Identification of Decomposition Products from Autoxidation of Methyl 4,7,10,13,16,19-Docosahexaenoate

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ABSTRACT

This is a continuation of a previous publication in which a number of aldehydes, aldehyde esters, hydrocarbons, and methyl esters was identified among the oxidation products of methyl docosahexaenoate. In the present paper, the identification of certain keto-esters, unsaturated ketones, cyclic esters, and furan derivatives also is reported. Mechanisms for the formation of these compounds are proposed.

INTRODUCTION

We have been investigating the autoxidation of highly unsaturated fatty acids by studying the volatile decomposition products of methyl docosahexaenoate after oxidation at 50 C for 28 hr. In a previous publication (1), we reported on the identification of a number of aldehydes, aldehyde esters, hydrocarbons, and methyl esters. It was proposed that these compounds are formed by cleavage of the 10 possible conjugated hydroperoxides. In the present paper, the identification of several other compounds, believed to form by mechanisms other than simple hydroperoxide scission, is reported.

EXPERIMENTAL PROCEDURE

Methyl 4,7,10,13,16,19-docosahexaenoate was purchased from Hormel Institute, Austin, Minn., and further purified by stripping under vacuum. The techniques used for the oxidation treatment and the analysis of the oxidation products were exactly the same as described previously (1).

RESULTS

Keto-Esters

Four keto-esters were identified: methyl 4-oxohexanoate, methyl 4-oxohexenoate, methyl 5-oxohexanoate, and methyl 8-oxononadienoate. The retention time of methyl 4-oxohexanoate on SE-30 silicone column coincides exactly with that of methyl 5-oxohexanoate; on the Carbowax column, however, it slightly precedes the Δ isomer. The mass spectrum (Fig. 1A) is similar to that published by Kawada, et al. (2). The base ion at m/e 57 is produced by cleavage α to the keto group at the 3,4 bond. α-Cleavage on the other side of the keto group at the 4,5 bond produces the intense M-29 ion. As in the C4:0 aldehyde ester (3), the fourth carbon is carbonyl-substituted, and only transfer of hydrogen atoms from the third or fifth carbons may produce the very small ion at m/e 74. However, the m/e 87 and M-31 fragments are typical of the ester.

The gas liquid chromatography (GLC) retention time and the mass spectrum of methyl 5-oxohexanoate were identical to those of the authentic compound. The base peak at m/e 43 (Fig. 1B) may result from cleavage of the 4, 5 bond (α to the keto group) and represents the $CH_3{-}C{\equiv}\overset{+}{O}$ fragment typical of methyl ketones (4). The fragment at m/e 43 also arises from the ions at m/e 112 and m/e 113, as indicated by the metastable peaks at m/e 16.36 $(113^+ \rightarrow 43^+ + 70)$ and at m/e 16.51 $(112^+ \rightarrow 43^+ + 69)$. The ions at m/e 112 and m/e 113 correspond to the loss of methanol and a methoxy group, respectively, from the molecular ion. A significant ion is observed at m/e 101 which corresponds to M-43 and also to cleavage γ to the methyl ester carbonyl. The ions at m/e 59, 74, and 87, characteristic of the methyl ester, are prominent. The metastable peak at m/e 63.94 indicates the loss of 28 mass units from the M-31 ion $(113^+ \rightarrow 85^+ + 28)$. The ion at m/e 84 is probably similarly produced from the M-32 ion indicated by the metastable peak at m/e 63.00 $(112^+ \rightarrow 84^+ + 28)$.

Methyl 4-oxo-5-hexenoate has a longer retention time on Carbowax columns than the oxohexanoates; on an SE-30 column, however, it elutes earlier. With the exception of the three fragments: M-27, M-28, and M-29, the mass spectrum (Fig. 1C) has a pattern very similar to the methyl 4-oxohexanoate discussed above. The m/e 74 ion is extremely small, resembling the ion intensity noted in the methyl 4-oxohexanoate and the C4:0 aldehyde ester, suggesting that the keto group is in the fourth position. Furthermore, if the keto group were on the third carbon, intense ions at m/e 58 and M-58 would be expected (5). If the carbonyl group were in the fifth position, the m/e 74 ion would be intense, regardless of the double bond position, for, in the 2- and 3-hexenoates, the formation of an ion at m/e 74 is not supressed (6). No peak is observed at M-43 which would be expected from cleavage α to the carbonyl group of a 5-oxoester. The assumption that the double bond is in the terminal positon is consistent with the loss of 27 mass units. The fragments M-28 and M-27 would be formed by elimination of ethylene or the $CH_2{=}CH{-}$ moiety, respectively. The base peak at m/e 55 is produced by the 3,4 bond cleavage α to the keto group. A metastable ion is observed at m/e 62.06 for the expulsion of 28 mass units from the acylium ion at M-31 $(111^+ \rightarrow 83^+ + 28)$. The ion at m/e 72 could be formed by a 2,3 bond cleavage with hydrogen rearrangement to the ketone fragment and charge retention on the ester fragment. The ion at m/e 74 produced by this cleavage is supressed as mentioned earlier.

The mass spectrum of methyl 8-oxononadienoate (Fig. 1D) shows fragments at M, M-31, M-32, M-74, and M-87 and at m/e 59 and 74, as typical of a methyl ester. The intense ion at m/e 43 and the small peak at M-15 suggest that the keto group is located on the eighth carbon. Cleavage of the 7,8 bond would produce the m/e 43 ion, characteristic of methyl ketones. The small ions at M-42 and M-43 are produced by the same cleavage, with and without hydrogen rearrangement, with the charge retained on the ester fragment. The base ion at m/e 95 is most likely produced by cleavage γ to the ester carbonyl. A metastable peak at m/e 64.08 corresponds to the production of the intense ion at M-74 by the 2,3 bond cleavage with hydrogen rearrangement $(182^+ \rightarrow 108^+ + 74)$. The strong ion at m/e 109 corresponds to the same 2,3 bond cleavage with no hydrogen rearrangement. The IR spectrum was consistent with this interpretation. Absorption was observed at 1740 cm^{-1} and 1180 cm^{-1} as typical of esters and in the 1680-1640 cm^{-1} region suggesting a conjugated ketone.

Cyclic Esters

On polar columns, aromatic esters elute much later than

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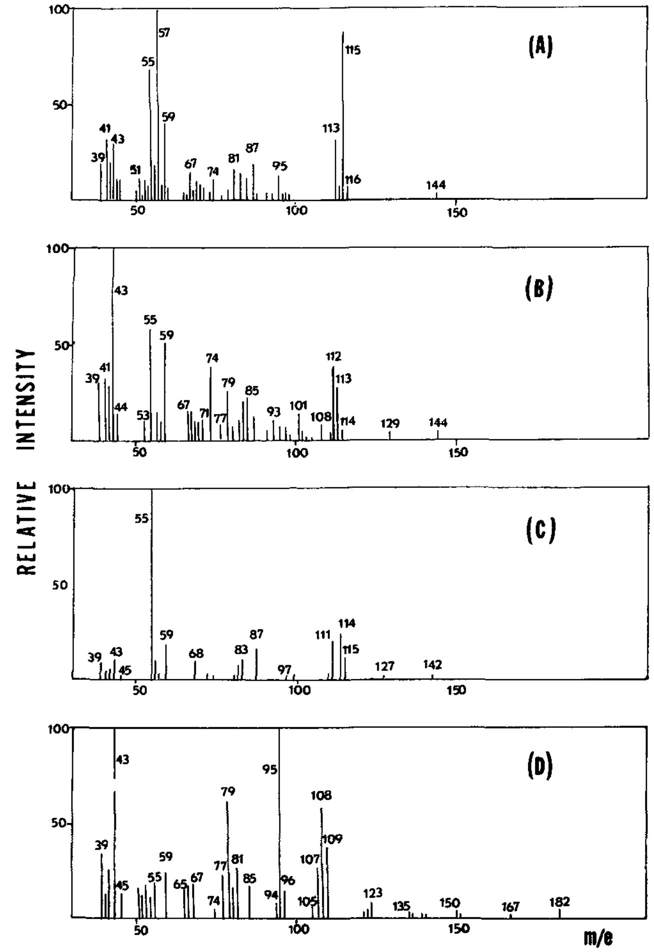
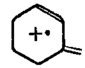


FIG. 1. Mass spectra of components identified as keto-esters. (A) Methyl 4-oxohexanoate; (B) methyl 5-oxohexanoate; (C) methyl 4-oxohexenoate; and (D) methyl 8-oxononadienoate.

the straight chain methyl esters of the same carbon number; on OV-1 however, the aromatic esters have a retention time just slightly longer than the retention time of straight chain methyl esters of equivalent carbon number (7). Two members of this class were identified: methyl 2-cyclohexenyl acetate and methyl 5-cyclohexenyl pentenoate.

As typical of cyclic and aromatic compounds, the molecular ion in the mass spectrum of methyl 2-cyclohexenylacetate (Fig. 2A) is more intense than the straight chain C9:2 ester discussed earlier (1). The characteristic methyl ester fragment M-31 is present, but the ions at m/e 59 and 74 are very small due to the greater ability of the ring structure to distribute the positive charge. The base peak at m/e 81 is produced at the expense of the ion at m/e 74 by cleavage α to the ring. The fragment at m/e 53 arises from the ion at m/e 81 as indicated by a metastable peak at m/e 34.8 ($81^+ \rightarrow 53^+ + 28$). The very intense ion at m/e 94 is formed by cleavage β to the ring with hydrogen rearrangement to the carbomethoxy fragment with charge retention on the ring: . The ion at m/e 95 would arise from the same cleavage without hydrogen rearrangement.

The component identified as methyl 5-cyclohexenylpentenoate showed GLC retention times and mass spectral characteristics similar to the C8:1 cyclic methyl ester in relation to that of the straight methyl ester of the same mol wt. Thus, the retention time of this compound is greater on Carbowax but shorter on silicone than that of the C12:3 methyl ester. In the mass spectrum (Fig. 2B), the fragments M-31 and M-32 are present, while the ions at m/e 59, 74, and 87 are very small. The base peak at m/e 120 may be produced via a McLafferty rearrangement with the charge retention on the ring moiety. Again, the low intensity of the ion at m/e 74 is due to the location of the charge on the ring, rather than the ester fragment. This is confirmed by the presence of a metastable ion at m/e 74.23 corresponding to $194^+ \rightarrow 120^+ + 74$. A metastable ion at m/e 69.01, corresponding to $120^+ \rightarrow 91^+ + 29$, also was observed.

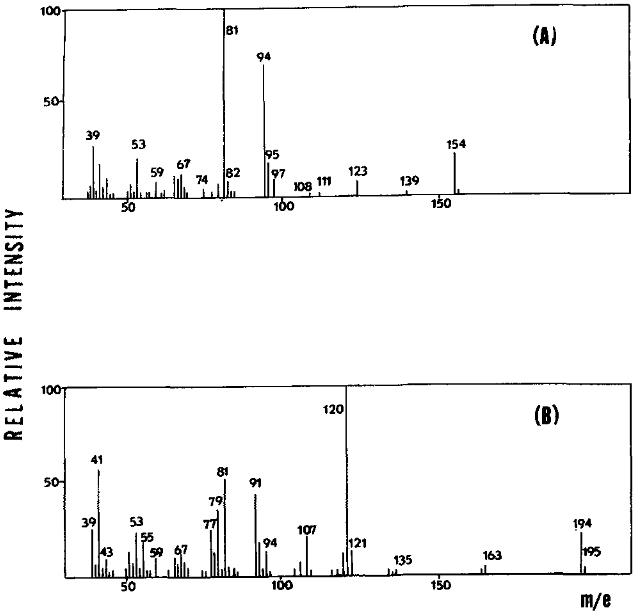


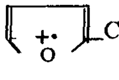
FIG. 2. Mass spectra of components identified as cyclic esters. (A) Methyl 2-cyclohexenyl acetate and (B) methyl 5-cyclohexenyl pentenoate.

Unsaturated Methyl Ketones

The component identified as 3,5-octadien-2-one gave a mass spectrum (Fig. 3B) identical to that published by Yamanishi, et al. (8) for the authentic compound. The major fragments are also in accord with those reported by Bondarovich, et al. (9). A similar pattern is shown for the compound identified as undecatrien-2-one (Fig. 3C).

Furans

Among the furan derivatives identified in this work were 2-pentylfuran, 2-butylfuran, and 2-pentenylfuran. The mass spectrum of 2-pentylfuran and its retention times on Carbowax and silicone columns were identical with those of an authentic sample synthesized in this laboratory. The mass spectrum of 2-pentylfuran also has been reported by Bondarovich, et al., (9) Heyns, et al., (10) and Chang, et al. (11).

The compound identified as 2-pentenylfuran is eluted immediately after the saturated compound on both Carbowax and SE-30 columns. The mass spectrum (Fig. 3A) is very similar to that reported by Heyns, et al., (10) for alkenyl furans. The molecular ion and fragments at M-28 and M-29 are of equal intensity. Cleavage Δ to the ring with the loss of 29 mass units yields the ion at m/e 107. This structure, if conjugated, undergoes the loss of CO to contribute to the intense ion at m/e 79. A metastable peak at m/e 58.1 confirming the transition $107^+ \rightarrow 79^+ + 28$ was observed in this spectrum and also was reported by Heyns, et al., for 2-propenylfuran. The ion at m/e 79 further loses two hydrogen atoms to form the fragment at m/e 77, as indicated by a metastable peak at m/e 75.1 ($79^+ \rightarrow 77^+ + 2$). A metastable peak at m/e 34.7 corresponds to the expulsion of CO from the ion at m/e 81 ($81^+ \rightarrow 53^+ + 28$). Cleavage α to the ring with hydrogen rearrangement produces the base peak at m/e 68. The peak at m/e 94 corresponds to the ion  $CH{=}CH_2$ which may be

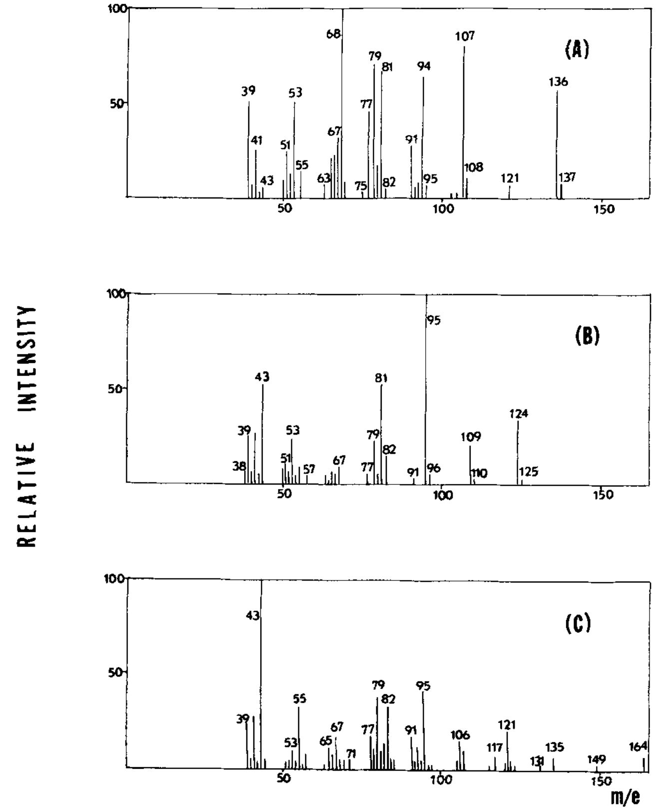


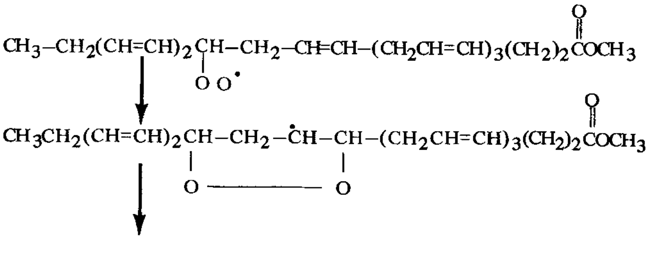
FIG. 3. Mass spectra of components identified as: (A) pentenylfuran; (B) 3,5-octadien-2-one; and (C) undecatrien-2-one.

formed by cleavage γ to the ring with hydrogen rearrangement. A metastable ion at m/e 46.3 indicates the loss of CO from the fragment at m/e 94 ($94^+ \rightarrow 66^+ + 28$). The ion at m/e 65 is either produced by loss of CHO from the ion at m/e 94 or loss of H from the ion at m/e 66 (10). The double bond appears to be located α,β to the ring on the basis of the conjugation requirement in the fragment at m/e 107 for elimination of CO, the production of the ion at m/e 94, and decreased intensity of the m/e 81 ion. However, hydrogen rearrangements, prevalent in alkenes, which result in the migration of radical sites along the chain, may occur here in the molecular ion (4). Thus, the site of unsaturation cannot be conclusively pinpointed.

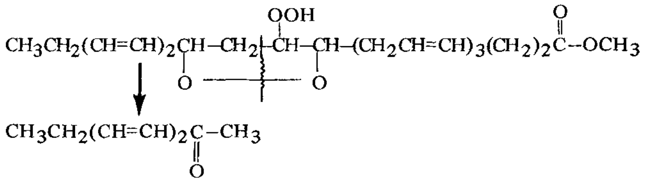
The mass spectrum of the compound identified as 2-butylfuran was identical to that published by Kawase, et al. (12), and similar fragmentation patterns led to tentative identification of some other furan derivatives, i.e. 2-ethylfuran, heptenylfuran, octenylfuran, and octadienylfuran.

DISCUSSION

A mechanism for the formation of the unsaturated methyl ketones may be via scission of cyclic dihydroperoxides similar to those identified by Begemann, et al., (13) from autoxidized methyl linolenate. Thus, 3,5 octadienone can be formed from the 16-peroxy ester as follows:



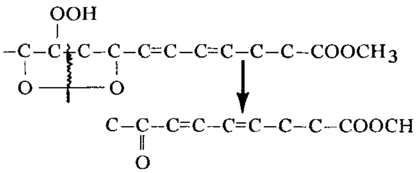
$$CH_3{-}CH_2(CH{=}CH)_2\underset{OO^\bullet}{CH}{-}CH_2{-}CH{=}CH{-}(CH_2CH{=}CH)_3(CH_2)_2\overset{O}{\overset{\|}{C}}OCH_3$$
$$\downarrow$$
$$CH_3CH_2(CH{=}CH)_2\underset{O}{CH}{-}CH_2{-}\dot{C}H{-}\underset{O}{CH}{-}(CH_2CH{=}CH)_3(CH_2)_2\overset{O}{\overset{\|}{C}}OCH_3$$
$$\downarrow$$



$$CH_3CH_2(CH{=}CH)_2CH{-}CH_2{-}\underset{O}{CH}{-}\overset{OOH}{CH}{-}\underset{O}{CH}(CH_2CH{=}CH)_3(CH_2)_2\overset{O}{\overset{\|}{C}}{-}OCH_3$$
$$\downarrow$$
$$CH_3CH_2(CH{=}CH)_2\underset{O}{\underset{\|}{C}}{-}CH_3$$

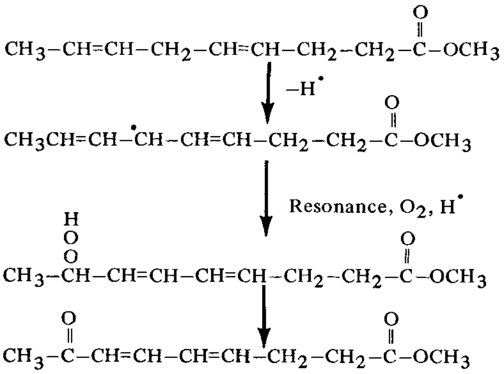
Undecatrienone would similarly be produced from the 13-peroxy ester.

The same mechanism also may explain the production of keto-esters. For example, 8-oxononadienoate can be formed as follows:



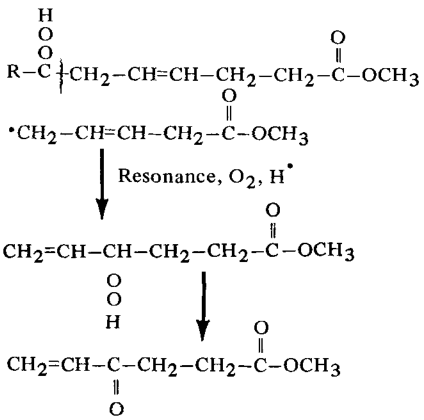
$$\overset{OOH}{-C{-}C}{-}\underset{O\ \ \ \ O}{C{-}C}{-}C{=}C{-}C{=}C{-}C{-}C{-}COOCH_3$$
$$\downarrow$$
$$C{-}\underset{O}{\underset{\|}{C}}{-}C{=}C{-}C{=}C{-}C{-}C{-}COOCH_3$$

Keto-esters, however, can be produced by several alternative mechanisms. The compound 8-oxononadienoate may result from further oxidation of methyl nonadienoate:



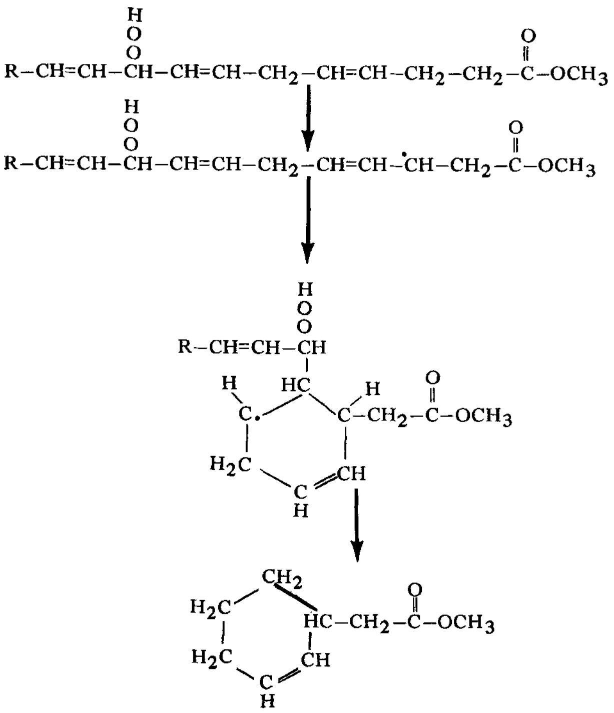
$$CH_3{-}CH{=}CH{-}CH_2{-}CH{=}CH{-}CH_2{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}OCH_3$$
$$\downarrow -H^\bullet$$
$$CH_3CH{=}CH{-}\dot{C}H{-}CH{=}CH{-}CH_2{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}OCH_3$$
$$\downarrow \text{Resonance, } O_2,\ H^\bullet$$
$$CH_3{-}\overset{\overset{H}{\overset{O}{O}}}{CH}{-}CH{=}CH{-}CH{=}CH{-}CH_2{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}OCH_3$$
$$\downarrow$$
$$CH_3{-}\overset{O}{\overset{\|}{C}}{-}CH{=}CH{-}CH{=}CH{-}CH_2{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}OCH_3$$

and methyl 4-oxohexenoate from the 7-hydroperoxy ester:



$$R{-}\overset{\overset{H}{\overset{O}{O}}}{C}{-}CH_2{-}CH{=}CH{-}CH_2{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}OCH_3$$
$$^\bullet CH_2{-}CH{=}CH{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}OCH_3$$
$$\downarrow \text{Resonance, } O_2,\ H^\bullet$$
$$CH_2{=}CH{-}\underset{\underset{H}{\underset{O}{O}}}{CH}{-}CH_2{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}OCH_3$$
$$\downarrow$$
$$CH_2{=}CH{-}\underset{O}{\underset{\|}{C}}{-}CH_2{-}CH_2{-}\overset{O}{\overset{\|}{C}}{-}OCH_3$$

The cyclic esters probably are produced by intramolecular addition of a free radical to a double bond prompting the cyclization to form the six-membered ring. Thus, methyl 2-cyclohexenyl acetate would arise from the 9-hydroperoxy ester, as shown below. The mechanisms would involve abstraction of hydrogen from position 3 and addition of the resulting radical to the 7,8 double bond. The cyclic ester then is formed by cleavage α to the hydroperoxide group and hydrogen abstraction:



By a similar mechanism, the compound 5-cyclohexenylpentenoate can be produced from the 12-hydroperoxy ester. Similar mechanisms, but not involving hydroperoxide formation, were reported by Hutchison and Alexander (14) and Michael (15) for the production of cyclic compounds from linolenates. It should be pointed out that the mechanisms proposed by these workers involve, as in our case, an allylic proton abstraction outside the 1,4-pentadiene system.

The furan compounds identified in this study may have been formed via dehydration of the dienolic form of γ dialdehydes or γ diketones as postulated by Chang and coworkers (11) for the formation of 2-pentylfuran.

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