Carbonyls in Oxidizing Fat. IX. Aldehydes Isolated from Autoxidized Methyl Arachidonate

SUMMARY

The monocarbonyl compounds developed by oxidation of methyl arachidonate under mild conditions were measured by three methods of isolation. Aldehydes from scission of hydroperoxides of the pentadiene system furthest removed from the carboxyl group accounted for 80% of the total, and the same ones are characteristic of oxidized linoleate. The C_6 alkanal was the major aldehyde and C_7 alkanal, a compound not readily accountable for by conventional mechanisms of autoxidation, was second in quantity. The mild quantitatively equal Girard T and Schwartz methods broke down some precursors, but in a qualitatively different manner. The Pool and Klose alumina chromatographic method decomposed precursors, but not to the extent that has been observed in autoxidized lard. Two unsaturated aldehydes isolated by this method may be alk-2,4,6-trienals.

INTRODUCTION

Arachidonic acid is present in animal depot fats and tissue phospholipids. Hornstein *et al.* (1961) found 0.1% in the triglycerides of beef and pork and 19.2% and 16.3%, respectively, in their phospholipids. The oxidation of this highly susceptible fatty acid component of lipids apparently has not been extensively studied. According to Badings (1960) the methyl ester oxidizes at a rate approaching twice that of methyl linolenate.

Little appears to have been done on determination of the carbonyl compounds formed in the autoxidation of arachidonic acid and its esters. Sulzbacher *et al.* (1963) reported examination of the monocarbonyls obtained by steam distillation at 100°C of an oxidized sample of methyl arachidonate and found that the aldehydes were qualitatively rather similar to those observed from methyl linoleate by Ellis *et al.* (1961). Notable exceptions were the C_7 alkanal, C_{11} enal, and C_{11}, C_{12} dienals. This work was undertaken to determine the quantitative relationships between the monocarbonyl compounds formed by methyl arachidonate. Since recent investigations concerned with the isolation of free aldehydes have shown a sensitivity of precursors to method of isolation, with resultant quantitative and sometimes qualitative effects (Gaddis *et al.,* 1965), several different methods of isolation were used: the Girard T method of Gaddis *et al.* (1964), the Schwartz procedure (Schwartz *et al.,* 1963), and the Pool and Klose method (Keith and Day, 1963). An isolation of volatile aldehydes would also have been desirable, but it was feared that results would be complicated by the short path of the Lea method (Lea and Swoboda, 1962), which might result in some collection of primary oxidation products. The triglyceride of the fatty acid, which would have been suitable, was not available.

EXPERIMENTAL

Solvents, reagents, materials, and methods. Solvents were purified as described in previous reports (Gaddis *et al.,* 1964, 1965). The methyl arachidonate (lot 5M) used, obtained from the Hormel Foundation, Austin, Minnesota, had an estimated purity of higher than 90%. One gram of methyl arachidonate was oxidized for 24 hr at 23°C under artificial light, by which time it had a peroxide value of 550 meq/1000 g. Samples varying from 0.12 to 0.19 g were weighed out exactly, and duplicate isolations of carbonyl compounds were made simultaneously by the Girard T method, the Schwartz method, and the Pool and Klose method. Monocarbonyl derivatives, isolated by the alumina reaction method of Pool and Klose, were separated from the fat by the Seasorb method of Schwartz *et al.* (1963). Carbonyl compounds isolated by the Girard T and Schwartz methods were fractionated to simple monocarbonyl compounds by chromatography on alumina. Before paper chromatography, all monocarbonyl fractions were further purified by the ion-exchange method of Schwartz *et al.* (1962). Isolated monocarbonyl derivatives were measured by paper chromatographic separations into classes and individual compounds by previously reported methods and applications (Ellis, Gaddis, and Currie, 1958; Gaddis and Ellis, 1959; Ellis and Gaddis, 1959; Gaddis *et al.,*

1961). The $\lambda_{max.}$ of the classes and values used for conversion to μm are described by Gaddis *et al.* (1964). Corrections were applied to the classes isolated by the Pool and Klose method. All results were calculated on the basis of 10.00 g of methyl arachidonate. Absorbance values represent the amount of 2,4-dinitrophenylhydrazones in 100 ml of carbon tetrachloride. Suitable corrections were made for blanks.

RESULTS AND DISCUSSION

Differences between mild methods of isolation. The Girard T and Schwartz methods give quantitative reaction with aldehydes (Gaddis *et al.*, 1964; Schwartz *et al.*, 1963; Gaddis *et al.*, 1965). Table 1 shows that the Girard T and Schwartz monocarbonyl compounds had the same total absorbance values. However, the class compositions were considerably different. In total μm, the Girard T hydrazones were 93.5% of the Schwartz hydrazones. The Schwartz aldehydes had higher amounts of alkanal and enal classes, and only a trace of the dienal class. On the other hand, the Girard T hydrazones had nearly as much alk-2,4-dienal as alk-2-enal class. In lard oxidized at 23°C, higher Schwartz alkanal and enal class isolation than the Girard T values has been found characteristic (Gaddis *et al.*, 1965). This has been assumed to indicate some precursor breakdown by the Schwartz reaction conditions. Dienal values for the two methods were similar for autoxidized lard. In the case of the oxidized arachidonate, it would appear that the Girard T method contributed to a dienal precursor breakdown. The availability of an appreciable amount of dienal precursors may be an abnormal condition (Gaddis *et al.*, in ms.). Comparison of these results with a vacuum distillation technique would have been helpful in interpretation, but this could not be used, for reasons already stated.

The proportions of saturated aldehydes were similar, but the enal aldehydes differed because of higher proportions of the C_8 and

Table 1. Comparison of monocarbonyl isolation methods.

	Girard T			Schwartz			Pool and Klose		
	Amounts	% each class	% total	Amounts	% each class	% total	Amounts	% each class	% total
Total absorbance values	19.59			19.60			53.84		
Class absorbance values									
Alkanal	14.13		72.1	16.67		85.0	34.73		64.5
Enal	2.51		12.8	2.93		15.0	5.55		10.3
Dienal	2.95		15.1	trace			7.86		14.6
Unknown	....		....	....		....	5.70		10.6
μm/10 g									
Alkanal	68.94		80.2	81.32		88.5	169.42		80.5
Enal	9.09		10.6	10.62		11.5	20.11		9.5
Dienal	7.91		9.2	trace			21.06		10.0
Unknown	....		....	....		....	?		
Total	85.94			91.94			210.58		
Individual aldehydes									
Alkanal C_2	2.62	3.8	3.0	2.93	3.6	3.2	4.47	2.6	2.1
C_6	57.45	83.3	66.9	68.49	84.2	74.5	131.30	77.5	62.4
C_7	8.87	12.9	10.3	9.90	12.2	10.8	33.65	19.9	16.0
Enal C_7	1.70	18.8	2.0	1.88	17.7	2.0	3.97	19.8	1.9
C_8	3.87	42.5	4.5	5.17	48.7	5.6	5.96	29.6	2.8
C_9	1.93	21.2	2.3	3.57	33.6	3.9	7.08	35.2	3.3
C_{11}	1.59	17.5	1.8	trace			3.10	15.4	1.5
Dienal C_9	1.18	14.9	1.4				trace		
C_{10}	5.27	66.7	6.1				13.87	65.9	6.6
C_{11}	0.91	11.5	1.1				4.09	19.4	1.9
C_{12}	0.55	6.9	0.6				3.10	14.7	1.5

C_9 enals and negligible amounts of C_{11} enal in the Schwartz method. The major alkanals and enals isolated by both methods were the C_6 and C_7 alkanals and C_8 enal. The C_{10} dienal in the Girard T method exceeded the C_8 enal.

Aldehydes isolated by the Pool and Klose method. Aldehydes extracted by the hydrated alumina method (Keith and Day, 1963) exceeded the mild methods considerably, although not nearly to the extent observed in oxidized lard (Gaddis *et al.,* in ms.). This was probably due to a low proportion of monomeric hydroperoxides in the oxidized arachidonate. Early work on method comparison by Gaddis *et al.* (1960) showed that the monocarbonyl compounds isolated by the Pool and Klose method (Pool and Klose, 1951) were higher than those isolated by the Lea mild vacuum distillation method (Lea and Swoboda, 1958). Lea and Jackson (1964) have also demonstrated that Keith and Day (1963) modification is invalid for free aldehydes due to a partial conversion of hydroperoxides to monocarbonyl compounds.

There was an unknown class present of about the same magnitude as the enal class. This had properties similar to those of unknown classes isolated by the method from oxidized lard (Gaddis *et al.,* in ms.). The maximum of the unknown class in carbon tetrachloride was 390–95 mμ, and in alkali there was no secondary maximum or fading in the course of 30 min. Such properties suggest an unsaturated aldehyde with a conjugated triene system, an alk-2,4,6-trienal. The class composition of the known aldehydes was similar to that of the Girard T aldehydes, except that dienals exceeded the enals.

The proportions of individual aldehydes differed from the two "mild" methods. In the alkanal class the C_7 compound was higher and C_6 aldehyde lower, and in the enals the C_9 aldehyde was the major compound. There was little difference between the Schwartz and Pool and Klose amounts of the C_8 enal.

The unknown unsaturated class was separated by paper chromatography into two compounds which retained the maximum in carbon tetrachloride at 390–95 mμ. These compounds were related quantitatively in the ratio of 1:3. The major compound had an R_F value close to that of a C_{11} dienal, and the minor compound that of a C_{12} dienal. The compounds are probably of longer carbon chain than these dienals, since mobility increases with the number of conjugated double bonds (Gaddis and Ellis, 1959).

Parent primary oxidation products. The polyunsaturated unconjugated fatty acids encountered in meat animal lipids are composed of pentadiene units, each with a methylene group between two double bonds. Farmer's theory of autoxidation (Frankel, 1962) favors initial attack at the methylene group and formation of a resonance hybrid of three valence bond structures. Since the products would be resonance-stabilized by a conjugated diene system, preferential reaction with oxygen at the end positions of the pentadiene system might be expected. Such a mechanism is indicated by reports that 90% of linoleate hydroperoxides are conjugated (Privett *et al.,* 1953). Frankel *et al.* (1961) found only 4 isomeric linolenate hydroperoxides of a possible 6 isomers. These were products of end-group attacks in the two pentadiene systems. The structure of methyl arachidonate is shown in Table 2 with the sensitive methylene groups labeled *a, b,* and *c*. The monohydroperoxides theoretically possible, a total of nine, are shown with their aldehyde decomposition products. If the oxygen attack is mainly at the end carbons of the pentadiene systems, the major hydroperoxides present would be the 15, 11, 12, 8, 9, and 5. Referring back to Table 1, it will be noted that hydroperoxide products of the *a* methylene group pentadiene system were the sources of the major known aldehydes measured. These scission products were 77.5% in the Girard T isolates, 80.1% in the Schwartz, and 71.8% in the Pool and Klose aldehydes of the total aldehydes. It should also be observed that the hydroperoxide the greatest distance from the carboxyl group furnished the major amount of aldehyde. Similar results have been observed in the case of autoxidized linoleate and linolenate (Gaddis *et al.,* 1961). It is not certain whether this is due to the formation of

Table 2. Hydroperoxides and aldehydes possible in the autoxidation of methyl arachidonate.

Methyl arachidonate	Methylene group	Isomeric hydroperoxides	Main aldehydes expected
$CH_3-(CH_2)_4-\underset{15}{CH}=CH-\overset{a}{\underset{13}{CH_2}}-\underset{12}{CH}=$	a	15-hydroperoxy $\Delta^{5,8,11,13}$	C_6-al
		13-hydroperoxy $\Delta^{5,8,11,14}$	C_8-Δ^2-enal
		11-hydroperoxy $\Delta^{5,8,12,14}$	C_{10}-$\Delta^{2,4}$-dienal
$\underset{11}{CH}-\overset{b}{\underset{10}{CH_2}}-\underset{9}{CH}=\underset{8}{CH}-\overset{c}{\underset{7}{CH_2}}-CH=$		12-hydroperoxy $\Delta^{5,8,10,14}$	C_9-Δ^3-enal
$CH-(CH_2)_3-COOCH_3$	b	10-hydroperoxy $\Delta^{5,8,11,14}$	C_{11}-$\Delta^{2,5}$-dienal
		8-hydroperoxy $\Delta^{5,9,11,14}$	C_{13}-$\Delta^{2,4,7}$-trienal
		9-hydroperoxy $\Delta^{5,7,11,14}$	C_{12}-$\Delta^{3,6}$-dienal
	c	7-hydroperoxy $\Delta^{5,8,11,14}$	C_{14}-$\Delta^{2,5,8}$-trienal
		5-hydroperoxy $\Delta^{6,8,11,14}$	C_{16}-$\Delta^{2,4,7,10}$-tetraenal

larger amounts of such hydroperoxides, or to differences in stability. The observation of Frankel *et al.* (1961) that the 15-hydroperoxy $\Delta^{9,12,14}$ linolenate was 48% of the total hydroperoxides isolated may be extremely significant. Evidence concerned with autoxidized lard (Gaddis *et al.*, in ms.) suggested that the 13-hydroperoxy $\Delta^{9,11}$ linoleate is the major primary oxidation product of linoleate, but that conditions, such as heat, produced its isomer, the 9-hydroperoxy $\Delta^{10,12}$ compound. The aldehydes possible from *b* and *c* methylene systems are difficult to relate to the remaining aldehydes because they may contain unconjugated double bonds which, as indicated by Badings (1960), cannot be detected by the methods used in this work. However, some of these might have undergone shifts to conjugated positions. The predicted C_9 3-enal could have shifted to form the C_9 2-enal found; the C_{11} 2,4-dienal might have been measured as a C_{11} enal or shifted to give the C_{11} 2,4-dienal found. The predicted C_{12} 3,6-dienal may have shifted to give the C_{12} 2,4-dienal determined. The two unknown compounds with properties similar to trienals separated by the Pool and Klose method could have come from the two trienals, or the tetraenal predicted by theory. This leaves unexplained the C_2, C_7 alkanal, C_7 enal, and C_9 2,4-dienal compounds measured. These, except for the C_7 alkanal, were present in very minor quantities and could be explained by the numerous side reactions possible. The C_7 alkanal was the second largest fraction and, in comparison with aldehydes developed by autoxidized oleate, linoleate, and linolenate (Gaddis *et al.*, 1961), is apparently quite characteristic of arachidonate. It increased more proportionally than any other compound in the hydroperoxide-destructive Pool and Klose method (Gaddis *et al.*, in ms.). This would appear to suggest that it comes from a primary oxidation product, but there is no assurance that its source is arachidonate since a highly unsaturated impurity might be present.

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