

### The Enzymic Conversion of 13-Hydroperoxy-*cis*-9-*trans*-11-Octadecadienoic Acid into 13-Hydroxy-12-Oxo-*cis*-9-Octadecenoic Acid

By G. A. VELDINK, J. F. G. Vliegenthart and J. BOLDINGH. (*Laboratory of Organic Chemistry, State University of Utrecht, Utrecht, The Netherlands*)

The formation of hydroperoxides of unsaturated fatty acids containing a *cis,cis*-1,4-pentadiene system is catalysed by lipoxygenase (EC 1.13.1.13). The metabolic role in the plant of the hydroperoxides is still unknown. Zimmerman (1966) reported that flax seed should contain an enzyme that is able to isomerize linoleic acid hydroperoxides to oxo-hydroxy compounds. The structure of these substances has not been completely proved (e.g. the u.v. spectrum is not in accordance with the proposed structures). Following the work of Zimmerman (1966) we tried to prepare these substances as follows. A mixture of the linoleic acid hydroperoxides was obtained from linoleic acid by incubation with soya-bean lipoxygenase (EC 1.13.1.13). After acidification and extraction the hydroperoxides were isolated by thin-layer chromatography on silica gel. The molar ratio of 13-hydroperoxy-*cis*-9-*trans*-11-octadecadienoic acid to 9-hydroperoxy-*trans*-10-*cis*-12-octadecadienoic acid was 10:1. This mixture of hydroperoxides was incubated with a 0.1 M-phosphate buffer, pH 7.4,

extract of an acetone-dried powder of flax seed. After acidification and extraction the reaction products were separated by thin layer chromatography on silica gel. The main spot was analysed. With the aid of i.r. and u.v. spectroscopy, nuclear-magnetic-resonance analysis of this substance, and by mass-spectrometric analysis of the methyl ester and its toluene-*p*-sulphonyl derivative, the compound was identified as 13-hydroxy-12-oxo-*cis*-9-octadecenoic acid. This structure was further confirmed by ozone and periodate degradation. The optical activity of this component was determined in chloroform as solvent [ $(\alpha)_D^{20} - 10.4$ ]. In contradiction to Zimmerman (1966), we found that the crude enzyme fraction reacts only with the 13-hydroperoxy-*cis*-9-*trans*-11-octadecadienoic acid component of the mixed linoleic acid hydroperoxides.

Further investigations of the substrate specificity with the peroxides of other unsaturated fatty acids are in progress. We attempted to prepare 13-hydroxy-12-oxo-*cis*-9-octadecenoic acid by the oxidation of vernolic acid (*cis*-12,13-epoxy-*cis*-9-octadecenoic acid) with dimethylsulphoxide (Tsuji, 1966). However, the reaction product turned out to be 12-hydroxy-13-oxo-*cis*-9-octadecenoic acid.

Tsuji, T. (1966). *Tetrahedron Lett.* no. 22, p. 2413.

Zimmermann, D. C. (1966). *Biochem. biophys. Res. Commun.* **23**, 398.