The Enzymic Formation of Colneleic Acid, a Divinyl Ether Fatty Acid: Experiments with [(9*S*)-$^{18}O_2$]Hydroperoxyoctadeca-(10*E*),(12*Z*)-dienoic Acid

Leslie Crombie, David O. Morgan, and Elisabeth H. Smith

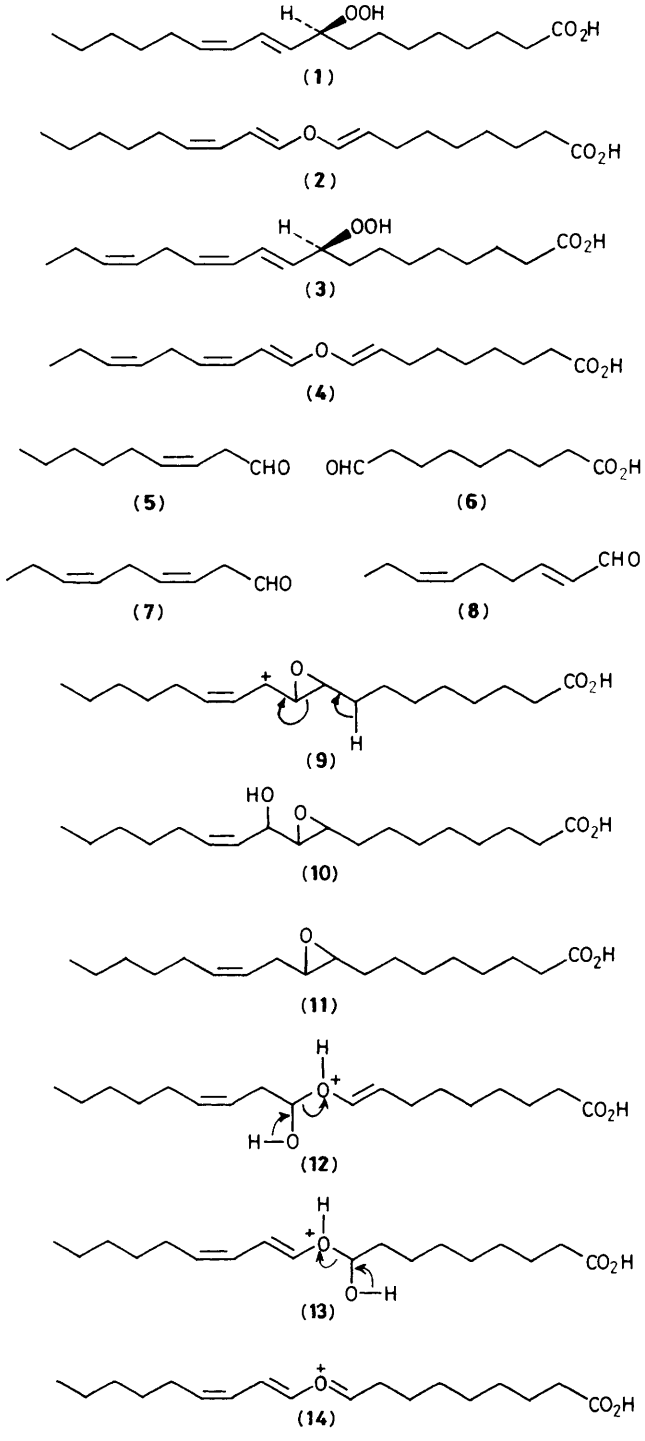
Department of Chemistry, The University, Nottingham NG7 2RD, U.K.

Contrary to earlier reports, the oxygen inserted enzymically between carbons 9- and 10- of the C-18 chain in forming colneleic acid originates from $^{18}O_2$ gas *via* the 9-hydroperoxide group of linoleic acid; a mechanism is proposed.

The organoleptic quality of natural and processed food is greatly influenced, either beneficially or adversely, by short-chain saturated and unsaturated aldehydes recognised as originating from the 9- and 13-hydroperoxides derived from linoleic and linolenic acids.[1] However, little is known of the processes by which the 18-carbon chain is fractured. Galliard and his colleagues[2] made the interesting discovery that an enzyme from potato having a pH optimum near 9.0 was capable of converting linoleic acid 9-hydroperoxide (**1**) into colneleic acid (**2**), and linolenic 9-hydroperoxide (**3**) into colnelenic acid (**4**). These divinyl ethers may be cleaved enzymically or under acid conditions to give an unsaturated aldehyde and an aldehyde–acid. Thus colneleic acid gives non-(3*Z*)-enal (**5**) [also converted further enzymically or by acid into non-(2*E*)-enal and other products],[3,4] and 9-oxononanoic acid (**6**).[5] Colnelenic acid gives (**6**) and (**7**) or (**8**). The status of colneleic acid however has been enigmatic as experiments with $^{18}O_2$ indicated that the divinyl ether oxygen did not come from either of the hydroperoxide oxygens of (**1**) but possibly came from water.[6] We have re-examined the origin of the divinyl ether oxygen of colneleic acid, as formed by potato enzyme, and find that, contrary to the earlier results, it originates from the oxygen gas forming the 9-hydroperoxide.

Potato lipoxygenase[7] was isolated from potato homogenate in sodium phosphate buffer (pH 7.2) containing sodium metabisulphite which was treated with ammonium sulphate at 0 °C to 50% saturation. Centrifugation at 4 °C (15 000 *g*) followed by resuspension of the pellet and dialysis against water (48 h, 4 °C) gave a cloudy solution which was freeze-dried to give partially purified lipoxygenase as a white powder. This, (100 mg) in sodium acetate buffer (pH 5.5) (20 ml), was added to linoleic acid (50 mg) which had been dissolved in sodium borate buffer (pH 9.0) (20 ml) containing Tween 20 and then diluted with the sodium acetate buffer (160 ml). Buffer solutions had been thoroughly degassed under vacuum by three thaw–melt procedures and kept under argon. Reaction was then allowed to proceed under an $^{18}O_2$ atmosphere (80 atom%) for 1 h with stirring. Work-up by addition of methanol, chloroform, and citric acid solution followed by evaporation and esterification (CH_2N_2) gave, after preparative t.l.c., [(9*S*)-$^{18}O_2$]hydroperoxyoctadeca-(10*E*),(12*Z*)-dienoic acid. Mass spectrometry showed 80 atom% of $^{18}O_2$ in the hydroperoxy group. Data from an accompanying unlabelled run showed that the product was actually a mixture of 9- and 13-hydroperoxides in the ratio 92:8 [C_{18}-reversed phase h.p.l.c., eluting with methanol–water (4:1)].

A partially purified preparation of the potato enzyme which forms colneleic acid[2a] was made from homogenate in sodium phosphate buffer (pH 7.2) containing mercaptobenzothiazole. The precipitate from 45—65% ammonium sulphate saturation was centrifuged and resuspended in 2 M ammonium sulphate, dialysed against 0.1 M sodium phosphate buffer (pH 7.2) at 4 °C (48 h), and used immediately. [(9*S*)-$^{18}O_2$]Hydroperoxyoctadeca-(10*E*),(12*Z*)-dienoic acid (**1**) (10 mg) in borate buffer (pH 9.0) (200 ml) was shaken with the enzyme preparation at 25 °C for 20 min and then worked up and esterified by a procedure similar to that described for the



hydroperoxide. After reversed phase h.p.l.c. the product, methyl colneleate, had a ^{1}H n.m.r. spectrum identical with an authentic sample and mass spectrometry showed 83% ^{18}O incorporation. In a preliminary experiment with hydroperoxide of 40 atom% $^{18}O_2$, and using crude potato homogenate as the enzyme source, very low (4%) incorporation was recorded, largely due to dilution by endogenous material.

In view of the origins of the divinyl ether oxygen from the hydroperoxide (**1**) which in its turn is derived from oxygen gas, a mechanism may be proposed for the origins of colneleic acid (**2**) (and similarly for colnelenic acid). Protonation of the hydroperoxy group and loss of water leads to the epoxycarbonium ion (**9**) which undergoes cleavage of the 9,10-bond as shown. Removal of the C-8 hydrogen is doubtless enzyme assisted and it is of interest that the enzyme has a pH optimum of 9.0 which suggests that a suitable basic grouping needs to be free. At acid pH, using a crude enzyme preparation, the intermediate (**9**) does not cleave but is stabilised as (**10**),[8] though whether the colneleic-forming enzyme or another is involved seems unclear. Administration experiments using potato extract with a specimen of [1-^{14}C]coronaric acid (**11**), made by epoxidation of [1-^{14}C]linoleic acid, show that it is not involved in colneleic acid formation. Whilst a radical mechanism might be written leading to a radical analogue of (**9**), the extensive work of Gardner[9] on *in vitro* hydroperoxide decomposition makes a carbonium ion pathway more attractive.

Decomposition of colneleic and colnelenic acids to aldehydes can be acid or enzyme catalysed and for the former example doubtless involves (**12**) or (**13**). Such intermediates could also arise without the intervention of (**2**) through S_N2'-type attack at C-9 on (**9**) instead of elimination, or by rearrangement to (**14**) with OH^- attack at C-9.

We thank the Ministry of Agriculture, Fisheries, and Food (MAFF) for supporting this work.

Received, 8th October 1986; Com. 1435

References

1 *Inter alia* B. A. Vick and D. C. Zimmerman, *Plant Physiol.*, 1976, **57**, 780; T. Galliard and D. R. Phillips, *Biochim. Biophys. Acta*, 1976, **431**, 278; T. Galliard, D. R. Phillips, and J. Reynolds, *ibid.*, 1976, **441**, 181; A. Hatanaka, T. Kajiwara, J. Sekiya, and Y. Kido, *Phytochemistry*, 1977, **16**, 1828; T. Galliard and J. A. Matthew, *ibid.*, 1977, **16**, 339; 1978, **17**, 1043; D. R. Phillips and T. Galliard, *ibid.*, 1978, **17**, 355; A. Hatanaka, T. Kajiwara, J. Sekiya, and S. Inouye, *ibid.*, 1982, **21**, 13.

2 T. Galliard and D. R. Phillips, *Biochem. J.*, 1972, **129**, 743; T. Galliard, D. R. Phillips, and D. J. Frost, *Chem. Phys. Lipids*, 1973, **11**, 173.

3 A. Hatanaka, T. Kajiwara, and T. Harada, *Phytochemistry*, 1975, **14**, 2589.

4 D. R. Phillips, J. A. Matthew, J. Reynolds, and G. R. Fenwick, *Phytochemistry*, 1979, **18**, 401.

5 L. Crombie, D. O. Morgan, and E. H. Smith, unpublished work.

6 T. Galliard and J. A. Matthew, *Biochim. Biophys. Acta*, 1975, **398**, 1.

7 T. Galliard and D. R. Phillips, *Biochem. J.*, 1971, **124**, 431.

8 T. Galliard, D. R. Phillips, and J. A. Matthew, *Biochim. Biophys. Acta*, 1975, **409**, 157.

9 H. W. Gardner, D. Weisleder, and E. C. Nelson, *J. Org. Chem.*, 1984, **49**, 508; H. W. Gardner, E. C. Nelson, L. W. Tjarks, and R. E. England, *Chem. Phys. Lipids*, 1984, **35**, 87; H. W. Gardner and R. D. Plattner, *Lipids*, 1984, **19**, 294.