, Westport, 1962, p. 269.
- 2) N. Matsuo, *ibid.*, p. 321.
- 3) M. Yoshioka and T. Kaneda, *J. Japan Oil Chem. Soc.*, **21**, 316 (1972).
- 4) H. Kaunitz, *Food Tech.*, **21**, 278 (1967).
- 5) E. N. Frankel, C. D. Evans, D. G. McConnell and E. P. Jones, *J. Am. Oil Chem. Soc.*, **38**, 134 (1961).
- 6) B. D. Sully, *Analyst (London)*, **79**, 86 (1954).
- 7) K. Oette, *J. Lipid Res.*, **6**, 450 (1965).
- 8) C. O. Willits, C. Ricciuti, H. B. Knight and D. Swern, *Anal. Chem.*, **24**, 785 (1952).
- 9) C. O. Willits, C. Ricciuti, C. L. Ogg, S. G. Morris and R. W. Riemschneider, *J. Am. Oil Chem. Soc.*, **30**, 420 (1953).
- 10) L. R. Dugan, B. W. Beadle and A. S. Henick,

- ibid.*, **26**, 681 (1949).
- 11) O. S. Privett, O. W. Lundberg, N. A. Kahn, W. E. Tolberg and D. H. Wheeler, *J. Am. Oil Chem. Soc.*, **30**, 61 (1953).
- 12) J. E. Jackson, R. F. Paschke, W. Torberg, H. M. Boyd and D. J. Wheeler, *ibid.*, **29**, 229 (1952).
- 13) "Oil Chemists Handbook," ed. by Japan Oil Chem. Soc., Maruzen, 1971, p. 407.
- 14) D. Chapman, *J. Am. Oil Chem. Soc.*, **42**, 353 (1965).
- 15) J. L. Bolland and H. P. Koch, *J. Chem. Soc.*, **1945**, 445.
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