Metal Ion-catalysed Oxidation of Linoleic Acid. Lipoxygenase-like Regioselectivity of Oxygenation

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Summary Haem-proteins and Cu^{II} and Fe^{II} ions catalyse the oxygenation of linoleic acid in aqueous buffer to yield mainly the 13-OOH positional isomers of linoleate hydroperoxide, a regioselectivity that is similar to those of lipoxygenases.

AUTOXIDATION of methyl linoleate (**1**; R = Me) yields a complex mixture of four diene hydroperoxide isomers [(**2**)—(**5**); R = OH] which has recently been determined quantitatively by h.p.l.c. as the corresponding hydroxy derivatives [(**2**)—(**5**); R = H].[1] Equal proportions were formed of the 9-OOH and 13-OOH isomers each of which was present as two geometrical isomers [(**2**) and (**3**) for the 9- and (**4**) and (**5**) for the 13-isomer, R = OH].

$$\overset{c}{\text{Me}[\text{CH}_2]_4\text{CH}}=\text{CH}-\text{CH}_2-\overset{c}{\text{CH}}=\text{CH}[\text{CH}_2]_7\text{CO}_2\text{R}$$
(**1**)

$$\overset{c}{\text{Me}[\text{CH}_2]_4\text{CH}}=\text{CH}-\overset{t}{\text{CH}}=\text{CHCH(OR)}[\text{CH}_2]_7\text{CO}_2\text{Me}$$
(**2**)

$$\overset{t}{\text{Me}[\text{CH}_2]_4\text{CH}}=\text{CH}-\overset{t}{\text{CH}}=\text{CHCH(OR)}[\text{CH}_2]_7\text{CO}_2\text{Me}$$
(**3**)

$$\text{Me}[\text{CH}_2]_4\overset{t}{\text{CH(OR)CH}}=\text{CH}-\overset{c}{\text{CH}}=\text{CH}[\text{CH}_2]_7\text{CO}_2\text{Me}$$
(**4**)

$$\text{Me}[\text{CH}_2]_4\overset{t}{\text{CH(OR)CH}}=\text{CH}-\overset{t}{\text{CH}}=\text{CH}[\text{CH}_2]_7\text{CO}_2\text{Me}$$
(**5**)

c = *cis*, *t* = *trans*

Although the autoxidation of linoleic acid (**1**; R = H) is also non-regioselective (*i.e.*, equal proportions of 9- and 13-isomers are formed), the non-haem iron enzymes, lipoxygenases, catalyse the regioselective and enantioselective oxygenation of linoleic acid to form either the 9(*R*) enantiomer of (**2**; R = OH) or the 13(*S*) enantiomer of (**4**; R = OH) depending on the enzyme.[2] The oxidation of linoleic acid catalysed by metal ions or complexes (*e.g.*, haem-proteins and Cu^{II} and Fe^{II} ions) is also biologically important.[3] However, despite many investigations, the possible selectivity of this reaction has not been studied previously because of the difficulties of analysing the mixture of isomeric products. Using the rapid h.p.l.c. method which separates the four isomers [(**2**)—(**5**); R = H] as distinct components, we have determined the isomeric distribution from these oxidations.

An aqueous emulsion of linoleic acid (300 μl, 2·4 mM) or methyl linoleate was added to oxygen-saturated phosphate buffer (pH 6, 0·1 M, 2·7 ml). Haem-protein solution (30 μl, 6·4 mg ml^{-1}), ferrous or cupric sulphate (30 μl, 10^{-2} M) was then added and oxygen-uptake monitored at 25 °C by an oxygen electrode until 8—10 mole % (based on fatty acid or ester) was consumed. The contents of the cell were added to cold methanol (3 ml) and the hydroperoxides reduced by $NaBH_4$. The proportions of the isomers [(**2**)—(**5**); R = H] were determined by h.p.l.c.[1] after acidification, hexane extraction, and, in the case of the acid, methylation with diazomethane.

The Table shows the percentage compositions of the isomers [(**2**)—(**5**); R = H] obtained from catalysis by haemoglobin, myoglobin, and Fe^{II} and Cu^{II} ions. In the

TABLE

Percentage compositions of the isomers [(**2**)—(**5**); R = H] derived from the oxidation of linoleic acid and methyl linoleate catalysed by haemoglobin (Hb), myoglobin (Mb), and Cu^{II} and Fe^{II} ions.

Catalyst	Substrate	(**2**)	(**3**)	(**4**)	(**5**)	9 : 13 Isomeric ratio
Hb	(**1**; R = H)	27·6	7·9	52·0	12·5	35·5 : 64·5
Mb	"	23·5	5·9	61·8	8·8	29·4 : 70·6
Cu^{II}	"	24·1	9·4	51·8	14·7	33·5 : 66·5
Fe^{II}	"	18·1	13·1	55·5	13·2	31·2 : 68·7
Hb	(**1**; R = Me)	27·3	20·6	31·1	21·0	47·9 : 52·1
Mb	"	29·1	19·9	32·2	18·8	49·0 : 51·0
Cu^{II}	"	36·3	11·5	39·5	12·7	47·8 : 52·2
Fe^{II}	"	33·5	16·3	34·1	16·1	49·8 : 50·2

oxidation of linoleic acid, a strong preference for the 13-isomers was shown and in all cases over 50% of the mixture was the isomer (**4**). The assays for lipoxygenase positional specificity do not usually take into account the geometrical isomerism of the hydroperoxides but merely express the isomeric distribution as the 9 : 13 isomer ratio.[2] It is therefore significant that the 9 : 13 isomeric ratio (*ca.* 30 : 70) obtained from metal ion catalysis is similar to those of some lipoxygenases.[2,4] In striking contrast to the oxidation of linoleic acid, the oxidation of methyl linoleate (see Table) was non-selective and yielded a ratio of close to 1 : 1 in all cases.

The ratio of enantiomers of the 9- and 13-isomers formed from the metal ion-catalysed oxidation of linoleic acid was examined by the established procedure[5] of (i) forming the (−)-menthoxycarbonyl derivative of the mixture of isomers [(**2**)—(**5**); R = H], (ii) oxidative ozonolysis, (iii) methylation with CH_2N_2, and (iv) g.l.c. separation of the resultant diastereomeric (−)-menthoxycarbonyl derivatives of methyl 2-(*RS*)-hydroxyheptanoate (from the 13-isomers) and of dimethyl 2-(*RS*)-hydroxydecanedioate (from the 9-isomers). That the reaction was not enantioselective was shown by the *R* : *S* ratios of the 9- and 13-isomers which were 1 : 1·00 ± 0·02 in all cases.

The results reported here are an example of the regioselectivity in the non-enzymic, non-specific catalysis in the oxidation of a molecule that is biologically important. Since the conditions employed were similar to those used for enzymic oxidations it is a significant indication that positional selectivity is no longer a sufficient criterion of lipoxygenase activity as is often assumed; enantioselectivity appears to be the only valid criterion. The lack of regioselectivity in the metal ion-catalysed oxidation of methyl linoleate as compared with that of linoleic acid is likely to be a result of the different conformational preferences of these molecules in aqueous media. The significance of this will be discussed elsewhere.

(*Received, 9th November* 1977; *Com.* 1160.)

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