Carbonyls in Oxidizing Fat: XII.

The Isolation of Free Aldehydes From Autoxidized Triolein, Trilinolein and Trilinolenin

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Abstract

Methods of aldehyde isolation have been compared in the separation of free aldehydes from autoxidizing triglycerides of pure unsaturated fatty acids. Vacuum distillation was the mildest method of aldehyde isolation. Amounts of decomposed monocarbonyl precursors were directly related to acidity of extraction conditions. Analysis of autoxidizing trilinolenin showed, after attainment of an early maximum, a decrease in monocarbonyl values from vacuum distillation and Girard T reagent methods. Known aldehydes isolated by the acid celite methods increased progressively. These results were considered to be due to a decline in monomeric hydroperoxides as related to free aldehydes, and decomposition of not only monomeric peroxides but also polymers by the acid celite methods. Amounts of decomposed monocarbonyl precursors (broken down) were directly related to acidity of extraction conditions. Analysis of autoxidizing trilinolenin showed a decrease in monocarbonyl values from vacuum distillation and Girard T reagent values and an increase in these values with (those of) the acid celite reaction methods. This was considered to be due to a decline in monomeric hydroperoxides and decomposition of polymers by the acid celite methods. Studies with autoxidizing lard demonstrated several factors having a strong influence on the amount of aldehydes isolated by the phosphoric acid celite reaction method. Passage through the column decreased hydroperoxide values 10–15%. Amount of monocarbonyl compounds was directly related to the time of exposure on the column. The yield of aldehydes decreased with increase in the amount of oxidized material applied to the 2,4-dinitrophenylhydrazine reaction column.

Introduction

VARIOUS METHODS (1,13,14,19,21,25) have been described for the isolation of aldehydes from autoxidized fat. Most of these methods are comparable in efficiency of reaction with monocarbonyls from a quantitative standpoint, and each type possesses certain definite advantages. The celite column reaction methods (1,25) permit recovery of aldehydes as 2,4-dinitrophenylhydrazones from a solution of fat in nonpolar solvents. Use of the Girard T reagent (13) enables extraction of aldehydes under polar, aqueous, and slightly acid conditions. Short path vacuum distillation procedures (11,14,21) permit physical removal of volatile aldehydes without exposure of the labile hydroperoxides and other complex oxidation products to direct action of reagent, solvents, or the acid conditions usually necessary for quantitative formation of hydrazone derivatives.

The need for determination of free aldehydes has been discussed to some length in earlier publications (11,14,15,16). Determination of free aldehydes requires conditions involving isolation with minimum peroxide breakdown. The mildest methods, such as the Schwartz celite method (25), a Girard T method developed by Gaddis, Ellis, and Currie (13), and the Lea vacuum distillation method (21), have been compared and considerable differences found in the aldehydes isolated (14–16). The Girard T method (13), based purely on studies with autoxidized lard (14,16) seemed to be the best method because of lower amounts of isolated aldehydes. However, the method may be suspected because of a tendency toward removal of larger quantities of unsaturated compounds (14,16). These consist mainly of the enal class which seem to be to some extent abnormal hydroperoxide breakdown products. Also, the Girard T (14) has been repeatedly observed to extract additional quantities of aldehydes from freshly vacuum distilled fat. It appears that the vacuum distillation method may be the preferable one. The vacuum distillation procedure causes some hydroperoxide breakdown (21,22), but this is not great, and the decomposition taking place is amenable to control by shortening the time of distillation to a minimum, lowering the temperature, and use of hydroperoxide stabilizers (22).

Curious changes in relationship between the Schwartz and vacuum distillation methods (14,16) have been observed as lard autoxidation progressed. The characteristics of these changes have been fully discussed in previous publications (14,16). In brief, the Schwartz method values had an initial, much more rapid increase than those of the vacuum distillation method. At peroxide values of 100 meq/1000 g or higher, the vacuum distillation values accelerated at a faster rate to approach, equal or eventually exceed the Schwartz method values and to break away from the Girard T values. This puzzling behavior superficially appeared due to alteration in specific sensitivity of monocarbonyl precursors with stage of autoxidation. This explanation is difficult to accept, since a spontaneous acceleration of hydroperoxide decomposition by the vacuum distillation conditions appears to occur. Lea and Hobson-Frohock (22) have indicated that the amount of hydroperoxide breakdown at 50C in the vacuum distillation procedure is small. Nevertheless, it seems apparent that the change was in the vacuum distillation since the Girard T and Schwartz values continued to progress with little shift in relationship or rates. This is curious since autoxidation takes place at an accelerating rate. Consideration should be given to the possibility that accumulating oxidation products interfere with the Schwartz and Girard T reactions. During the initial oxidation stage of lard the main

TABLE I

Total Monocarbonyls and Classes Isolated from Triolein

		μm/10 g				
		Monocarbonyl				
		Total			Alk-2-enals	
Storage at 24 F	Peroxides		$\frac{(\%)\ \text{carbonyl}}{\text{peroxide}} \times 100$	Alkanals		% Total
10 Days						
Vacuum distillation	95	1.22	1.3	0.91	0.26	21.2
Girard T		1.31	1.4	0.80	0.52	39.3
Schwartz		2.77	2.9	1.98	0.79	28.6
Begemann-de Jong		11.92	12.5	5.16	6.75	56.7
24 Days						
Vacuum distillation	315	3.01	1.0	2.35	0.67	22.1
Heated, vacuum distillation[a]		16.98	5.4	9.40	7.58	44.6
Girard T		3.03	1.0	1.84	1.19	39.4
Schwartz		3.95	1.3	3.09	0.87	21.9
Begemann-de Jong		26.82	8.5	12.30	14.52	54.1
30 Days						
Vacuum distillation	455	4.35	1.0	3.15	1.20	27.6
Girard T		4.73	1.0	2.76	1.96	41.6
Schwartz		7.95	1.8	6.22	1.73	21.7
Begemann-de Jong		25.64	5.6	12.49	13.15	51.3

[a] 165C for 15 min under nitrogen.

cause of differences between the methods seemed to be due to acidity. However, the effect of acids on hydroperoxides is a controversial subject (2,14,18, 23,25,26,27), and diverse opinions run the full range of possibilities. The present study was undertaken in the expectation that application of the methods to autoxidized pure triglycerides might clarify the relationships, further define their performance, and conclusively indicate the method most suitable for isolation of free aldehydes.

Experimental

Solvents and reagents were the same as described in previous publications (6,12–15). Highly purified triolein, methyl oleate, trilinolein, and trilinolenin samples were procured from the Hormel Foundation, Austin, Minn. Lard was prepared and autoxidized as in earlier studies (14,15,16). Isolation of volatile aldehydes by vacuum distillation was conducted exactly as described by Lea and Swoboda (21). Reaction of distillate with 2,4-dinitrophenylhydrazine was made as indicated by Gaddis et al. (13). Isolation of carbonyl 2,4-dinitrophenylhydrazones with the Girard T reagent was made by the modification of Gaddis et al. (13). Carbonyl 2,4-dinitrophenylhy- drazones formed with oxidized triglycerides by the Schwartz (25) and Begemann-de Jong methods (1) and the Pool and Klose modification of Keith and Day (19) were separated from the fatty material by the Seasorb method of Schwartz et al. (25). Fractionation of carbonyl hydrazones to mono- carbonyl derivatives by column chromatography (24), monocarbonyl resolution into classes and in- dividual compounds by paper chromatography, and measurement of the various fractions by spectro- photometry at λ max were performed as described in earlier publications (3–6,9,13,15). All determina- tions on an oxidized sample were set up simulta- neously and duplicate analyses were made. Results were calculated on the basis of 10.00 g. Absorbance values at λ max represent the amount of 2,4-dinitro- phenylhydrazones in 100 ml of carbon tetrachloride. Suitable corrections for blanks were made. Data on μm are on the basis of 10 g of oxidized material. Peroxides were determined on 0.200-g samples by the method of Kenaston et al. (20) and expressed as meq/1000 g or μm/10 g. Hydroperoxides were reduced by a modified Mizuno and Chipault (23) method. The modification consisted principally of the use of hexane in place of benzene.

Results and Discussion

Triolein in the amount of 75.0 g was spread in a thin film and allowed to oxidize under artificial light at 24F. Samples were removed for analysis after 10, 24, and 30 days. Under these conditions, peroxide values of 19, 63, and 91 meq/1,000 g were obtained, and sample sizes used were 3.00 g, 2.00 g, and 2.00 g, respectively.

The total monocarbonyl compounds were separated into classes and into individual compounds by paper chromatography. An unknown fraction was isolated with the alkanal class. This fraction separated into two compounds with R_f values of C_7 and C_{18} carbon chains. The C_7-like compound was not an alkanal as judged by fading in alcoholic alkali. The amount of unknown compounds was directly related to the known total monocarbonyls. Data for total known monocarbonyls and classes are shown in Table I. The total monocarbonyls of the vacuum distillation and Girard T procedures were closely similar, but there was considerable difference in enals. The larger amount of enals isolated by the Girard T is character- istic and was observed also in autoxidized lard (14,16). The Schwartz was substantially higher, and the Begemann-de Jong method was considerably greater than any other. This indicates a strong effect of acid on the amount of aldehydes isolated. The Begemann-de Jong monocarbonyls exceeded the amount of aldehydes released by heating at 165C. Despite the differences in totals, the vacuum distilla- tion and Schwartz methods had similar proportions of classes.

Analysis of individual aldehydes showed the presence of those already reported (5,12) and ex- pected on theoretical considerations, as shown in Table II. Among the mild methods, the C_9 alkanal was the major monocarbonyl compound isolated, and based on total alkanals the vacuum distillation method had the highest proportion of this compound.

The amount of C_8 alkanal isolated by vacuum distillation and Girard T methods was small. This is an expected product of decomposition of isomeric oleate hydroperoxides. Schwartz and Begemann-de

TABLE II

Individual Aldehydes Isolated from Autoxidized Triolein

| | μm/10 g | | | | | |
| | Alkanal | | | | Alk-2-enal | |
	C_8	C_9	C_{10}	C_{11}	C_{10}	C_{11}
Peroxide 19[a]						
Vacuum distillation	......	0.81	......	0.15	0.13	0.13
Girard T	......	0.61	......	0.18	0.26	0.26
Schwartz	0.65	1.38	tr	tr	0.39	0.40
Begemann-de Jong	1.40	2.12	0.82	0.82	2.84	3.92
Peroxide 63[a]						
Vacuum distillation	0.45	1.41	......	0.49	0.28	0.39
Heated, vacuum distillation[b]	0.97	6.89	......	1.62	3.07	4.51
Girard T	0.31	0.91	0.14	0.47	0.51	0.68
Schwartz	0.79	1.96	0.72	0.53	0.42	0.44
Begemann-de Jong	2.95	6.64	0.72	0.98	7.26	7.26
Peroxide 91[a]						
Vacuum distillation	0.37	2.14	......	0.65	0.45	0.75
Girard T	0.87	1.52	......	0.38	0.81	1.15
Schwartz	1.18	3.19	1.05	0.80	0.93	0.80
Begemann-de Jong	2.92	6.72	1.46	1.40	6.25	6.90

[a] (meq/1000 g).
[b] 165C for 15 min under nitrogen.

Jong methods isolated considerable amounts of the C_8 alkanal as well as appreciable quantities of unpredicted C_{10} and C_{11} alkanals. Relationships between the three mild isolation methods with oxidized triolein were similar to those with oxidized lard during the initial oxidation stage (14,16). Vacuum distilled, heated triolein resembled the unheated sample in that no C_{10} alkanal was detected.

Autoxidized Methyl Oleate

A quantity of 71.5 g of methyl oleate was autoxidized under exactly the same conditions as the triglyceride for 17, 22, and 25 days, at which times peroxides were 30, 65, and 91.5 meq/1000 g. Samples taken for each method were 4, 3, and 3 g, respectively. The method of vacuum distillation was not used because of volatility of the methyl ester. Unknown fractions were isolated only by the Begemann-de Jong procedure. This fraction seemed to consist mostly of the C_7-like material which separated in the alkanal class, which had the proper λ max but did not fade with alcoholic alkali. As shown in Table III, results were on the whole similar to those observed with the triglyceride. In consideration of the relationship between the Girard T and Schwartz methods and the respective amounts of alkanal and enal, there appeared to be a pivotal difference in class

TABLE III

Monocarbonyl Classes Isolated from Autoxidized Methyl Oleate

| | Monocarbonyl | | | | |
	Total μm/10 g	Alkanal μm/10 g	Alkanal %	Enal μm/10 g	Enal %
Peroxide 30[a]					
Girard T	0.70	0.39	55.2	0.31	44.7
Schwartz	1.15	0.90	78.8	0.24	21.2
Schwartz on Girard T res.	0.94	0.68	72.7	0.26	27.3
Begemann-de Jong	16.74	6.92	41.3	9.82	58.7
Peroxide 65[a]					
Girard T	1.70	0.65	38.3	1.05	61.7
Schwartz	3.64	2.75	75.5	0.89	24.5
Schwartz on Girard T res.	1.80	1.42	78.9	0.38	21.1
Begemann-de Jong	24.90	11.24	45.2	13.66	54.8
Peroxide 91.5[a]					
Girard T	3.48	1.71	49.1	1.77	50.9
Schwartz	4.41	3.17	71.8	1.25	28.2
Schwartz on Girard T res.	2.89	2.35	81.3	0.54	18.7
Begemann-de Jong	41.37	19.05	46.0	22.33	54.0
Reduced SnCl₂ Begemann-de Jong	31.12	24.49	78.7	6.63	21.3

[a] (meq/1000 g).

composition. This indicated that the Girard T tended toward more breakdown of enal precursors, while the Schwartz method preferentially decomposed alkanal precursors. Somewhat analogous results were obtained with autoxidized methyl arachidonate (6). A particularly significant result was the amount and composition of aldehydes isolated by the Schwartz method from a sample of oxidized methyl oleate recovered immediately after the Girard T method was run. The amounts of monocarbonyls obtained would seem to be due to decomposition of precursors by the Schwartz method since it might be supposed that the Girard T would remove virtually all free aldehydes. It should be particularly observed that the proportions of classes tend toward similarity to the original Schwartz results. These results come as no surprise since in earlier work (14) the Schwartz method invariably isolated additional monocarbonyl compounds when applied to autoxidized lard recovered from vacuum distillation pot stills and from the Girard T reactions.

As further shown in Table III, a 3.0-g sample of the oxidized methyl oleate with a peroxide value of 91.5 meq/1000 g was reduced by a modified stannous chloride method of Mizuno and Chipault (23). After this treatment the peroxide value was 0.0. The Begemann-de Jong method was applied to the reduced methyl oleate. Considerable monocarbonyl was isolated amounting to 75% of the original before reduction. However, the composition of the monocarbonyls from the reduced material was quite different from the original. This was characterized by a higher amount of alkanal which was almost entirely the C_9 compound. In contrast, only about 10 μm of the original Begemann-de Jong isolate was C_9 alkanal. It is not clear what has occurred in this reduction, but the inference may be that aldehydes are formed by or during the reducing process. The approach of reduction to remove the interference of peroxides with the determination of carbonyls in autoxidized fats would seem to require closer study.

In measuring the individual monocarbonyl compounds, it was found that the alkanals isolated by the Girard T and Schwartz on Girard T residue, had similar proportions with C_9 the major alkanal. In considering differences between Schwartz and Girard T isolates, the values suggest there is a tendency to be additive. This seems to indicate a much greater alkanal precursor decomposing effect by the Schwartz method and little effect by the Girard T reagent. In the case of the enals, proportions differed and showed a much higher amount of C_{11} enal separated by the Girard T method. Values were not additive, showing a definite effect of the Girard T reagent on enal precursors.

Trilinolein

A 75.0-g quantity of trilinolein was allowed to autoxidize under artificial light at 24C. Exactly weighed 2.0-g portions were taken for analysis after 2, 3, and 7 days of oxidation when peroxide values were 82, 210, and 1110 meq/1000 g, respectively. Amounts of monocarbonyl classes isolated are shown in Table IV. Differences between vacuum distillation and the Schwartz method were as great as with oxidized oleate. The result is contrary to some reports (2,18,25) that acid does not influence the breakdown of linoleate hydroperoxides to monocarbonyls. The Girard T results were considerably higher than the vacuum distillation values. Fractionation of the

TABLE IV
Monocarbonyl Classes from Trilinolein

| | Peroxide | Monocarbonyls μm/10 g | | | | | |
| | | Total | | Alkanal | | | |
		Total	(%) carbonyl/peroxide ×100	Alkanal	% Total	Alk-2-enal	Alk-2,4-dienal
2 Days							
Vacuum distillation	410	1.71	0.4	1.09	64.1	0.44	0.18
Girard T		3.92	1.0	3.08	78.6	0.65	0.19
Schwartz		6.95	1.7	5.41	77.9	1.54	tr
Pool and Klose		11.71	2.9	8.29	70.8	1.24	2.18
Begemann-de Jong		15.55	3.8	13.43	86.4	2.12	tr
3 Days							
Vacuum distillation	1050	2.44	0.2	1.68	68.9	0.57	0.19
Girard T		3.48	0.3	2.61	76.0	0.73	0.15
Schwartz		7.45	0.7	5.89	79.0	1.56	tr
Pool and Klose		35.35	3.4	24.68	59.8	4.56	6.11
Begemann-de Jong		17.50	1.7	14.86	84.9	2.65	tr
7 Days							
Vacuum distillation	5550	5.83	0.1	3.87	66.4	1.49	0.48
Girard T		8.00	0.1	5.63	70.3	2.01	0.36
Schwartz		9.38	0.2	6.42	68.4	2.96	tr
Pool and Klose		69.72	1.3	49.41	70.8	7.60	12.77
Begemann-de Jong		141.00	2.5	125.19	88.8	15.81	tr

monocarbonyls indicated the presence of an unknown derivative or fraction which could not be readily identified. This fraction separated in the alkanal class, had R_f values similar to C_7 alkanal, but was not saturated. The fraction, therefore, resembled that found in oxidized triolein, but this was not further investigated. Amount of the unknown material seemed directly related to the severity of the isolation conditions. Clear-cut differences between the various methods in the amounts of known monocarbonyl compounds are shown by these data. The vacuum distillation values had a definitely lower proportion of alkanals than the Girard T and Schwartz methods. The Begemann-de Jong method for monocarbonyls had a high proportion of alkanals and amount of alk-2,4-dienals was very small. The Girard T values were consistently intermediate between vacuum distillation and the Schwartz method. This relationship of the Girard T method is entirely different from that found in autoxidation studies of lard (14,16) where the Girard T values tended to lag with greater autoxidation. Amounts of aldehydes isolated are compiled in Table V.

Determination of the individual aldehydes showed little differences in the alkanals, with C_6 alkanal

predominating. In the enal class, the method affected the proportions of C_7, C_8, and C_9 enals. The Schwartz method differed in that it isolated a major amount of C_9 enal. However, the major enal removed by vacuum distillation and Girard T methods was C_7 enal. A similar difference was observed between these methods with autoxidized lard. The amount of alk-2,4-dienals isolated by the three mild methods and the Begemann-de Jong method was very small and was composed of both C_9 and C_{10} dienal. Theory subscribes to the formation of major quantities of two conjugated linoleate hydroperoxide isomers (12,14,15,16). As discussed previously, most of the aldehyde formed under normal conditions is C_6 alkanal (12,14,15,16). This has led to the supposition that either the linoleate hydroperoxides are composed of nearly all one isomer or the isomeric hydroperoxide yielding C_{10} alk-2,4-dienal is very stable. Such evidence has been considered to suggest that very little of the C_{10} dienal precursor was present unless formed by heat or some other treatment. Similar conclusions might be developed here since the hydroperoxide destructive Begemann-de Jong method produced little dienal. However, the monocarbonyls formed or isolated by the method repre-

TABLE V
Aldehydes Isolated from Trilinolein

| | Monocarbonyls μm/10 g | | | | | | | |
| | Alkanal | | | Alk-2-enal | | | Alk-2,4-dienal | |
	C_2	C_3	C_6	C_7	C_8	C_9	C_9	C_{10}
Peroxide 82[a]								
Vacuum distillation	0.07	1.06	0.96	0.19	0.10	0.15	0.07	0.11
Girard T	0.10	0.31	2.66	0.37	0.12	0.16	0.09	0.09
Schwartz	......	0.66	4.76	0.44	0.29	0.80	tr	tr
Pool and Klose	......	......	8.29	0.63	0.36	0.25	2.18	tr
Begemann-de Jong	0.56	1.09	11.78	0.88	0.60	0.65	tr	tr
Peroxide 210[a]								
Vacuum distillation	tr	0.43	1.25	0.25	0.13	0.19	0.10	0.09
Girard T	tr	0.30	2.32	0.39	0.16	0.17	0.07	0.08
Schwartz	......	......	5.89	0.43	0.41	0.72	tr	tr
Pool and Klose	......	......	24.68	1.76	1.57	1.23	5.60	0.52
Begemann-de Jong	......	1.85	13.01	1.44	0.80	0.40	tr	tr
Peroxide 1110[a]								
Vacuum distillation	tr	tr	3.87	0.63	0.56	0.30	0.25	0.23
Girard T	......	0.37	5.26	0.76	0.87	0.39	0.26	0.16
Schwartz	......	......	6.42	0.52	0.91	1.53	tr	tr
Pool and Klose	......	......	49.41	3.15	2.53	1.91	10.63	2.14
Begemann-de Jong	1.59	16.34	107.26	5.65	5.33	4.92	tr	tr

[a] (meq/1000 g).

TABLE VI
Monocarbonyl Classes in Autoxidized Trilinolenin

µm/10 g

	Total monocarbonyl		(%) carbonyl/peroxide × 100	Alkanal		Alk-2-enal	Alk-2,4-dienal
	Peroxide				%		
47 Hours							
Vacuum distillation	615	9.44	1.5	8.33	88.3	tr	1.10
Girard T		21.78	3.5	16.35	75.1	3.88	1.55
Schwartz		12.63	2.1	10.45	82.7	2.18	tr
Begemann-de Jong		103.57	16.8	81.68	78.9	21.88	tr
71 Hours							
Vacuum distillation	1250	8.06	0.7	6.68	70.5	tr	2.38
Girard T		28.76	2.3	22.73	79.1	3.59	2.44
Schwartz		19.61	1.6	14.06	71.7	4.22	1.32
Begemann-de Jong		138.27	11.1	112.39	81.3	18.87	7.07
95 Hours							
Vacuum distillation	2600	7.07	0.3	3.25	45.9	1.76	2.07
Girard T		17.52	0.7	14.30	81.7	2.32	0.90
Schwartz		37.44	1.4	29.26	78.2	3.68	4.50
Begemann-de Jong		201.04	7.7	148.35	73.8	41.19	11.50

sented only a small proportion of the total hydroperoxides. The Pool and Klose method with total monocarbonyls usually less than that of the Begemann-de Jong method had a substantial alk-2,4-dienal class with predominant amounts of C_9 dienal. Similar results were also found in studies of autoxidized lard with the Pool and Klose method (15). However, oxidized arachidonate which is similar in many ways to linoleate did not convert to a C_9 dienal (6), as one would expect.

Trilinolenin

A 10.0-g quantity of linolenate triglyceride was autoxidized as a thin film at 24C with samples taken for analysis after 47, 71, and 95 hours. The duplicate samples drawn were weighed exactly in amounts of around 0.4, 0.3, and 0.3 g, respectively. The peroxide values at the sampling times were 123, 250, and 520 meq/1000 g, respectively. The monocarbonyl compounds isolated by the various methods were fractionated. The totals and classes separated as µm/10 g are shown in Table VI. Monocarbonyl totals and alkanals of the vacuum distillation method decreased with oxidation. However, alk-2-enals and alk-2,4-dienals tended to increase, becoming a higher proportion of the vacuum distillation aldehydes. Girard T total monocarbonyl compounds increased and then decreased as did also the monocarbonyl classes. The Girard T monocarbonyl compounds were much greater than the vacuum distillation at all stages. Schwartz monocarbonyls, including all classes, in-

creased with oxidation and exceeded Girard T values only in the last oxidation sample. The differences between vacuum distillation and Schwartz monocarbonyls increased with oxidation. All methods had a considerable dienal fraction, the vacuum distillation possessing an unusually high proportion. The Begemann-de Jong monocarbonyls had the highest values and contained a substantial amount of alk-2,4-dienals. The pattern of response to the methods was quite different from that with oleate, linoleate, and even arachidonate (6). This is possibly due to linolenic acid's high rate of oxidation and polymerization which has made its autoxidation mechanisms so difficult to follow (7,8). Rapid decrease of monomeric hydroperoxides and formation of polymeric substances may be responsible for the erratic and unorthodox results.

The individual aldehydes isolated, identified, and measured are listed in Table VII. The C_3 alkanal was the major saturated alkanal with all methods. Relative amounts of enals were fairly even but C_5 compound tended to be the major one in that class. The C_7 was the major dienal and amounts isolated by the methods varied, and consistent basis for comparison was lacking. However, the availability of dienal precursor seemed different from linoleate, and the acid methods were effective in causing the formation of C_7 dienal. The results indicate, in the range and state of autoxidation examined, an increase and then a decrease in the free aldehydes. Results with the Girard T reagent also increased and then de-

TABLE VII
Individual Aldehydes Isolated from Trilinolenin

	µm Alkanals				µm Alk-2-enals				µm Alk-2,4-dienals		
	C_1	C_2	C_3	C_4	C_4	C_5	C_6	C_7	C_6	C_7	C_9
Peroxide 123[a]											
Vacuum distillation	1.25	2.33	3.76	....	tr	tr	tr			0.75	0.35
Girard T	0.73	0.63	14.99	....	0.71	0.71	2.46			0.80	0.75
Schwartz	....	0.90	9.55	....	0.78	0.67	0.37	0.37		tr	tr
Begemann-de Jong	....	5.97	12.48	13.23	6.38	7.42	4.28	3.81		tr	tr
Peroxide 250[a]											
Vacuum distillation	....	....	5.68	....	tr	tr	tr	0.67		2.38	tr
Girard T	....	1.28	21.45	....	1.01	0.77	1.14	1.28		2.44	tr
Schwartz	....	1.18	12.89	....	0.61	1.26	1.07	2.42	tr	1.32	tr
Begemann-de Jong	....	19.19	80.29	12.91	7.26	5.56	3.63		tr	7.07	tr
Peroxide 520[a]											
Vacuum distillation	....	....	3.25	....	0.45	0.47	0.50	0.39	tr	2.07	tr
Girard T	....	....	14.30	....	0.53	0.70	0.60	0.49	tr	0.90	tr
Schwartz	....	0.69	28.57	....	0.67	1.12	0.85	1.05	tr	4.50	tr
Begemann-de Jong	....	....	148.35	....	7.82	18.94	9.04	5.39	tr	11.50	tr

[a] (meq/1000 g).

TABLE VIII

Decreases in PV on Schwartz Column

| | meq/1000 g | | | |
	Before passage	After passage	Difference	%
5 Grams oxidized	193.7	170.8	22.9	11.8
Lard, 5%	252.7	217.4	35.6	14.0
Solution in hexane				
at 25 ml/hr	484.8	411.4	73.4	15.1

creased, but some monocarbonyl precursor decomposition must have occurred. The values from acid column methods increased throughout the range of oxidation, and precursors decomposable into monocarbonyls therefore must have increased. Little monomeric hydroperoxide (8) forms in oxidized linolenate. Frankel et al. (7) have shown that yields of linolenate hydroperoxide determined by chromatography were markedly lower than linoleate hydroperoxides at equivalent levels of oxidation. The concentration of secondary polar decomposition products was correspondingly higher in linolenate than linoleate, and these differences were more pronounced at advanced stages of the reaction. Increasingly greater differences have been found to exist between hydroperoxides as measured by iodometric method and the amount of monomeric hydroperoxides isolated by chromatographic method, which soon leveled off and decreased. These research findings by Frankel et al. (7) explain satisfactorily the relationships found between the monocarbonyl isolation methods on autoxidized trilinolenin.

Celite Acid Hydrazine Reaction Columns

The preceding studies of free monocarbonyl compounds developed by pure unsaturated fatty acid triglycerides during autoxidation unquestionably indicate real differences between the various methods in carbonyl precursor breakdown. There was no evidence in these experiments of changing or reversing relationships such as found with autoxidizing lard in earlier investigations. The most puzzling relationship observed with autoxidizing lard was between the Schwartz column and vacuum distillation method. An examination was therefore made of factors involved in reaction of monocarbonyls and hydroperoxides with 2,4-dinitrophenylhydrazine on a dilute phosphoric acid-celite column. It has been reported that a methyl linoleate hydroperoxide concentrate was altered by passage through the celite reaction column with the effluent becoming incapable of oxidizing acidic KI (25). Also no monocarbonyls were produced by action of the column on the methyl linoleate hydroperoxide. This has been explained (19) on the basis of autoxidation mechanism theories predicting the formation of triols or triol esters on exposure of hydroperoxides to acids. On the other hand, Taufel, Heder, and Franke (26,27) have reported decomposition of hydroperoxides and formation of monocarbonyls to a degree dependent on time of exposure and strength of acid. Horikx (17) has reported quantitative breakdown of methyl oleate hydroperoxides to monocarbonyls on the Begemann-de Jong dilute HCl celite column. Experiments on oxidized lard in earlier work have shown no wholesale disappearance of hydroperoxide on the acid column, and that there is some formation of monocarbonyls through action of acid columns (10,11,14). An example is shown in Table VIII of the disappearance of hydroperoxides on the Schwartz column. The decrease is relatively small, but as repeatedly

TABLE IX

Effect of Rate of Flow through Column on Monocarbonyls Isolated

| | Absorbance λ max | | | | | |
| | Ml/hr | | | | | |
	20 ml	25 ml	35 ml	50 ml	75 ml	Difference
Lard oxidized in artificial light at 20 C[a]						
PV- 20[b]		0.40		0.38		0.02
97		0.57		0.42		0.15
214		1.10		0.84		0.26
426		2.46		2.00		0.46
574		7.60		5.40		2.20
PV- 32	0.42			0.39		0.03
83.3	0.79			0.65		0.14
201.9	1.28			0.75		0.53
Lard oxidized in dark						
PV- 25.1[b]	0.30			0.22		0.08
49.9	0.50			0.41		0.09
100.5	0.98			0.65		0.33
153.0	0.63			0.56		0.07
186.4	0.83			0.55		0.28
Lard oxidized ultra-violet						
PV- 90.5[b]	1.16	1.06	0.93	0.80		0.36(max)

[a] 5 g in 45 ml hexane.
[b] (meq/1000 g).

shown, this may mean two to three times more monocarbonyl isolated.

The rate of passage through the column, dilution of the fat samples, and amount of oxidized fat applied may be important factors in quantitative reaction of the carbonyls and amount of hydroperoxide breakdown. The effect of rate of flow through the column is shown in Table IX. These data demonstrate a very definite influence of time on the column. Presumably the differences were due to hydroperoxide breakdown. By the methods used, there has been no difficulty in quantitative isolation of known amounts of monocarbonyls from fresh lard (13,14). However, further study of recoveries from autoxidized fat needs consideration.

The effect of dilutions using 5.0 g of oxidized lard at a rate of 35 ml per hour was tested. As shown in Table X, there was a trend toward higher results at the lower dilutions. Schwartz et al. (25), in making quantitative tests, used 3–5% (50 ml volume) solutions with rates of 44.4 to 6.2 ml per hour for monocarbonyl contents of 4 to 40 μmoles. The recommendation appeared to be use of 20% (50 ml) solutions at a flow rate of about 25.9 ml per hour. The authors (14,16) have regularly used 10 g of lard in 5 to 10% dilutions at rates of 40–50 ml per hour. Such conditions in the case of unoxidized lard were found to give quantitative recoveries of known amounts of aldehydes.

In the earlier studies with the Schwartz method, 10.0 g of oxidized lard were used throughout as the peroxides increased. A typical performance with the Schwartz method has been that after a first stage rapid increase in monocarbonyls there is a slowing of the rate. The result has been that with the progression of autoxidation, the vacuum distillation

TABLE X

Effect of Dilution on Aldehydes Isolated

Rate 35 ml/hr, 5 g fat	λ mμ Absorbance
2.5%	0.72, 0.75 } 0.74
5.0%	0.79, 0.86 } 0.83
10.0%	0.67, 0.65 } 0.66
20.0%	0.71, 0.68 } 0.70

method values may eventually attain similar or higher values than those of the Schwartz procedure. A remaining factor to be examined is the influence of amount of hydroperoxide applied to the reaction column on the per cent of hydroperoxide decomposition. A suggestion that this might affect results has been indicated by the work of Horikx (17) with oxidized methyl oleate on the Begemann-de Jong column (1). A quantitative production of mono-carbonyl hydrazones was obtained at low peroxide levels, but with larger amounts of hydroperoxides on the column the yield of monocarbonyl derivatives decreased. This factor was tested by comparison of values obtained with 10.00 g oxidizing lard with those found when the quantity of sample applied to the Schwartz column was adjusted to keep the amount of hydroperoxide constant. The oxidizing lard examined had an initial peroxide value of 40.1. As shown in Fig. 1, where the amount of oxidized lard applied remained the same, the increasing amounts of hydroperoxides seem to depress the percentage of monocarbonyl derivatives isolated. This is believed to be due at least in part to a decreasing efficiency in the decomposition of hydroperoxides and possibly other primary oxidation products as these substances accumulate. Such an effect explains the puzzling changing relationships between the Schwartz and vacuum distillation methods. These results definitely indicate that the vacuum distillation procedure is the best method for free aldehydes.
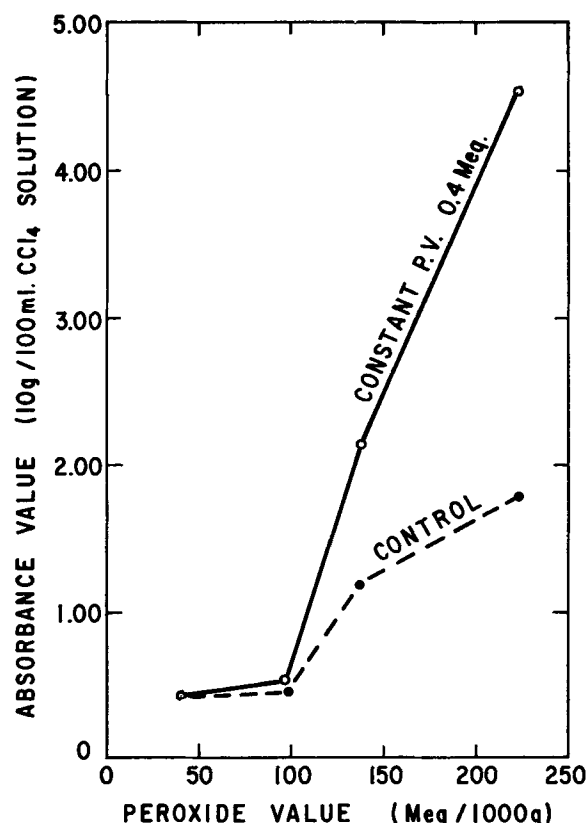

FIG. 1. Effect of amount of oxidized fat on the yield of aldehyde 2,4-dinitrophenylhydrazones isolated by the phosphoric acid-celite reaction column.

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