Note

Stereoselective Oxygenation of Linoleic Acid to 13-Hydroperoxide in Chloroplasts from *Thea sinensis*

Tadahiko KAJIWARA,

Nobuhiko NAGATA, Akikazu HATANAKA
and Yoshinobu NAOSHIMA*Department of Agricultural Chemistry,
Faculty of Agriculture,
Yamaguchi 753, Japan*Department of Chemistry, Faculty of Science,
Okayama University of Science,
Ridai-Cho, Okayama 700

Received July 12, 1979

We have previously demonstrated that *endogenous* linolenic acid in tea chloroplasts is cleaved into *cis*-3-hexenal (C_6) and 11-formyl-*cis*-9-undecenoic acid (C_{12}) via a very labile intermediate (e.g. dioxetane) by E_2 of an enzyme system E_2 (E_2 and $E_2' + E_2''$) bound to the lamellae membranes of chloroplasts under aerobic conditions. On the other hand, on *external addition* of a large amount of linoleic acid to chloroplasts, E_2' and E_2'' activities are newly induced in addition to E_2 activity and E_2' catalyzes the formation of 13-hydroperoxide and then the product is further cleaved into *n*-hexenal by E_2'' .¹⁾

In this paper is described how the enantiomeric composition of the 13-hydroperoxide produced by E_2' was determined by GLC and NMR analysis. After a large amount of linoleic acid was incubated with tea chloroplasts, a mixture of hydroperoxides (13-hydroperoxy-*cis*-9, *trans*-11-octadecadienoic acid/9-hydroperoxy-*trans*-10, *cis*-12-octadecadienoic acid = 84/16: **crude-I**) was isolated according to the method previously reported.¹⁾ The major hydroperoxide of the **crude-I** was identified as 13-L-hydroperoxy-*cis*-9, *trans*-11-octadecadienoic acid (L-I) containing a small amount of its enantiomer (D-I) by comparison of the NMR and IR spectra, and optical rotation $[\alpha]_D^{25} + 11.0$ (c. 1.33, EtOH) of methyl 13-hydroxy-*cis*-9, *trans*-11-octadecadienoate (**I'**) obtained after separation of methyl 9-hydroxy-*trans*-10, *cis*-12-octadecadienoate (**II'**), with those of authentic methyl 13-L-hydroxy-*cis*-9, *trans*-11-octadecadienoate (L-I')^{2,3)} [optical purity 90%; $[\alpha]_D^{25} + 12.2$ (c. 1.20, EtOH)].

i) *GLC analysis* The enantiomeric composition of **crude-I** was determined by GLC analysis of the (*R*)-(+)- α -methoxy- α -trifluoromethylphenylacetyl (MTPA) ester of methyl 2-hydroxyheptanoate

FIG. Enantiomeric Composition of Hydroperoxide (**crude-I**) Formed by E_2' in Tea Chloroplasts.

15% Silicone DC QF-1 on Chromosorb W (AW-DMCS) (Nihon Chromato Works, LTD.); glass column, 4 m; flow rate, 40 ml/min; column temp., 200~230°C (1°C/min) for MTPA-ester and 150~200°C (2°C/min) for MC-derivative.

(a) (from 13-hydroperoxide) and dimethyl 2-hydroxysebacate (b) (from 9-hydroperoxide) obtained after ozonolysis of the MTPA-ester of **crude-I'** (Fig.). The MTPA-esters of methyl 2-D-hydroxyheptanoate (D-a) and dimethyl 2-D-hydroxysebacate (D-b) were found to have shorter retention times than the derivatives of corresponding 2-L-hydroxy isomers (L-a, L-b), respectively, using authentic specimens.^{6,7)} Although the (–)-menthoxycarbonyl (MC)-derivative of 2-D-hydroxyheptanoate (D-a') showed a longer retention time than that of the L-isomer (L-a'), the same composition (L-I'/D-I' = 80/20) was obtained (Fig.).⁸⁾ The figure clearly indicates that E_2' in chloroplasts catalyzes the stereoselective oxygenation of linoleic acid to 13-L-hydroperoxy-*cis*-9, *trans*-11-octadecadienoic acid (L-I) accompanied by 9-hydroperoxy-*trans*-10, *cis*-12-octadecadienoic acid (D-II'/L-II = 58/42) (**II**). The enantiomeric composition of **II** was probably caused by autoxidation of linoleic acid during the incubation and isolation procedures.

ii) *NMR analysis* The NMR spectrum of a mixture of diastereomeric MTPA-esters of 13-hydroxy-dienoate (**I'**) can be used for the quantitative

analysis of the enantiomeric composition of the chiral **I'** using a lanthanide shift reagent such as Eu(DPM)₃.^{4,5)} Increasing the amount of Eu(DPM)₃ afforded a splitting of the OCH₃ signal of the MTPA moiety and the signal was well separated in two peaks with a molar ratio of 0.3 of Eu(DPM)₃/MTPA-ester. Integration of these peaks (L: 3.5; D: 4.7 ppm) gave the composition of L-**I'**/D-**I'** (80/20).

Applying this method to the determination of the enantiomeric composition of **crude-I'**, the composition of L-**I'**/D-**I'** (86/14) was observed. This composition is slightly different from that determined by GLC analysis, and this seems to depend on that NMR signals of the MTPA-esters of **I'** and **II'** are not sufficiently separated or the crucial signals overlap with other resonances.

EXPERIMENTAL

Formation of 13-L-hydroperoxy-cis-9,trans-11-octadecadienoic acid (I) from linoleic acid by E₂'. After tea chloroplasts (1 g) were incubated with linoleic acid (710 μmol) at 35°C for 10 min, crude hydroperoxide (**crude-I**) (50 μmol) was isolated according to the method previously reported.¹⁾

The **crude-I'** was obtained after reduction of the hydroperoxy group of the hydroperoxide with NaBH₄, purification by column chromatography (SiO₂; petroleum ether/diethyl ether/acetic acid=80/70/0.5) and then esterification with CH₂N₂. TLC separation (10% AgNO₃-SiO₂; benzene/dioxane=95/5) of **crude-I'** afforded **I'** (4.8 μmol). IR $\nu_{\text{max}}^{\text{film}}$ cm⁻¹: 3340 (-OH), 3010 (=CH), 1735 (-COOCH₃), 990 (t, -CH=CH-). NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$ ppm: 7.25 (1H, s, OH), 6.52~4.11 (5H, m, -CH=CH-CH=CH-, -HC-OH), 3.68 (3H, s, -COOCH₃), 1.33~1.14 (22H, m, CH₃-(CH₂)₄-, -(CH₂)₇-), 0.83 (3H, t, -CH₃) UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm: 234 (-CH=CH-CH=CH-). $[\alpha]_D^{25} = +11.0$ (c, 1.33, EtOH).

MTPA-ester of methyl 13-hydroxy-cis-9,trans-11-octadecadienoate (I'). The crude MTPA-ester of **I'** was prepared from **I'** (50 mg, 160 μmol) and (R)-(+)-MTPA (50 mg, 200 μmol) according to the usual method.⁴⁾ The crude diastereomeric MTPA-esters

of **I'** (20 mg, 40 μmol) were purified by column chromatography (SiO₂; benzene/dioxane=95/5). IR $\nu_{\text{max}}^{\text{film}}$ cm⁻¹: 3050 (=CH), 1750 (-COOCH₃), 995 (t, -CH=CH-). NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$ ppm: 7.32 (5H, s, -C₆H₅), 6.52~4.11 (5H, m, -CH=CH-CH=CH-, -HC-O-), 3.58 (3H, s, -OCH₃), 0.88 (3H, t, -CH₃).

Ozonolysis of MTPA-ester of methyl 13-hydroxy-cis-9,trans-11-octadecadienoate (I'). The MTPA-ester of **I'** (10 mg) was dissolved in methanol (10 ml) and ozone was bubbled through the methanol solution at -10°C for 2 hr and then the methanol was evaporated *in vacuo*. The residue was dissolved in glacial acetic acid (10 ml) and 35% hydrogen peroxide (15 ml) and the solution was stirred at 50°C for 18 hr. After the reaction mixture was extracted with ether, the extract was esterified with CH₂N₂ and then carefully concentrated *in vacuo*. The concentrate was subjected to column chromatography on SiO₂ (benzene/dioxane=95/5) and after evaporation of the solvent the MTPA-ester of methyl 2-hydroxyheptanoate (**a**)⁶⁾ was obtained. MNR $\delta_{\text{TMS}}^{\text{CDCl}_3}$ ppm: 7.32 (5H, s, -C₆H₅), 3.50 (3H, s, -OCH₃), 3.38 (3H, s, -COOCH₃), 1.33~1.14 (8H, m, -(CH₂)₄-), 0.88 (3H, t, -CH₃).

REFERENCES

- 1) A. Hatanaka, T. Kajiwara, J. Sekiya and K. Fujimura, *Agric. Biol. Chem.*, **43**, 175 (1979).
- 2) H. W. Gardner and D. Weisleder, *Lipids*, **7**, 191 (1971).
- 3) H. E. Pattee and J. A. Singleton, *J. Agric. Food Chem.*, **27**, 216 (1979).
- 4) J. A. Dale, D. L. Dull and H. S. Mosher, *J. Org. Chem.*, **34**, 2543 (1969).
- 5) S. Yamaguchi and F. Yasuhara, *Tetrahedron Lett.*, 89 (1977).
- 6) G. W. Moersch and M. L. Zwiesler, *Synthesis*, **9**, 647 (1971).
- 7) J. Sekiya, H. Aoshima, T. Kajiwara, T. Togo and A. Hatanaka, *Agric. Biol. Chem.*, **41**, 827 (1977).
- 8) M. Hamberg, *Anal. Biochem.*, **43**, 515 (1971).