Formation of Acyclic α- and γ-Ketols and 12-Oxophytodienoic Acid from Linolenic Acid 13-Hydroperoxide by a Flax Enzyme Preparation. Evidence for a Single Enzyme Leading to a Common Allene Epoxide Intermediate

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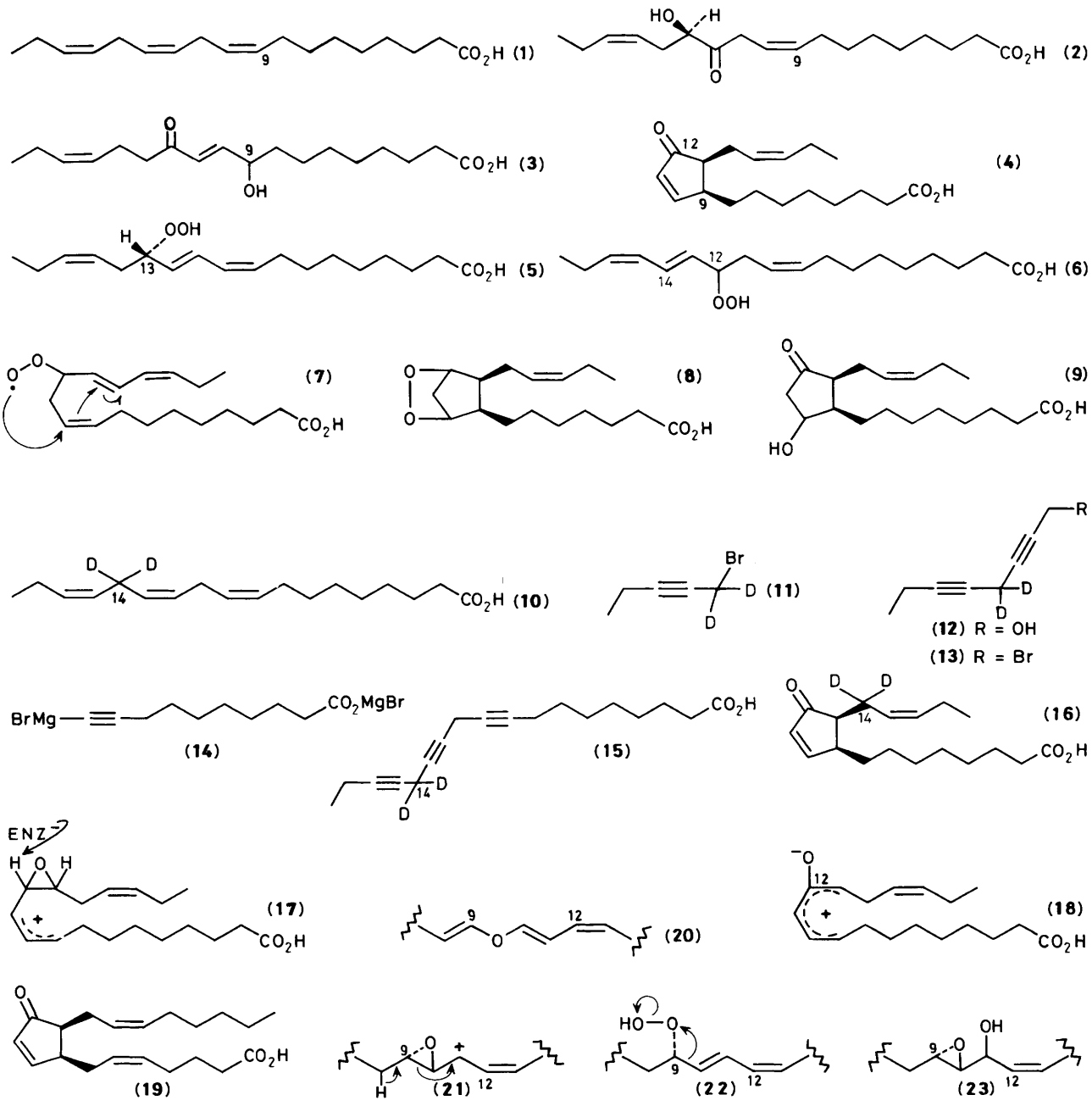
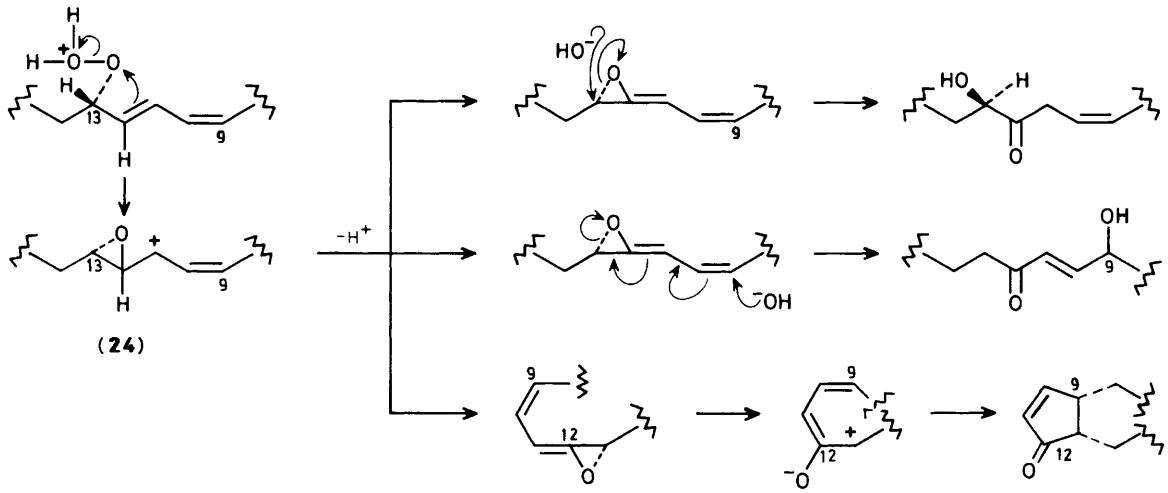
Experiments with [14-$^2H_2$]linolenic acid confirm the requirement for a 13-hydroperoxide for 12-oxophytodienoic acid (12-oxoPDA) formation by a flax enzyme preparation; an allene epoxide intermediate is capable of explaining the formation of the acyclic α- and γ-ketols as well as 12-oxoPDA, suggesting the involvement of a single enzyme.

Zimmerman *et al.* have described an enzyme preparation from flax seed capable of converting linolenic acid (**1**) into the α-ketol (**2**), the γ-ketol (**3**), and the prostaglandin (PGA_1)-like 12-oxophytodienoic acid (12-oxoPDA) (**4**).[1] This system is of considerable importance to plant physiology, and to food chemistry, as it is widespread among higher plants.[2] The precursor to (**4**) is considered to be the 13-hydroperoxide (**5**) derived from linolenic acid (**1**).[1] Were the biosynthesis of





Scheme 1. Allene-epoxide mechanism for the formation of the α- and γ-ketols, and 12-oxoPDA from linolenic acid 13-hydroperoxide and flax enzyme.

12-oxoPDA to follow a pathway resembling that of mammalian prostaglandins, a 12-hydroperoxide (**6**) would be involved in the pathway leading to (**4**) *via* (**7**), (**8**), and (**9**).[3] Since there is some evidence that a 12-hydroperoxide may be formed by flax seed lipoxygenase,[4] we administered h.p.l.c. pure [1-^{14}C]-13-hydroperoxyoctadeca-(9*Z*),(11*E*),(15*Z*)-trienoic acid (**5**) to the flax seed cyclase enzyme. Although satisfactory incorporation into 12-oxoPDA was eventually obtained, there were initial difficulties which led to the experiment below. This demonstrates in a different way that the 13- and not the 12-hydroperoxide is involved.

[14-$^2H_2$]Linolenic acid (**10**) was synthesised as follows. But-1-yne (as its Grignard complex) was converted into [1-$^2H_2$]pentynol by treatment with deuterioformaldehyde (made by deuteriation of dibromomethane, conversion into [$^2H_2$]methylene diacetate, and hydrolysis).[5] [1-$^2H_2$]Pentynol was converted (PBr_3) into the bromide (**11**) and coupled (copper catalyst)[6] with the Grignard reagent from tetrahydropyranyl-protected prop-2-ynyl alcohol to give (**12**), itself converted into the bromide (**13**). The latter was coupled under copper cyanide catalysis with the acetylenic bis-Grignard (**14**). Triyne-acid (**15**) was esterified (CH_2N_2), reduced with di-isopentylborane,[7] and hydrolysed to give [14-$^2H_2$]linolenic acid (**10**). Treatment with flax seed cyclase enzyme,[1] followed by chromatographic work-up, gave 12-oxoPDA as its methyl ester (CH_2N_2). This retained both deuterium atoms (M^+ 306 + 2, $M^+ - OMe$ 275 + 2 *etc.*) (**16**). The ^{1}H n.m.r. spectrum of (**16**), when compared with that of the undeuteriated material, showed that both deuteriums were still located on the 14-methylene (**16**). Thus the multiplet at δ 2.51 (1H, 14-H_a) and the ddd at 2.15 (1H, 14-H_b) were not present: the erstwhile ddd at 2.44 (1H, 13-H) now appeared as a clean doublet and the olefinic multiplet at 5.40 (H-15 and H-16) was simplified. It is clear that the 13-, not the 12-, hydroperoxide must be the precursor of 12-oxoPDA, with oxygen migration from C-13 to C-12 occurring in the biosynthesis.

Zimmerman[8] has proposed that the cyclase enzyme leads to an epoxy-carbonium ion (**17**) from which enzymic loss of a proton leads to zwitter-ion (**18**) which undergoes cyclisation to give (**4**). Corey has lately proposed a similar antarafacial pericyclic closure to explain the formation of preclavulone A (**19**),[9] produced by treating arachidonic acid with an homogenate of the coral *Clavularia viridis*, and has pointed out a chemical analogy for the process.[10,11] This compound is also a *cis*-compound unlike the mammalian prostaglandin series.

The three products from the flax enzyme system and linolenic acid can now be assembled into a single mechanistic scheme in which the central feature is the formation of a protonated allene epoxide (**24**) (Scheme 1), deprotonated to the allene epoxide itself. One branch leads to the α-ketol,[12] one to the γ-ketol,[13] and a third to 12-oxoPDA as shown. It has not been possible to resolve the flax enzyme preparation into an isomerase and a cyclase enzyme,[14] and the present chemical knowledge leads us the view that these functions merge and that a single enzyme is involved in initiating these processes.

The potato enzyme system[15,16] which operates on the hydroperoxides of linoleic and linolenic acid has some similarities. Formation of the colneleic/colnelenic system (**20**) at pH 7—9 has earlier been shown by us to involve (**21**) though it could also be represented as a less likely Baeyer–Villiger C → O type shift (**22**). However, at pH 5—7 it may be significant that the same enzyme leads to (**21**) in hydroxy-trapped form (**23**).[17]

We thank the Ministry of Agriculture, Fisheries, and Food (M.A.F.F.) for supporting this work, and Dr. G. Shearer (M.A.F.F.) for his interest in its progress.

Received, 12th November, 1987; Com. 1651

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