An Isotopic Study (^2H and ^{18}O) of the Enzymic Conversion of Linoleic Acid into Colneleic Acid with Carbon Chain Fracture: the Origin of Shorter Chain Aldehydes

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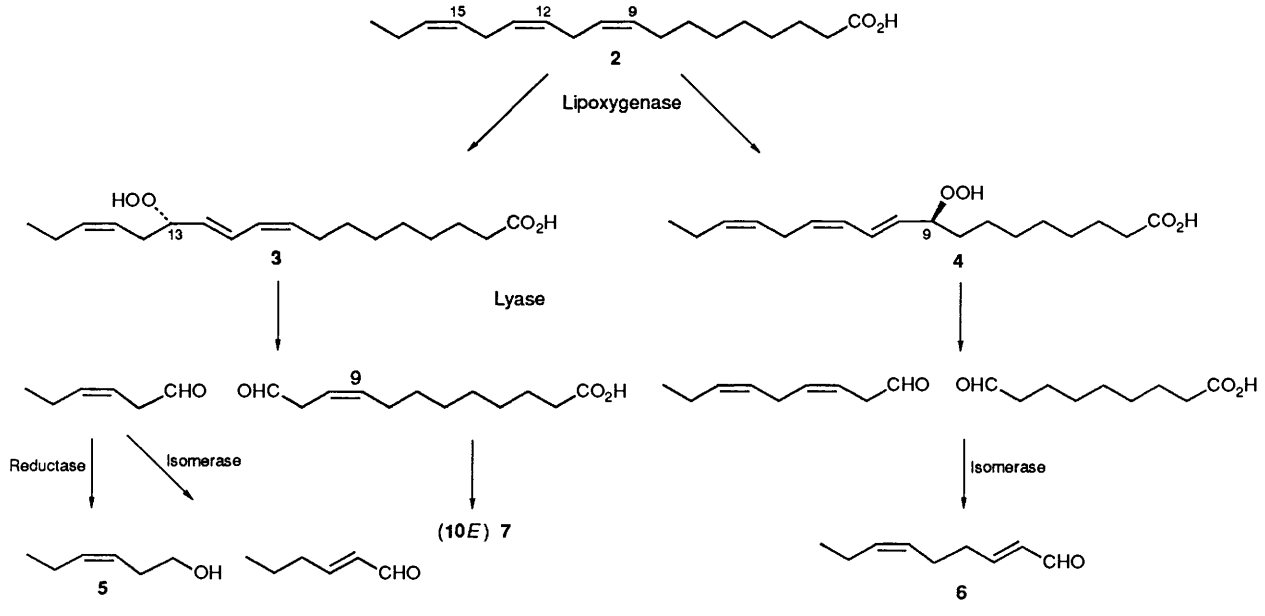
Contrary to earlier reports, the divinyl 9-ether oxygen of colneleic acid is shown by experiment with $^{18}O_2$ to originate from oxygen, not water. Using [9,10,12,13-$^2H_4$]-9(*S*)-hydroperoxyoctadeca-10(*E*), 12(*Z*)-dienoic acid, made enzymically from synthetic [9,10,12,13-$^2H_4$]linoleic acid, it is found that the distribution of deuterium as determined by NMR and mass spectrometry in the fractured carbon chain of colneleic acid formed by potato enzyme, is consistent with the intervention of an epoxy-carbonium ion intermediate. Though divinyl acids such as colneleic and colnelenic acid give the expected shorter chain aldehydes on treatment with aqueous acid, it is likely that the latter are formed in most plants by trapping of a monovinyl oxonium ion rather than by rehydration of colneleic and colnelenic acid.

Linoleic **1** and linolenic **2** acids are important fatty acids in the metabolism of plants and their degradation products are, in turn, important in influencing the flavouring of fruits, vegetables and other foods.[1] Damage, such as crushing, cutting, or freezing causes the activation of enzymes which release free fatty acids by the lipolysis of glycerides, glycolipids and phospholipids. Lipoxygenase (LOX), a non-heme iron-containing dioxygenase enzyme, then catalyses the oxidation of the two unsaturated acids leading, *via* an antarafacial process, to hydroperoxides which become the starting points for a series of reactions forming a cascade of products, among them short-chain aldehydes, alcohols, acids, cyclopentanoids, epoxides, ketols and other products. Some lipoxygenases, under appropriate conditions, give mainly the (13*S*)-hydroperoxide **3** (*e.g.* soya bean, flax, cotton, cucumber fruit), others mainly (9*S*)- **4** (potato, tomato, rice embryo) and others mixtures of (13*S*)- and (9*S*)- (gooseberry, bakers yeast): the homogeneity of the product depends on factors such as isoenzymes, pH, temperature *etc.*[2]

These hydroperoxides are decomposed by a lyase to form aldehydes as illustrated in Scheme 1 for linolenic acid **2**. The odorous short-chain aldehydes are then isomerised, reduced and oxidised and the acids and alcohols formed are esterified to give a range of products which contribute to odours and flavourings.[1] Leaf alcohol **5** and nonadienal **6** have well-known uses in perfumery, whilst traumatin **7** and its oxidation product traumatic acid are wound hormones.[3] A similar scheme can be written for linoleic acid, hexanal and 11-formylundec-9(*Z*)-t-enoic acid being the products from the (13*S*)-hydroperoxide and non-3(*Z*)-enal and 9-oxononanoic acid are the immediate products from the (9*S*)-. Many of the products have attractive or acceptable odours though some, such as hexanal which is responsible for the 'beany' odour of soya products, are considered deleterious.

Little direct experimental information has been available on the processes by which the 18-carbon chains of linoleic and linolenic acid are fractured in Nature and our interest turned to the work of Galliard and his colleagues who discovered that a potato enzyme preparation having a pH optimum near 9.0 was capable of converting linoleic (9*S*)-hydroperoxide **8** into a divinyl ether fatty acid **9** which they named colneleic acid:[4] analogously, the (9*S*)-hydroperoxide of linolenic acid **4** gave colnelenic acid **10**.[5] Colneleic and colnelenic acid occurring



Scheme 1 Enzymic formation of unsaturated aldehydes from linolenic acid

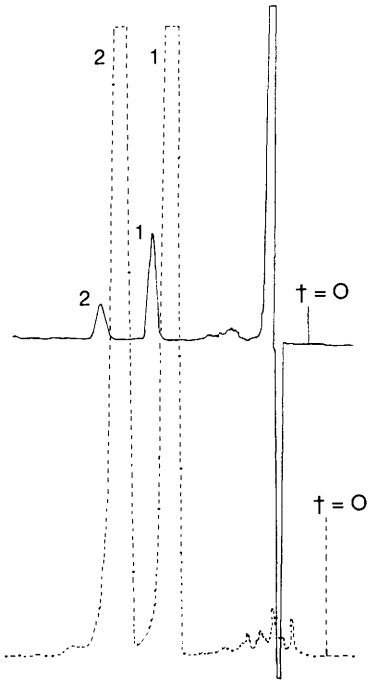


Fig. 1 Reverse-phase HPLC separation of a mixture of methyl colneleate **9** (peak 2) and methyl colnelenate **10** (peak 1) obtained by incubating linolenic acid **2** with crude homogenates of potato:— = refractive index and --- = UV. Column: 8mm 10μ C18 Radpak for Z module; solvent methanol-water (9:1); solvent flow 2 ml min^{-1}; chart speed 5 mm min^{-1}.

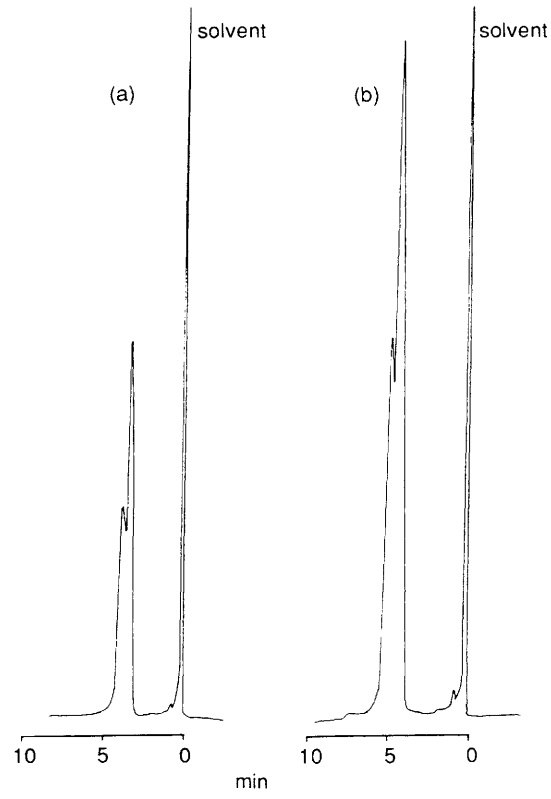


Fig. 2 GLC analysis of (a) methyl colneleate **9** and (b) methyl colnelenate **10**. Column: 2% Silar 10C on Chromosorb W (80–100 mesh); oven temp. 200 °C; injector temp. 250 °C; detector temp. 250 °C; Nitrogen flow 40 ml min^{-1}.

together in potato homogenate can be separated by HPLC (Fig. 1), a method superior to the earlier[5] silver nitrate procedure. GLC work on methyl colneleate was earlier reported[4] to be frustrated by on-column decomposition. Under our conditions methyl colneleate gave two peaks (Fig. 2) but both compounds gave identical mass spectra which were essentially the same as pure methyl colneleate. One of the components possibly results from *Z*/*E* isomerisation about the 3′-4′ double bond. Similar results were obtained with methyl colnelenate.

Such divinyl ethers are capable of cleavage at acid pH to give the short chain aldehydes of Scheme 1. However the relationship of colnelenic acid to the latter Scheme has been enigmatic, since experiments using ^{18}O-labelled linoleic acid (9*S*)-hydroperoxide showed no incorporation into colneleic acid and the divinyl ether oxygen was thought to have come from water.[6] Particularly since $^{18}O_2$ of rather low abundance (10%) had been used in the experiments, it seemed desirable that the matter should be re-examined and the present paper gives an account of our work and its consequences.

Both tomato[7] and potato[8] were used as sources of (9*S*)-lipoxygenase, but the latter was found to be cleaner and easier to work with, particularly when isotopic oxygen experiments were involved. Potato lipoxygenase was isolated from potato homogenate as described by Galliard *et al.*[8] and partially purified by ammonium sulphate precipitation and dialysis. The preparation was freeze-dried and in that condition retained activity at −15 °C for some months. The enzyme in sodium acetate buffer pH 5.5 was added to linoleic acid **1** dissolved in sodium borate buffer (pH 9.0) containing Tween-20 and then diluted with the sodium acetate buffer. All buffer solutions were thoroughly degassed under vacuum by three freeze–thaw procedures and then kept under argon. Reaction was allowed to proceed under an 80% $^{18}O_2$ atmosphere for 1 h with stirring. Work-up and esterification of a portion with diazomethane, followed by preparative TLC, (operations being conducted under an inert gas atmosphere), then gave methyl [(9*S*)-$^{18}O_2$]-hydroperoxyoctadeca-10(*E*),12(*Z*)-dienoate, **8**. Mass spectral examination showed 80 atoms % $^{18}O_2$ in the hydroperoxide grouping. The selectivity of hydroperoxidation at the 9-position was not total however, since HPLC on a C_{18}-reversed phase column (eluent methanol–water, 4:1) showed a 92:8 distribution between the 9- and 13-positions. Potato lipoxygenase and the enzyme forming colneleic acid from the 9-hydroperoxide have different pH optima and this allows convenient separation of the two steps.

A partially purified preparation of the unstable enzyme which converts the 9-hydroperoxy acid into colneleic acid[4,5] was made from potato homogenate in phosphate buffer (pH 7.2) containing mercaptobenzothiazole. Ammonium sulphate precipitation and dialysis were used for the purification and the product was employed immediately. [(9*S*)-$^{18}O_2$]-hydroperoxyoctadeca-10(*E*), 12(*Z*)-dienoic acid (80 atom % $^{18}O_2$) in borate buffer (pH 9.0) was then shaken with the enzyme preparation for 20 min at 25 °C, worked up and esterified (CH_2N_2). After reversed-phase HPLC purification, the specimen had a ^1H NMR spectrum identical with an authentic specimen of unlabelled methyl colneleate made by Galliard's method from linoleic acid: (experiments with [^{14}C]labelled oleic acid showed that the necessary dehydrogenases were not present for it to be used as a starting material). ^1H NMR spectral data for methyl colneleate and colnelenate are shown in Tables 1 and 2 and ^{13}C NMR data, based on carbon–proton 2D-correlation spectroscopy, are given in the Experimental section. Mass spectroscopic examination of the ^{18}O-labelled specimen showed 83 atoms % of ^{18}O in the ether oxygen. It is thus clear that contrary to what had been reported earlier,[6] the divinyl ether oxygen of colneleic acid arises from the oxygen of the hydroperoxidised linoleic acid.[8] In a preliminary experiment using linoleic hydroperoxide containing 40 atom % $^{18}O_2$ and using crude potato enzyme, the incorporation was low (4%), though significant, largely due to dilution with endogenous material. Similar difficulties may have

Table 1 ^{1}H NMR data (250 MHz) for methyl colneleate *cf.* **9**

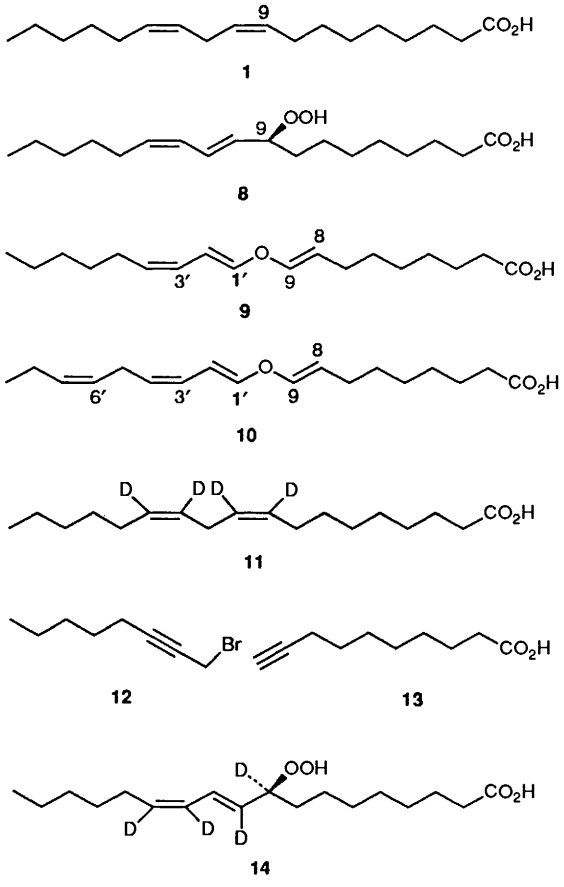
Chemical Shift δ	Multiplicity	Number of Protons	Coupling constant (Hz)	Assignment
6.52	d	1	11.7	1′-H
6.27	dd	1	12.1, 1.1	9-H
6.01	td	1	11.7, 0.7	2′-H
5.85	ddt	1	11.7, 10.5, 1.5	3′-H
5.30	dt	1	10.5, 7.4	4′-H
5.15	dt	1	12.1, 7.4	8-H
3.67	s	3		OCH_3
2.32	t	2	7.6	2-H_2
2.11	m	2		5′-H_2
1.95	m	2		7-H_2
1.61	m	2		3-H_2
1.31	m	12		4-H_2, 5-H_2, 6′-H_2, 7′-H_2, 8′-H_2
0.88	br t	3	7	9′-H_3

Table 2 ^{1}H NMR data (250 MHz) for methyl colnelenate *cf.* **10**

Chemical Shift δ	Multiplicity	Number of Protons	Coupling constant (Hz)	Assignment
6.53	d	1	11.7	1′-H
6.27	dt	1	12.1, 1.5	9-H
6.02	td	1	11.7, 0.8	2′-H
5.88	ddt	1	11.7, 10.3, 1.6	3′-H
5.48–5.09	m	4		8-H, 6′-H, 4′-H, 7′-H
3.68	s	3		OCH_3
2.86	t	2	7.0	5′-H_2
2.31	t	2	7.6	2-H_2
2.10	quintet	2	7.2	8′-H_2
1.96	m	2		7-H_2
1.61	m	4		6-H_2, 3-H_2
1.32	m	4		4-H_2, 5-H_2
0.99	t	3		9′-H_3

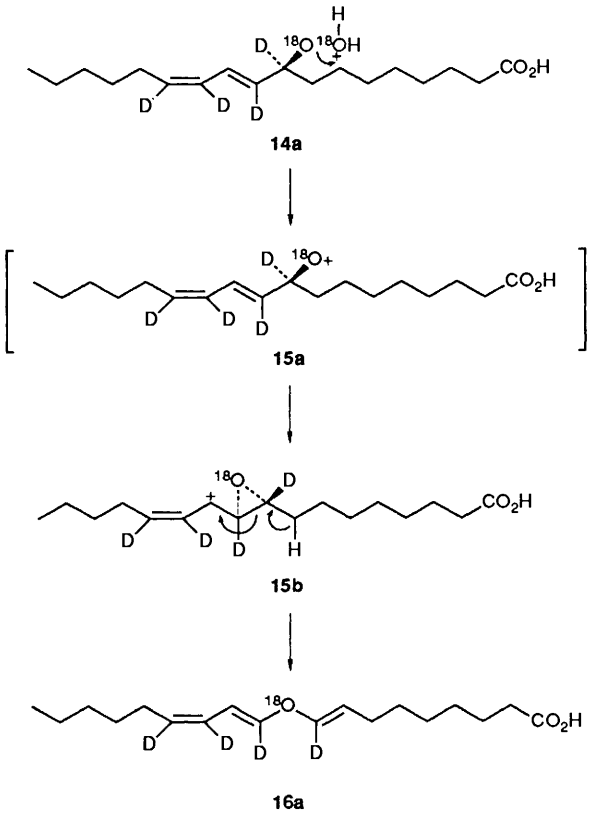
attended the original experiment[6] which used oxygen of low abundance.

With the origins of the divinyl ether oxygen now clear,[9] further experimental information was obtained by following the path of olefinic deuterium in the conversion of [9,10,12,13-$^2H_4$]-octadeca-9(*Z*)′,12(*Z*)-dienoic acid **11** into colneleic acid. The former acid was made by coupling[10] the bis-Grignard derivative of dec-9-ynoic acid **13**[11] with oct-2-ynyl bromide **12** in the presence of copper(I) cyanide. *cis*-Addition of deuteriated diisoamylborane (from sodium borodeuteride, BF_3–diethyl ether and 2-methylbut-2-ene)[12,13] to the diyne so formed, followed by addition of [$^2H_2$]ethylene glycol and then deuterioacetic acid, gave the required tetradeuterio acid with 98 atom % $^2H_4$ at the four olefinic sites as determined by NMR (Omission of the [$^2H_2$]ethylene glycol gave a high isotope incorporation, but the [9,10,12,13-$^2H_4$]linoleic formed was accompanied by 5–10% of conjugated material). The tetradeuteriolinoleic acid **11** was then converted into [9,10,12,13-$^2H_4$]-(9*S*)-hydroperoxyoctadeca-10(*E*),12(*Z*)-dienoic acid **14** using the lipoxygenase enzyme described above. The resulting hydroperoxide was converted into deuteriated colneleic acid by the above enzyme procedure. After esterification (CH_2N_2) and purification by HPLC, mass spectral analysis of the latter showed that all the four deuterium atoms of the original linoleic acid and its hydroperoxide were retained in the colneleic acid. They were located as follows. Comparison of the ^{1}H NMR of the tetradeuterio-ester with an undeuteriated standard of methyl colneleate showed the following proton absences: 1′-H [δ 6.52 (1 H), d, *J*/Hz 11.7]; 9-H [δ 6.27 (1 H), dd, *J*/Hz 12.1, 1.1], 3′-H [δ 5.85 (1 H) ddt, *J*/Hz 11.7, 10.5, 1.5] and 4′-H [δ 5.30 (1 H), dt, *J*/Hz 10.5, 7.4]. The double triplet at 6.01 (2′-H) had become a broadened singlet and the triplet of doublets at 5.15 (8-H) had now the appearance of a broadened triplet. On these grounds, the isotope positions in colneleic acid derived from **11** are as indicated in **16**.

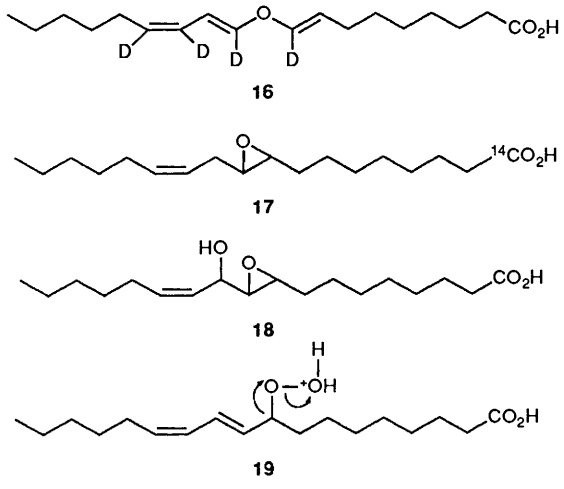


With this isotope information to hand, a mechanism (Scheme 2) can be proposed for the formation of colneleic acid **9** and by the same token, colnelenic acid **10**. Protonation and loss of water may lead to the addition of positively charged oxygen to the adjacent double bond to form an epoxycarbonium ion **15b** which would be stabilised relative to **15a**. Coronaric acid **17**, a naturally occurring fatty acid,[14,15] can be regarded as the reduction product of the proposed epoxy-carbonium ion **15b**, but this acid is not connected with colneleic acid in the sense of being an intermediate. Thus, a specimen of (±)-[1-^{14}C]coronaric acid was made by partial epoxidation of linoleic acid[16] and separated from the mixture of isomeric mono-epoxide (vernolic acid), bis-epoxide and unchanged material: on administration to potato extract, there was no incorporation into colneleic acid. The epoxycarbonium ion **15b** is viewed as undergoing cleavage as shown, removal of the C-8 proton being enzyme assisted*. The enzyme has a pH optimum of 9.0, suggesting that a suitable assisting base on the enzyme needs to be free. It is perhaps significant that at acid pH, using a crude potato enzyme preparation, colneleic acid is not formed and an epoxy-alcohol **18** results,[17] suggesting that without the necessary free base on the enzyme to initiate proton elimination the carbon–carbon σ bond is not fractured but the epoxy-

* Note added in proof: It has recently been shown that the hydrogen removed in the formation of colneleic acid from (9*S*)-hydroperoxyoctadeca-(10*E*,12*Z*)-dienoic acid is C-8 pro-*R*. (P. Fahlstudius and M. Hamberg, *J. Chem. Soc., Perkin Trans. 1*, 1990, 2027.



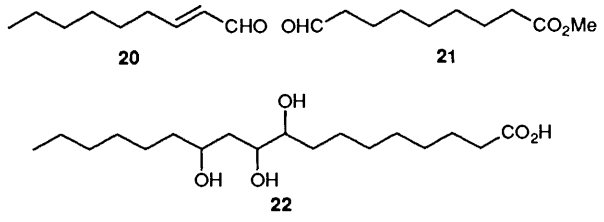
Scheme 2 Isotopic labelling and mechanism for enzymic formation of colneleic acid



carbonium ion is trapped by nucleophilic attack with water. The carbon–carbon bond cleavage could be represented as the product of a direct Baeyer–Villiger rearrangement **19**[18] but in our opinion this is a less attractive representation. Nucleophilic attack by the π-system is energetically more acceptable than direct attack by the σ bond and apart from the epoxy alcohol evidence mentioned above, it will be seen[19] that the epoxy-carbonium ion[20] is central to a range of enzymic products derived from hydroperoxides derived, in turn, from linoleic and linolenic acids. It was also postulated in Gardner's *in vitro* study of the acid catalysed decomposition of such hydroperoxides, in which the products formed resemble enzymic products much more than do the products of radical decomposition.[21]

For examination of their chemical and spectroscopic properties, colneleic **9** and colnelenic **10** acids were produced enzymically using an homogenate of potatoes which contains both the 9-peroxidase and the divinyl ether forming enzyme, working at a compromise pH of 7.5. Purification was initially by silica-gel chromatography followed by separation of colneleic and colnelenic acids as their methyl esters by reversed phase HPLC (see above). The quantities of the two acids can be increased by the addition of linoleic and linolenic acids to the crude potato extract at a rate of about 10 mg of fatty acid per 100 g of potato tuber: yields also depend on the variety, age and condition of the tubers.

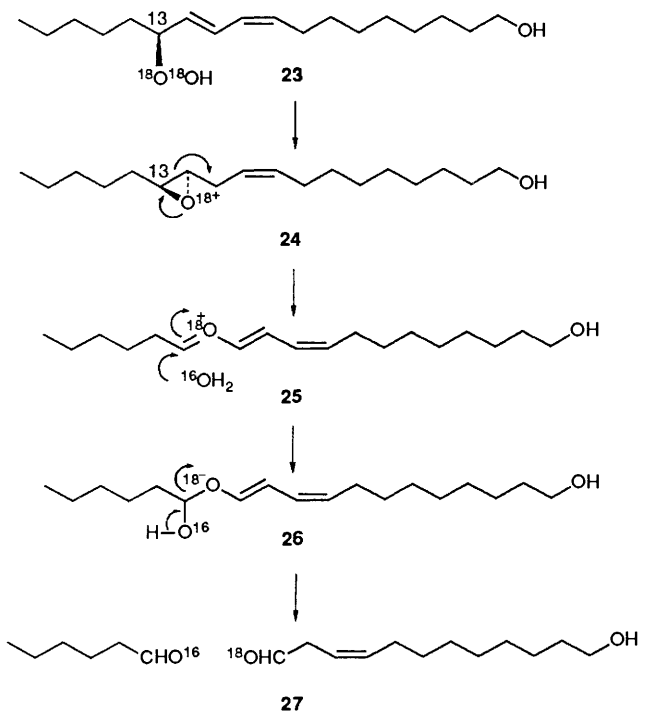
As would be expected of a divinyl ether, colneleic acid is sensitive to acid. The rate determining step in vinyl ether hydrolysis is protonation, not on oxygen, but at the β carbon to form an intermediate oxocarbonium ion.[22] Treatment of methyl colneleate with a drop of acid in methanol gave non-2(*E*)-enal **20** [presumably *via* isomerisation of the labile non 3(*Z*)-enal first formed] and methyl 9-oxononanoate **21** which were identified by comparison with authentic specimens made from castor oil.[28] The oil was hydroxylated using peracetic acid and



hydrolysed to give 9,10,12-trihydroxystearic acid **22** which was then cleaved with lead tetraacetate: the two aldehydes were separated and purified by distillation. In a similar fashion, methyl colnelenate gave nona-2(*E*),6(*Z*)-dienal **6** [presumably from isomerisation of the labile nona-3(*Z*),6(*Z*)-dienal] and methyl 9-oxononanoate **21**. The former was identical with a synthetic specimen made from hexa-1,5-diyne by a literature procedure.[23] Kept in acetate buffer (pH 5.0) for 20 h in the presence of a little Tween 20, followed by extraction and examination by GLC, methyl colneleate gave non-2(*E*)-enal and methyl 9-oxononanoate. The free acid itself also underwent decomposition in 0.1M acetate buffer at pH 5.5 (24 h) but was stable in buffers at pH 7.2 and 9.0 (72 h).

Colneleic and colnelenic acids throw considerable light on the chain fracture processes involving the 9-hydroperoxides of linoleic and linolenic acids and by acid catalysed decomposition can lead to the characteristic aldehydes of their enzymic lysis. On the other hand, as is known at present, colneleic and colnelenic acids are unique to the potato and even in this tuber the synthase does not act on the corresponding 13-hydroperoxides of linoleic and linolenic acid.[6] Enzyme extracts prepared in the manner used for potato were obtained from tomato, cucumber and the cotyledons, hypocotyls and root sections of 6-day old water melon seeds showed no evidence of divinyl ether–acid utilisation as judged by UV spectrophotometric methods (λ_{max} 250 nm): using TLC criteria, crude extracts of green bananas, pears and aubergines also appeared not to degrade colneleic acid.

In a revealing experiment Hatanaka and his colleagues[24] have shown that in the enzymic degradation of [13*S*-$^{18}O_2$]-hydroperoxyoctadeca-9(*Z*),11(*E*)-dienol **23** using tea chloroplasts, the lyase forms two products, hexanal and 12-hydroxy-(3*Z*)-dodecenal **27**, the ^{18}O labelling being retained entirely by the latter. This suggests that the epoxy-carbonium ion (cf. **24**) is formed as in a colneleic acid route but does not lose a 14-proton to stabilise itself, as would happen in a colneleic acid type of pathway. Instead, the oxonium ion **25** is attacked by water nucleophile at the erstwhile C-13 position as shown (Scheme 3). Decomposition of the hemiacetal **26** then produces the two



Scheme 3 ^{18}O Distribution in aldehyde products from decomposition of $^{18}O_2$-hydroperoxylinoleyl alcohol using tea chloroplast lyase

aldehydes with ^{18}O in only one of them **27**. Such a route may suggest why the divinyl acids are apparently by-passed in most plants, the proton loss and rehydration during conversion of the hydroperoxy acid precursor into aldehyde fragmentation products being energetically more wasteful. Unless for colneleic acid the rehydration was enzyme controlled and totally regiospecific, isotope would be found distributed between the two aldehyde oxygens in such an $^{18}O_2$ experiment. The divinyl acids themselves may therefore be viewed as probable accumulation products rather than as widely distributed reactive metabolic intermediates. Nevertheless, apart from what their structures reveal about the chain fracture process, it is possible that colneleic and colnelenic acids, or their metabolic products, have other, as yet unrecognised, functions in the potato. Colneleic acid for example, is reported to be a very effective competitive inhibitor of potato lipoxygenase.[25]

Experimental

Fatty acid hydroperoxides decompose, exchange, or isomerise and were handled under inert gas in dilute solutions, complete removal of solvent being carried out immediately prior to use. Storage was avoided if at all possible, but if unavoidable was carried out at −25 °C in solvents under argon or nitrogen. *J* Values are in Hz.

Fatty Acid Substrate Solutions.—A mixture of Tween 20 (0.5 ml) and sodium borate buffer (pH. 9.0; 5 mmol dm^{-3}; 10 ml) was added to the fatty acid (0.5 g). Aqueous sodium hydroxide (1 mol dm^{-3}; 1.3 ml) was added with agitation until a clear solution was obtained. Sodium borate buffer. (pH 9.0; 5 mmol dm^{-3}; 90 ml) was added and the total volume was made up to 200 ml with water. The substrate solution was stored below 0 °C under a nitrogen atmosphere prior to use. The above procedure was scaled up or down as required.

(9S)-9-*Hydroperoxyoctadeca*-10(E),12(Z)-*dienoic acid* **8** *from Crude Homogenates of Potato.*—Potato tubers were peeled and cut into small pieces. The potato tissue (100 g) was washed with ice-cold water and homogenised with sodium acetate buffer (pH 5.5; 0.1 mol dm^{-3}; 200 ml). Linoleic acid **1** substrate solution (9 mmol dm^{-3}; 20 ml) (see above) was added and the mixture was mechanically shaken in an open vessel at 25 °C (45 min). Methanol (600 ml) and chloroform (300 ml) were added and the mixture was gently stirred under a nitrogen atmosphere at room temperature (30 min). Citric acid (0.1 mmol dm^{-3}; 300 ml) was added. The organic phase was separated, dried (Na_2SO_4) and concentrated to a small volume. The concentrate was fractionated by preparative thick layer chromatography (silica gel) with light petroleum (b.p. 60–80 °C)–ether (1:1) as eluent. The band at R_f 0.51 (UV) was collected, giving (9*S*)-9-hydroperoxyoctadeca-10(*E*),12(*Z*)-dienoic acid **8** (20–30 mg) which was stored as a dilute solution in ether at −25 °C prior to use. For analysis a small sample was esterified with ethereal diazomethane. Reverse-phase HPLC with methanol–water (4:1) as eluent showed the 9- to 13-hydroperoxide ratio to be 92:8.

The hydroperoxide had $\nu_{max}(CHCl_3)/cm^{-1}$ 1743 (ester C=O); δ_H(250 MHz; $CDCl_3$) 7.75 (1 H, s, OOH), 6.58 (1 H, dd, *J* 15.8 and 10.8, 11-H), 5.98 (1 H, t, *J* 10.8, 12-H), 5.51 (2 H, m, 10-H and 13-H), 4.34 (1 H, M component of ABMX, *J* 7.5, 9-H), 3.67 (3 H, s, OCH_3), 2.29 (2 H, t, *J* 7.9, 2-H_2), 2.18 (2 H, q, *J* 7.5, 4-H_2), 1.70–1.20 (18 H, m, 3-H_2 to 8-H_2 and 15-H_2 to 17-H_2), and 0.90 (3 H, t, *J* 7.5, 18-H_3); *m/z* 308 ($M^{\bullet+}$ − H_2O, 20%), 293 ($M^{\bullet+}$ − OOH, 6), 276 ($M^{\bullet+}$ − H_2O − MeOH, 4), 237 ($M^{\bullet+}$ − H_2O − C_5H_{11}, 17), 185 ($M^{\bullet+}$ − H_2O − C_9H_{15}, 32), 166 ($M^{\bullet+}$ − H_2O − $C_8H_{14}O_2$, 37) and ($M^{\bullet+}$ − H_2O − $C_9H_{17}O_2$, 39).

9-[*Nona*-1′(E),3′(Z)-*dien-yl-oxy*]*non*-8(E)-*anoic Acid* (*Colneleic Acid*) **9** *and* 9-[*Nona*-1′(E),3′(Z)6′(Z)-*trienyloxy*]*non*-8(E)-*anoic Acid* (*Colnelenic Acid*) **10** *from Crude Potato Homogenates.*—Potato tissue (200 g) was homogenized with sodium phosphate buffer (pH 7.2; 0.1 mol dm^{-3}; 400 ml). Linoleic acid substrate solution (8 ml) was added to the homogenate and the mixture was mechanically shaken in an open vessel at 25 °C (15 min). Methanol (600 ml) and chloroform (300 ml) were added and the mixture was gently stirred at room temperature under a nitrogen atmosphere (45 min). Chloroform (300 ml) and citric acid (0.1 mol dm^{-3}; 960 ml) were added. The organic phase was separated, dried (Na_2SO_4) and evaporated. The residue was esterified with ethereal diazomethane and fractionated by EDTA (disodium salt; 0.1 mol dm^{-3})–washed silica gel (50 g) column chromatography with light petroleum (b.p. 60–80 °C)–ether (19:1) as eluent. Fractions (7 ml) were collected. Fractions (26–32) were combined and evaporated and the resultant mixture of methyl colneleate **9** and methyl colnelenate **10** was separated by C_{18}- reversed-phase HPLC (methanol–water, 9:1).

Methyl colnelenate **10** (2 mg) was eluted first. It had $\nu_{max}(CHCl_3)/cm^{-1}$ 1728 (ester C=O), 1645 (C=C), 1604 (C=C) and 918 (*trans* substituted vinyl ether): δ_H(250 MHz; $CDCl_3$) see Table 2; δ_C(250 MHz; $CDCl_3$) 175.35 (C-1), 147.39 (C-1′), 142.97 (C-9), 132.17 (C-4′), 127.12 (C-6′ or C-7′), 126.93 (C-6′ or C-7′), 123.60 (C-3′), 111.11 (C-8), 106.57 (C-2′), 51.38 (OCH_3), 34.11 (C-2), 29.83, 28.96, 28.62, 27.17 (C-7), 25.99, 24.93 (C-3), 20.59 and 14.23 (C-9′); *m/z* 306 ($M^{\bullet+}$, 12%), 137 [$M^{\bullet+}$ − CH=CH$(CH_2)_6CO_2Me$, 11], and 121 [$M^{\bullet+}$ − OCH=CH-$(CH_2)_6CO_2Me$, 38] (Found: *m/z* 306.2166. $C_{19}H_{30}O_3$ requires *m/z* 306.2137).

Methyl colneleate **9** (5 mg) was obtained as a colourless oil: $\nu_{max}(CHCl_3)/cm^{-1}$ 1728 (ester C=O), 1645 (C=C), 1606 (C=C), 1162 (C–O) and 918 (*trans* substituted vinyl ether): δ_H(250 MHz; $CDCl_3$) see Table 1: δ_C(250 MHz; $CDCl_3$) 174.18 (C-1), 146.98 (C-1′), 143.06 (C-9), 129.42 (C-4′), 123.33 (C-3′), 110.87 (C-8), 106.93 (C-2′), 51.40 (OCH_3), 34.11 (C-2), 31.52, 29.85, 29.37, 28.97, 28.62, 27.71 (C-5′), 27.18 (C-7), 24.94 (C-3), 22.56 and

14.03 (C-9'); *m/z* 308 ($M^{\bullet+}$, 52%), 251 ($M^{\bullet+} - C_4H_9$), 137 [$M^{\bullet+} - CH{=}CH(CH_2)_6CO_2Me$, 17] and 123 [$M^{\bullet+} - OCH{=}(CH_2)_6CO_2Me$, 27] (Found: *m/z* 308.2357 $C_{19}H_{32}O_3$ requires *m/z* 308.2362).

The proportion of methyl colnelenate produced can be increased by substituting linolenic acid for linoleic acid in the above procedure.

Partial Purification of Lipoxygenase from Potato Tubers.—Potato tubers were peeled and diced. The tissue (100 g) was washed with ice-cold water, lightly blotted and homogenized with cold sodium phosphate buffer (pH 7.2; 50 mmol dm^{-3})–sodium metabisulphite (2 mmol dm^{-3}; 200 ml). The homogenate was centrifuged (25 000 × g) at 4 °C (90 min). The supernatant (175 ml) was carefully collected, cooled to 0 °C and treated with ammonium sulphate to 50% saturation (50.93 g). The mixture was left at 0 °C (2 h) and then centrifuged (15 000 × g) at 4 °C (30 min). The supernatant was discarded and the pellet suspended in ammonium sulphate (2 mol dm^{-3}; 10 ml) and stored at 4 °C. The mixture was dialysed against water (1 l) at 4 °C (48 h) with water being replaced after 24 h. The resulting cloudy solution was freeze-dried to give a white powdery solid (120–150 mg) which was stored at −15 °C before use as an enzyme source.

Partial Purification of the Enzyme which Converts (9S)-9-*Hydroperoxyoctadeca*-10(E),12(Z)-*dienoic Acid* **8** *into Colneleic Acid* **9**.—Potato tissue (100 g) was washed with ice-cold water, lightly blotted and homogenized with cold sodium phosphate buffer (pH 7.2; 0.1 mol dm^{-3})–mercaptobenzothiazole (0.2 mmol dm^{-3}; 200 ml). The homogenate was filtered through silk cloth and then centrifuged (15 000 × g) at 4 °C (30 min). The supernatant (255 ml) was cooled to 0 °C and treated with ammonium sulphate to 45% saturation (65.79 g). The mixture was left at 0 °C (90 min) and then centrifuged (15 000 × g) at 4 °C (30 min). The supernatant (265 ml) was cooled at 0 °C and treated with ammonium sulphate from 45 to 65% saturation (32.60 g). The mixture was left at 0 °C (2 h) and then centrifuged (15 000 × g) at 4 °C (30 min). The supernatant was discarded and the pellet suspended in ammonium sulphate (2 mol dm^{-3}; 10 ml) and stored at 4 °C. The mixture was dialysed against sodium phosphate buffer (pH 7.2; 0.1 mol dm^{-3}; 1 l) at 4 °C (48 h). The buffer was replaced after 24 h. The resulting straw coloured solution was immediately used as an enzyme source.

[(9S)-$^{18}O_2$]-9-*Hydroperoxyoctadeca*-10(E),12(Z)-*dienoic Acid* **8**.—A mixture of linoleic acid substrate solution (20 ml) (see above) and sodium acetate buffer (pH 5.5; 0.1 mol dm^{-3}; 160 ml) was degassed in the following manner. The mixture was placed in a round-bottomed flask (500 ml) and the flask evacuated (oil pump), while the mixture was frozen in liquid nitrogen. The solution was then allowed to thaw under an atmosphere of argon. This procedure was repeated twice more. The flask was evacuated at room temperature and $^{18}O_2$ (80 atom % ^{18}O) was introduced. The mixture was allowed to equilibrate (20–30 min) and then adjusted to atmospheric pressure with nitrogen. A solution of partially purified potato lipoxygenase (100 mg) in degassed sodium acetate buffer, (pH 5.5; 0.1 mol dm^{-3}; 20 ml) was added and the reaction mixture was magnetically stirred at room temperature with occasional manual shaking (1 h). Methanol (100 ml) was added and the mixture opened to the atmosphere. Methanol (500 ml) and chloroform (300 ml) were added and the mixture gently stirred under a nitrogen atmosphere at room temperature (30 min). Citric acid (0.1 mol dm^{-3}; 300 ml) and chloroform (300 ml) were added. The organic phase was separated, dried (Na_2SO_4) and concentrated to a small volume. The concentrate was fractionated by preparative thick layer chromatography (silica gel) with light petroleum (b.p. 60–80 °C)–ether (1:1) as eluent. The band at R_f 0.5 (UV) was collected to give [9*S*-$^{18}O_2$]-9-hydroperoxyoctadeca-10(*E*),12(*Z*)-dienoic acid **8** (80 atom % ^{18}O), which was stored as a dilute solution in ether. For analysis a small sample was esterified with ethereal diazomethane: *m/z* 310 ($M^{\bullet+} - H_2{}^{18}O$; 11%), 294 ($M^{\bullet+} - {}^{18}O^{18}OH$, 4), 278 ($M^{\bullet+} - H_2{}^{18}O$ − MeOH, 5), 239 ($M^{\bullet+} - H_2{}^{18}O - C_5H_{11}$, 11), 187 ($M^{\bullet+} - H_2{}^{18}O - C_9H_{15}$, 25), 168 ($M^{\bullet+} - H_2{}^{18}O - C_8H_{14}O_2$, 28) and 153 ($M^{\bullet+} - H_2{}^{18}O - C_9H_{17}O_2$, 40).

Enzymic Formation of [^{18}O]*Colneleic acid* **9** *from* [(9S)-$^{18}O_2$]-9-*Hydroperoxyoctadeca*-10(E),12(Z)-*dienoic Acid* **8**.—A mixture of [(9*S*)-$^{18}O_2$]-9-hydroperoxyoctadeca-10(*E*),12(*Z*)-dienoic acid (80 atom % ^{18}O) (*ca.* 10 mg) and Tween 20 (10 μl) was dispersed in sodium borate buffer, (pH 9.0; 0.1 mol dm^{-3}; 10 ml). Aqueous sodium hydroxide (2.0 mol dm^{-3}) was added until a clear solution was obtained. This was diluted with sodium borate buffer, (pH 9.0; 0.1 mol dm^{-3}; 190 ml) and partially purified enzyme preparation (10 ml) (see above) was added. The mixture was mechanically shaken in an open vessel at 25 °C (20 min). Methanol (600 ml) and chloroform (300 ml) were added and the mixture was gently stirred at room temperature under a nitrogen atmosphere. Chloroform (300 ml) and citric acid (0.1 mol dm^{-3}; 300 ml) were added. The organic phase was separated, dried (Na_2SO_4) and evaporated. The residue was esterified with ethereal diazomethane and then fractionated by EDTA (disodium salt; 0.1 mol dm^{-3}), washed silica gel column chromatography with light petroleum (b.p. 60–80 °C)–ether (98:2) as eluent. Fractions containing methyl colneleate were combined and evaporated. The product was further purified by reversed-phase HPLC, using methanol–water (9:1), giving methyl [^{18}O]colneleate (83 atom % ^{18}O). The ^{1}H NMR was identical with an authentic sample of methyl colneleate **9**: *m/z* 310 ($M^{\bullet+}$, 38%) and 253 ($M^{\bullet+} - C_4H_9$, 13).

When a crude potato homogenate was employed as the enzyme source with the 9-hydroperoxide **8** (40 atom % ^{18}O), a low but significant (4%) incorporation was observed, due to the diluting effect of endogenous material.

Oct-2-yn-1-ol.—A Grignard reagent was prepared from magnesium (12.2 g, 0.51 mol), dry ether (250 ml), a crystal of iodine and bromoethane (71 g, 0.65 mol) in dry ether (50 ml). The reagent was cooled to −5 °C and hept-1-yne (48 g, 0.50 mol) in dry ether (30 ml) was added dropwise (1 h) with stirring. The reaction mixture was refluxed (1 h) and then cooled to −10 °C in an ice–salt bath. Gaseous formaldehyde, generated from dry paraformaldehyde (45 g) at 180 °C (oil bath temperature), was swept into the flask with nitrogen through a wide bore tube reaching to within 1 cm of the stirred reaction mixture (2 h). The reaction mixture was refluxed (30 min) and then cooled to −5 °C in an ice–salt bath and treated with a mixture of crushed ice (100 g) and 10% sulphuric acid (100 ml). Work-up, with addition of hydroquinone (50 mg) and distillation, gave oct-2-yn-1-ol as a colourless oil (26.2 g, 42%), b.p. 75–77 °C at 2 mmHg (lit.,[26] 80–81 °C at 5 mmHg, lit.,[27] b.p. 75–80 °C at 2 mmHg); ν_{max}(liquid film)/cm^{-1} 3390 (OH), 2280 (C≡C) and 2220 (C≡C); δ_H(90 MHz; $CDCl_3$) 4.27 (2 H, m, 1-H_2), 3.27 (1 H, br s, OH), 2.21 (2 H, m, 4-H_2), 1.40 (6 H, m, 5-H_2, 6-H_2 and 7-H_2) and 0.90 (3 H, t, 8-H_3).

1-*Bromooct-2-yne* **12**.—A mixture of oct-2-yn-1-ol (21 g, 0.167 mol), dry ether (25 ml) and dry pyridine (3.5 ml) was cooled to −8 °C. Phosphorus tribromide (18 g, 0.066 mol) was added dropwise (30 min) with stirring at −8 °C. The reaction mixture was refluxed with stirring (3 h), cooled to room temperature and treated with water (35 ml). The organic phase was separated and the aqueous phase extracted with ether (3 × 30 ml). The combined ethereal extracts were dried

($MgSO_4$) and evaporated. The residue was distilled to give 1-bromooct-2-yne **12** (19.89 g, 63%) as a colourless oil, b.p. 41.43 °C at 0.2 mmHg (lit.,[27] b.p. 69.5–70.5 °C at 1 mmHg); ν_{max}(liquid film)/cm^{-1} 2235 (C≡C); δ_H(90 MHz; $CDCl_3$) 3.97 (2 H, t, 2.5 Hz, 1-H_2), 2.26 (2 H, m, 4-H_2), 1.41 (6 H, m 5-H_2, 6-H_2 and 7-H_2) and 0.92 (3 H, t, 8-H_3); *m/z* 109 ($M^{\bullet+}$ − Br; 66%) (Found: *m/z* 109.1024 C_8H_{13} requires *m/z* 109.1094).

1,1-*Diphenylundec*-1-*en*-10-*yne*.—A Grignard reagent was prepared from magnesium turnings (7.4 g, 0.31 mol), dry ether (250 ml) and bromobenzene (48.2 g, 0.31 mol) in dry ether (50 ml). The reagent was cooled to −5 °C and methyl undec-10-ynoate (20 g, 0.10 mol) prepared by a literature method,[26] was added dropwise (2 h) with stirring. The reaction mixture was refluxed (2 h). Work-up gave 1,1-diphenyl-undec-10-yn-1-ol (36.8 g).

The latter was dehydrated by heating at 220–230 °C (silicone oil bath temperature) (1 h). Work-up gave 1,1-diphenylundec-1-en-10-yne (29.4 g, 95%), b.p. 158–162 °C at 0.05 mmHg (lit.,[11] 174–180 °C at 0.2 mmHg).

Dec-9-*ynoic Acid* **13**.—A mixture of 1,1-diphenylundec-1-en-10-yne (25 g, 83 mmol) and acetic acid (250 ml) was warmed to 60 °C. A solution of chromium trioxide (17 g, 170 mmol) in water (20 ml) was added dropwise (2 h) with stirring, the temperature being maintained at 60 °C. The reaction mixture was stirred at room temperature (17 h) and then evaporated and worked up. The resulting oil was purified over silica gel (500 g) by dry column chromatography. The column was eluted with light petroleum (b.p. 60–80 °C)–ether (9:1) (2500 ml) to remove polar products and the polarity of the solvent was gradually increased to light petroleum (b.p. 60–80 °C)–ether (1:4) to elute the required product. Dec-9-ynoic acid **13** (8.9 g, 64%) was a colourless oil, b.p. 110–111 °C at 0.4 mmHg (lit.,[26] 95–98 °C at 0.5 mmHg); ν_{max}(liquid film)/cm^{-1} 3299 (alkyne CH), 2120 (C≡C and 1705 (acid C=O); δ_H(90 MHz; $CDCl_3$) 11.99 (1 H, s, OH), 2.31 (4 H, m, 2-H_2 and 8-H_2), 1.98 (1 H, t, 2.5 Hz, 10-H) and 1.85–1.28 (10 H, m, 3-H_2, 4-H_2, 5-H_2, 6-H_2 and 7-H_2); *m/z* 108 ($M^{\bullet+}$ − $C_2H_4O_2$; 44%) (Found: *m/z* 108.0945 C_8H_{12} requires *m/z* 108.0950).

Octadeca-9,12-*diynoic Acid*.—Dec-9-ynoic acid (6.72 g, 40 mmol) was added dropwise (45 min) with stirring at 0 °C to a Grignard reagent prepared from magnesium (1.95 g, 30 mmol) and bromoethane (9.59 g, 20 mmol) in tetrahydrofuran (30 ml). The reaction mixture was stirred at room temperature (2 h). Anhydrous copper(I) cyanide (0.20 g) was added and the mixture stirred (10 min). A solution of 1-bromooct-2-yne **12** (3.78 g, 20 mmol) in dry tetrahydrofuran (10 ml) was added dropwise (15 min) with stirring at 20 °C. The reaction mixture was refluxed (23 h), cooled, worked up and fractionally distilled to give recovered 1-bromooct-2-yne and dec-9-ynoic acid followed by octadeca-9,12-diynoic acid (3.29 g, 60%) as white crystals, b.p. 165–168 ° at 0.1 mmHg (lit.,[10] b.p. 143–154 °C at 0.01 mmHg); ν_{max}($CHCl_3$)/cm^{-1} 3040br (OH) and 1705 (acid C=O); δ_H(90 MHz; $CDCl_3$) 14.50 (1 H, br s, CO_2H), 3.17 (2 H, br m, 11-H_2), 2.28 (6 H, br m, 2-H_2, 8-H_2, 14-H_2), 1.80–1.20 (16 H, m, 3-H_2 to 7-H_2 and 15-H_2 to 17-H_2) and 0.94 (3 H, br t, 18-H_3).

Methyl Octadeca-9,12-*diynoate*.—Octadeca-9,12-diynoic acid (400 mg, 145 mmol) in ether (10 ml) was treated with ethereal diazomethane at 0 °C to give, after silica gel chromatography, eluting with light petroleum (b.p. 60–80 °C)–ether (95:5), methyloctadeca-9,12-diynoate (399 mg, 95%); ν_{max}(liquid film)/cm^{-1} 1736 (ester C=O); δ_H(90 MHz; $CDCl_3$) 3.72 (3 H, s, OCH_3), 3.14 (2 H, quintet, *J* 2.2, 11-H_2), 2.22 (6 H, m, 2-H_2, 8-H_2 and 14-H_2), 1.80–1.10 (16 H, m, 3-H_2 to 7-H_2 and 15-H_2 to 17-H_2) and 0.90 (3 H, t, 18-H_3).

Methyl [9,10,12,13-$^2H_4$]*Linoleate*.—Sodium borodeuteride (98 atom %D; 84 mg, 2 mmol) was dissolved in dry diglyme (4 ml) under a nitrogen atmosphere. 2-Methylbut-2-ene (350 mg, 5 mmol) was added and the mixture cooled to 0 °C in an ice-bath. Boron trifluoride–ether (0.34 ml, 2.7 mmol) was added dropwise with stirring at 0 °C. The reaction mixture was kept at 0 °C (2 h). Methyloctadeca-9,12-diynoate (290 mg, 1 mmol) was added dropwise and the mixture was stirred at 0 °C (30 min) and then at room temperature (2 h); it was then cooled to 0 °C in an ice-bath. [$^2H_2$]Ethylene glycol (H/D exchanged 3 times with D_2O) (0.5 ml) was added followed by [$^2H_4$]acetic acid (99.5 atom % ^2H; 0.5 ml). The reaction mixture was stirred at room temperature (16 h) and then diluted with water (10 ml) and extracted with hexane 4 × 10 ml). The combined hexane extracts were dried ($MgSO_4$) and evaporated. The residue was subjected to column chromatography over silica gel (18 g) eluting with light petroleum (b.p. 60–80 °C)–ether (99:1). Fractions (10 ml) were collected. Fractions (17–24) were combined and evaporated to give methyl [9,10,12,13-$^2H_4$]linoleate (158 mg, 53%) (98 atom %D); δ_H(90 MHz; $CDCl_3$) 3.70 (3 H, s, OCH_3), 2.79 (2 H, br s, 11-H_2), 2.32 (2 H, t, 2-H_2), 2.06 (4 H, m, 8-H_2 and 14-H_2), 1.80–1.20 (16 H, m, 3-H_2 to 7-H_2 and 15-H_2 to 17-H_2) and 0.90 (3 H, t, 18-H_3); *m/z* 298 ($M^{\bullet+}$ 29%).

[9,10,12,13-$^2H_4$]*Linoleic Acid* **11**.—Methyl [9,10,12,13-$^2H_4$]linoleate (110 mg, 0.4 mmol), potassium hydroxide (1 g), ethanol (8 ml) water (2 ml), was refluxed under nitrogen (90 min). The mixture was then worked up and subjected to preparative thick layer chromatography (silica gel), with light petroleum (b.p. 60–80 °C)–ether, 3:2 as eluent, to give, as a band at R_f 0.51 (visualized with *p*-anisaldehyde spray) [9,10,12,13-$^2H_4$]linoleic acid **11** (76 mg, 70%); δ_H(90MHz; $CDCl_3$) 11.08 (1 H, br s, CO_2H), 2.79 (2 H, br s, 11-H_2), 2.36 (2 H, t, 2-H_2), 2.06 (4 H, m, 8-H_2 and 14-H_2), 1.80–1.20 (16 H, m, 3-H_2 to 7-H_2 and 15-H_2 to 17-H_2) and 0.90 (3 H, t, C18-H_3).

[9,10,12,13-$^2H_4$]-(9S)-9-*Hydroperoxyoctadeca*-10(E),12(Z)-*dienoic Acid* 14a.—[9,10,12,13-$^2H_4$]Linoleic acid **11** (50 mg) and Tween 20 (50 mg) were dispersed in sodium borate buffer (pH 9.0; 5 mmol dm^{-3}; 10 ml) and sodium hydroxide (2 mol dm^{-3}) was added dropwise until a clear solution was obtained. The solution was diluted with sodium acetate buffer (pH 5.5; 0.1 mol dm^{-3}; 200 ml). Partially purified potato lipoxygenase (150 mg) (see above) was added and the mixture was mechanically shaken in an open vessel at room temperature (45 min). The reaction mixture was worked up and the product isolated and stored in ether at −25 °C.

[9,1′,3′,4′,-$^2H_4$]*Colneleic Acid* **16a**.—[9,10,12,13-$^2H_4$]-(9*S*)-9-Hydroperoxyoctadeca-10(*E*),12(*Z*)-dienoic acid (*ca.* 10 mg) and Tween 20 (10 mg) were dispersed in sodium borate buffer (pH 9.0; 5 mmol dm^{-3}; 5 ml). Aqueous sodium hydroxide (2.0 mol dm^{-3}) was added dropwise until a clear solution was obtained. The solution was diluted with sodium borate buffer (pH 9.0; 0.1 mol dm^{-3}; 200 ml). A partially purified enzyme preparation (10 ml) (see above) was added and the mixture was mechanically shaken in an open vessel at 25 °C (20 min). The reaction was worked up and the product isolated and purified as its methyl ester.

Comparison of the ^1H NMR spectrum with that of authentic methyl colneleate **9** showed the absence of the d at 6.52, the dd at 6.27, the ddt at 5.85 and the dt at 5.30. The td at 6.02 (2′-H) now has the appearance of a broad singlet and the dt at 5.15 (C8-H) now appears as a broadened triplet. Thus, the product had deuterium atoms at C-9, C-1′, C-3′ and C-4′. It had *m/z* 312 ($M^{\bullet+}$), 255 ($M^{\bullet+}$ − C_4H_9) and 126 [$M^{\bullet+}$ − $ODC{=}CH(CH_2)_6CO_2Me$].

9,10,12-*Trihydroxystearic Acid* **22**.—A 30% hydrogen peroxide solution (20 ml) and acetic acid (82 ml) was heated at 80–85 °C (1 h). The mixture was cooled to room temperature and castor oil (containing 80% ricinoleic acid) (31.1 g) was added in one portion. The warm mixture was left at room temperature (1 h) and then heated to 70 °C and allowed to slowly cool to room temperature. Acetic acid was removed by steam distillation and the residual oil was refluxed with aqueous sodium hydroxide (2 mol dm^{-3}; 120 ml) (2 h). The hot solution was acidified with hydrochloric acid (2 mol dm^{-3}) and then cooled to room temperature. The aqueous solution was decanted from the solid, and the solid refluxed with water (100 ml) (10 min). The mixture was cooled to room temperature and the water decanted. The solid residue was recrystallised from ethanol in the presence of decolourising charcoal to give white crystals of 9,10,12-trihydroxystearic acid **22** (7.0 g), m.p. 105–106 °C (lit.,[28] m.p. 108–109 °C).

Non-2(E)-*enal* **20** *and Methyl* 9-*Oxononanoate* **21**.—9,10,12-Trihydroxystearic acid **22** (6.64 g, 20 mmol) was dissolved in warm acetic acid (50 ml). Trilead tetraoxide (red lead) (15.10 g, 22 mol) was added in portions (1 g) at 60–65 °C with stirring over 90 min. The red colour was allowed to discharge before the addition of the next portion. The reaction mixture was stirred at 65 °C (15 min) and then cooled to room temperature. Water (50 ml) was added and the mixture steam distilled until no more oil passed over. The distillate (300 ml) was extracted with ether (1 × 100 ml and 2 × 50 ml), worked up and distilled to give non-2(*E*)-enal **20** (0.12 g) as a colourless oil, b.p. 67 °C at 0.5 mmHg (lit.,[28] b.p. 56–58 °C at 0.1 mmHg), ν_{max}(liquid film)/cm^{-1} 1686 (α,β-unsaturated aldehyde C=O), 1634 (C=C) and 976 (*trans* C=C); *m/z* 139 ($M^{\bullet+}$ − 1, 4%) and 138 ($M^{\bullet+}$ − 2, 19): (Found *m/z* 139.1110 $C_{19}H_{15}O$ requires *m/z* 139.1097). The substantial pot residue from the distillation was non-2(*E*)-enoic acid.

The residue from the steam distillation was extracted with ether (3 × 50 ml). The combined ethereal extracts were washed with water (5 × 30 ml), dried ($MgSO_4$), evaporated and esterified with diazomethane. Distillation gave methyl 9-oxononanoate **21** (1.33 g, 36%), b.p. 125 °C at 1 mmHg (lit.,[29] b.p. 111–112 °C at 3 mmHg): ν_{max}(liquid film)/cm^{-1} 1737 (ester C=O) and 1726 (aldehyde C=O): *m/z* 186 ($M^{\bullet+}$, 0.4%), 185 ($M^{\bullet+}$ − 1, 3) and 155 ($M^{\bullet+}$ − 31, 25) (Found: *m/z* 186.1254 $C_{10}H_{18}O_3$ requires *m/z* 186.1252).

Both non-2(*E*)-enal **20** and methyl 9-oxononanoate **21** were single pure components when analyzed by GLC [column: 2% Silar 10C on Chromosorb W (80–100 mesh); oven temperature: 100 and 130 °C respectively; nitrogen flow: 40 ml/min].

Nona-2,6-*diynal Diethyl Acetal*.—As prepared from octa-1,5-diyne (2.19 g, 21 mmol) by Sondheimer's method nona-2,6-diynal diethyl acetal (2.82 g, 66%) had b.p. 70–72 °C at 0.1 mmHg (lit.,[23] b.p. 104–105 °C at 0.3 mmHg); ν_{max}(liquid film)/cm^{-1} 2248 (C≡C) (Found: *m/z* 208.1422. $C_{13}H_{20}O_2$ requires *m/z* 208.1381).

Nona-2(Z),6(Z)-*dienal Diethyl Acetal*.—Nona-2,6-diynal diethyl acetal (2.50 g, 12 mmol), palladium–barium sulphate catalyst (5% Pd; 250 mg), and ethyl acetate (20 ml) were shaken in hydrogen until 24 mmol had been absorbed (3 h). The catalyst was removed and the residue distilled to give nona-2(Z),6(Z)-dienal diethyl acetal (1.64 g, 64%), b.p. 60–64 °C at 0.1 mmHg (lit.,[23] b.p. 67–70 °C at 0.2 mmHg); *m/z* 167 ($M^{\bullet+}$ − OEt; 52%). (Found: *m/z* 167.1429. $C_{11}H_{19}O$ requires *m/z* 167.1423).

Nona-2(E),6(Z)-*dienal* **6**.—Nona-2(*Z*),6(*Z*)-dienal diethyl acetal (1.4 g, 7 mmol), sulphuric acid (2 mol dm^{-3}; 25 ml) and acetone (25 ml) were heated at 70 °C with stirring (1 h) and then worked up. The residue was chromatographed on silica gel with hexane–ether (19:1) as eluent. Fractions (7–10 ml) were collected. Fractions (29–40) were combined to give nona-2(*E*),-6(*Z*)-dienal **6** (230 mg, 25%), b.p. 100–104 °C at 11 mmHg (lit.,[23] b.p. 94–95.5 °C at 18 mmHg); ν_{max}(liquid film)/cm^{-1} 1687 (aldehyde C=O), 1648 (C=C), 978 (*trans* C=C) and 725 (*cis* C=C); δ_H(250 MHz; $CDCl_3$) 9.47 (1 H, d, 5.0, 1-H), 6.82 (1 H, dt, 10.4 and 4.6, 3-H), 6.11 (1 H, dd, 10.4 and 5.0, 2-H), 5.38 (2 H, m, 6-H and 7-H), 2.40 (2 H, m, 4-H_2), 2.27 (2 H, m, 8-H_2), 2.05 (2 H, m, 5-H_2) and 0.95 (3 H, t, 6.8, 9-H_3); δ_C(250 MHz; $CDCl_3$) 193.84 (C-1), 157.95 (C-3), 133.33 (C-2), 126.79 (C-6 and C-7), 32.76 (C-4), 25.52 (C-5), 20.61 (C-8) and 14.17 (C-9); *m/z* 138 ($M^{\bullet+}$; 8%) and 137 ($M^{\bullet+}$ − 1, 26) (Found: *m/z* 138.10132. $C_9H_{14}O$ requires *m/z* 138.0982).

The ^1H and ^{13}C NMR indicated the presence of several minor impurities. GLC analysis [column: 2% Silar 10C on Chromosorb W (8–100 mesh); oven temperature: 100 °C; nitrogen flow: 40 ml/min] showed these minor components were present in a total of 20%.

Breakdown of Methyl Colneleate **9** *under Acid Conditions*.—Methyl colneleate (2 mg), methanol (2 ml) and hydrochloric acid (2 mol dm^{-3}; 1 drop) were stirred at room temperature (16 h). The reaction mixture was evaporated and the residue was analysed by GLC [column: 2% Silar 10C on Chromosorb W (80–100 mesh); oven temperature: 100 and 130 °C; nitrogen flow: 40 ml/min] and found to be a mixture of non-2(*E*)-enal **20** and methyl 9-oxononanoate **21** identical with synthetically prepared samples. This result was confirmed by GC-MS.

Breakdown of Methyl Colnelenate under Acid Conditions.—A Methyl colnelenate (3 mg), methanol (3 ml) and hydrochloric acid (2 mol dm^{-3}; 2 drops) was stirred at room temperature (18 h). The reaction mixture was evaporated and the residue was analysed by GLC [column: 2% Silar 10C on Chromosorb W (80–100 mesh); oven temperature: 100 and 130 °C; nitrogen flow: 40 ml/min] and found to be a mixture of nona-2(*E*),6(*Z*)-dienal **6** and methyl 9-oxononanoate **21**, identical with synthetically prepared samples.

(±)-*Coronaric* **17** *and* (±)-*Vernolic Acid*.—Linoleic acid **1** (25 mg, 0.089 mmol) and *m*-chloroperbenzoic acid (16.94 mmol, 0.09 mmol, 1 equiv.) were dissolved in chloroform (5 ml) and stirred for 20 min at room temperature. After evaporation of the reaction mixture under diminished pressure, the acids were purified by column chromatography on silica with light petroleum (60–80 °C)–ether–formic acid (80:20:0.1) as eluent. The two mono-epoxides coronaric and vernolic acid were inseparable by HPLC, but after methylation by ethereal diazomethane and normal phase HPLC [light petroleum (60–80 °C)–ether (23:2), 8 mm 10 μ silica RADPAK] (±)-methyl coronate **17** was obtained in pure form, with δ_H(250 MHz, $CDCl_3$), 5.49 (2 H, m, 12-H, 13-H), 3.67 (3 H, s, OCH_3), 2.93 (2 H, m, 9-H, 10-H), 2.20 (6 H, m, 2-H_2, 11-H_2, 14-H_2), 1.33 (16 H, m, 17- to 15-H_2, 3- to 8-H_2) and 0.89 (3 H, t, 18-H_3).

[1-^{14}C]Linoleic acid (50 Ci) was similarly epoxidised and the mixture of [1-^{14}C]labelled coronaric and vernolic acids administered to the colneleic forming enzyme preparation. After methylation and work-up of the products using C_{18}-reversed phase HPLC [elution methanol–water (17:3)], no radio-labelled methyl colneleate could be found.

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