

The Conversion of Linoleic Acid (13*S*)-Hydroperoxide into (13*R*)-Hydroxy-12-oxo-octadec-(9*Z*)-enoic Acid and 9-Hydroxy-12-oxo-octadec-(10*E*)-enoic Acid by a Flax Enzyme. Isotopic Evidence for Allene Epoxide Intermediates

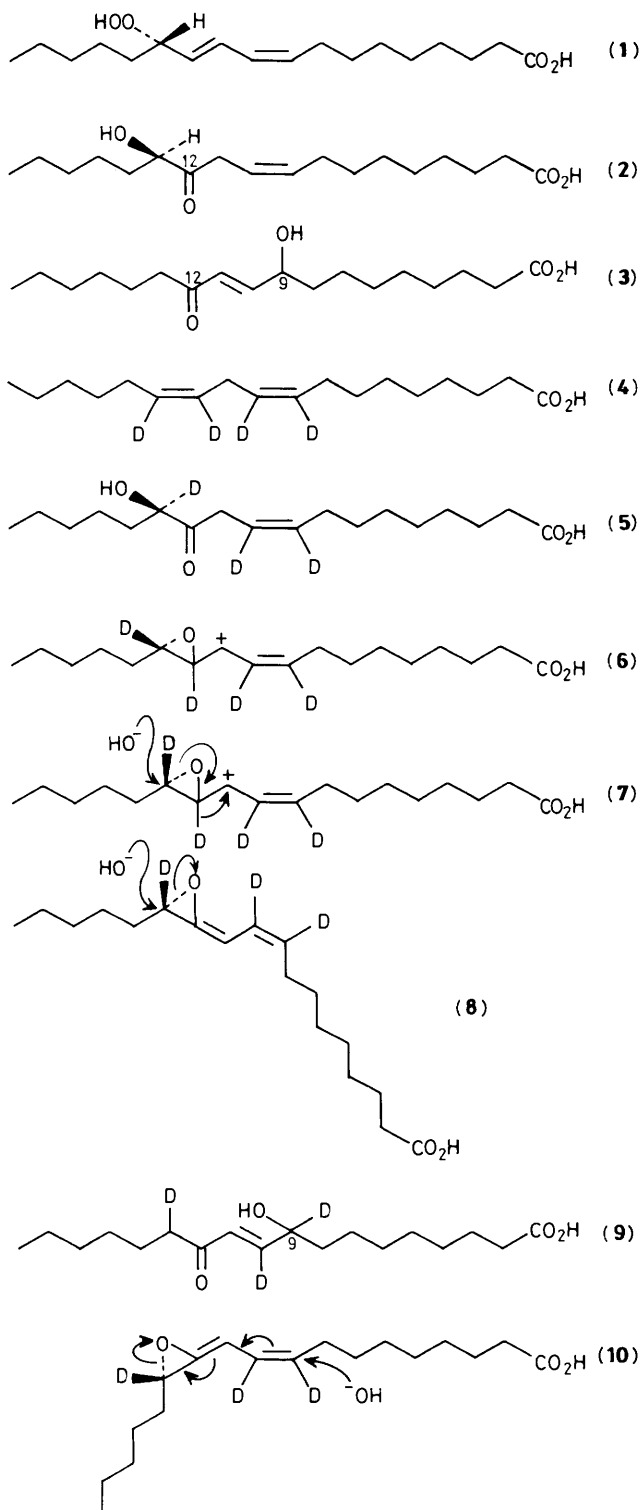
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Conversion of [9,10,12,13-²H₄]linoleic acid, *via* its 13-hydroperoxide, into the γ -ketol (**9**) by a flax enzyme, with loss of C-12-[²H], can only satisfactorily be explained by the intervention of an allene epoxide intermediate; α -ketol (**2**) formation involves a similar mechanism.

Many plants including corn germ, alfalfa, and flax seeds or seedlings, contain an isomerase enzyme capable of converting the 13-hydroperoxide derived from linoleic acid, (13*S*)-hydroperoxyoctadeca-(9*Z*),(11*E*)-dienoic acid (**1**), into (13*R*)-hydroxy-12-oxo-octadeca-(9*Z*)-enoic acid (**2**) (the

α -ketol)¹ and 9-hydroxy-12-oxo-octadec-(10*E*)-enoic acid (**3**) (the γ -ketol).² Linolenic acid gives analogous products.³ It is known from experiments using [¹⁸O₂]-labelled 13-hydroperoxide that the 12-oxo groups are derived from hydroperoxide oxygen (*i.e.* that there has been a 1,2-shift of oxygen from



C-13 to C-12) and that the 13-hydroxy of (2) and the 9-hydroxy of (3) are derived from water, with inversion in the former case.⁴ Intervention of an epoxy-intermediate has been suggested to explain the 1,2-oxygen transfer.⁵

Recently we reported a study of the formation of the α -ketol (2) employing [9,10,12,13-²H₄]linoleic acid (4).⁶ The final labelling pattern after administration to the flax seed enzyme preparation was as in (5) in which the loss of one deuterium from C-12 could be explained *via* formation of (6). Loss of the C-12 deuterium then occurs through the formation of an allene epoxide (8) or 1,2-shift of deuterium to C-11 followed by exchange with buffer. Our further experiments on the deuterium exchange reaction with buffer have shown us that it is not possible to explain the *total* loss of C-12 deuterium by exchange from C-11 and thus provides strong evidence for an allene epoxide intermediate (8). Study of the minor product from the isomerase enzyme, the γ -ketol (3), now provides still stronger evidence for the allene epoxide intermediate (8).⁷

Administration of tetra-deuteriolinoleic acid (4)⁶ to flax seed enzyme followed by isolation of the γ -ketol as its methyl ester gave a product containing three, rather than four, deuterium atoms (M^+ absent, $M^+ - \text{OMe}$ 295 + 3, $M^+ - \text{C}_5\text{H}_{10}$ 256 + 3 *etc.*) The ¹H n.m.r. spectrum showed the labelling pattern (9). Thus the broad quartet at δ 4.32 (1H, H-9), the double doublet at 6.78 (1H, H-10), and one of the pair of hydrogens at 2.56 (1H, H-13), all of which were present in the unlabelled γ -ketol (3),[†] were not present. The H-11 resonance, formerly a double doublet at δ 6.30 (J 15.9 and 1.6 Hz) now appeared in singlet form at δ 6.29. Explanation of the loss of a deuterium atom by exchange with buffer is no longer an acceptable possibility, and the formation of (9) is now readily explained by the intervention of (6) followed by loss of D-12 to give the allene epoxide (10). The process is completed by γ -attack (10), analogous to α -attack (8).

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[†] Structure evidence for (3) was limited, but has been fully confirmed in the present investigation.