Use of Labeled Compounds to Study the Mechanism of Flavor Formation in Oxidizing Fats[1,2]

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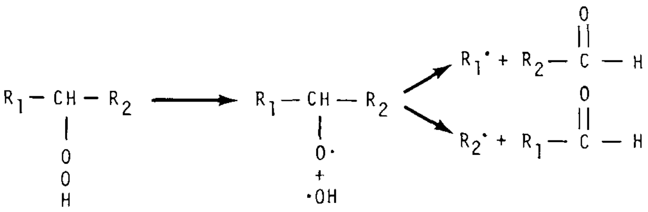
ABSTRACT

2-^{14}C-Hexanal, *trans, trans*-5-^{14}C-2,4-decadienal, 4-^{14}C-1-octen-3-ol, 4-^{14}C-1-octen-3-one and 1-^{14}C-1-pentanol were synthesized and added to freshly deodorized soybean oil in concentrations ranging from 7-125 ppm. The soybean oil was oxidized, and the fate of the labeled compounds was followed. Hexanal was converted to hexanoic acid especially at 50 C or higher. The 2,4-decadienal was converted to 2,4-decadienoic acid at room temperature, and heptenal, 2-octanal, 2-nonanal, glyoxal and malonaldehyde were found among the labeled products. 1-Octen-3-ol was converted to 1-octen-3-one at room temperature, but the 1-octen-3-one formed a stable end product that produced no other labeled compounds. 1-Pentanol was converted to pentanoic acid at 50 C or higher.

INTRODUCTION

The oxidation of fats and oils yields a complex mixture that includes aldehydes, ketones, hydrocarbons, acids and alcohols, all arising from scission of the fatty acyl chains. The chain-splitting mechanism widely accepted today was first proposed by Bell et al. (1) and is represented by the equations:



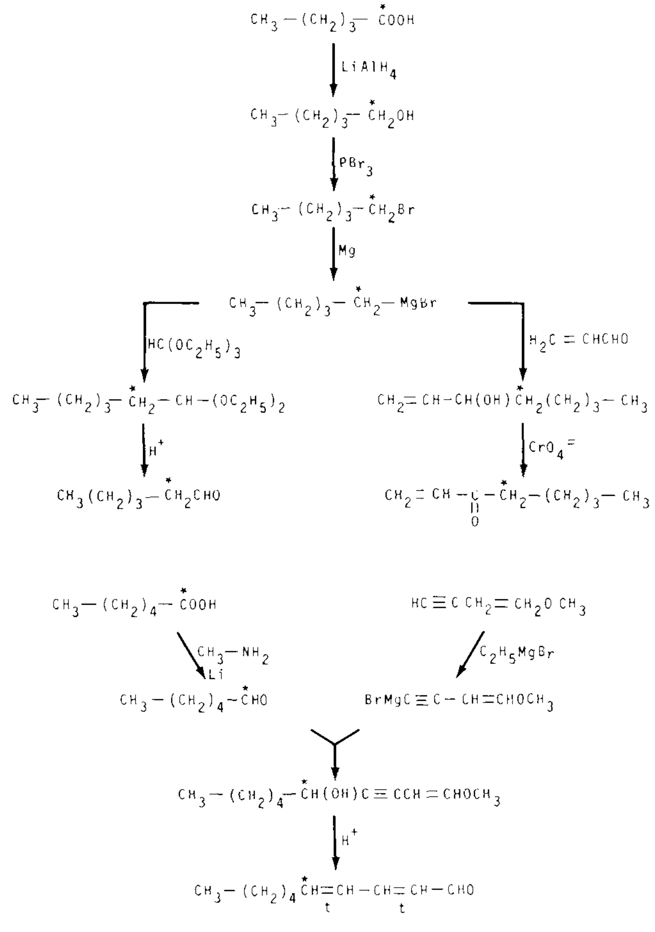
The alkyl free radicals can gain a hydroxy free radical to form alcohols and lose or gain a hydrogen free radical to form alkanes, alkenes or alkynes.

The evidence for this scission mechanism is indirect. The products formed during the oxidation of various fatty acids have been examined, and the major scission products identified can usually be accounted for by this mechanism. But some products have been identified that are not readily attributable to this mechanism, starting with the hydroperoxides believed present. For example, the vinyl ketones and viny alcohols reported in oxidized fats require a more complex mechanism, and several have been proposed (2-4). It has also been suggested that some of the products difficult to account for by the scission mechanism of Bell et al. arise from continued oxidation of the primary scission products (5-7).

A study of the scission reaction is hampered by very low yields and the instability of intermediates. The ease and sensitivity with which quantitative analyses can be made by using ^{14}C-labeled compounds make radio tracers an attractive approach to the study of certain questions of mechanism. We have already reported on the use of labeled compounds to study the contribution of saturated acyl groups to scission products (8). In this paper we have used labeled compounds to study the fate of primary scission products after they are formed.

METHODS

The synthetic methods shown for hexanal (9), 1-octen-3-one (10), 1-octen-3-ol (11), 1-pentanol and 2,4-decadienal



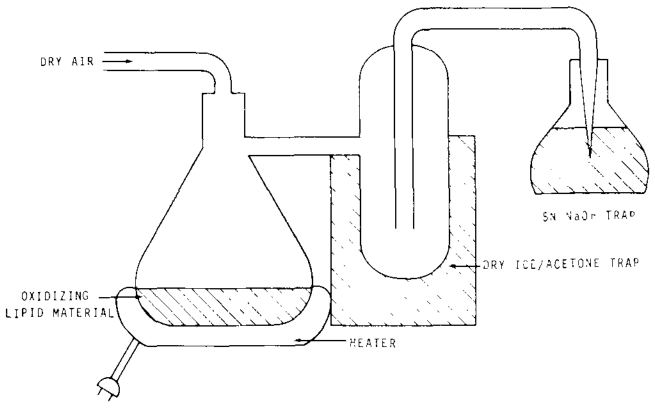


FIG. 1. Apparatus for the oxidation of soybean oil.

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[2] Journal Paper No. J-7082 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa 50010. Project 1856.

TABLE I

Oxidation of 2-^{14}C-Hexanal and *Trans,trans*-5-^{14}C-2,4-Decadienal

Temperature, C	Days	P.V.	Counts per minute					Per cent conversion to acid
			In 10 ml sample	Recovered as carbonyl	Recovered as acid	Fat	Cold trap	
Hexanal								
25	1	0.27	236,179	233,980	None	2178	Negative	None
50	25	30.3	237,800	153,250	73,000	6785	Negative	30.70
50	34	47.8	236,430	None	229,230	7180	Negative	96.95
2,4-Decadienal								
25	0	0.31	1,710,000	1,700,000	None	6110	—	None
25	11	28.2	1,676,480	1,650,000	2750	22,433	—	0.16
25	17	51.6	1,722,000	1,500,000	219,504	41,382	—	12.38
25	25	79.5	1,655,000	902,000	751,440	33,800	—	34.52
25	38	175.6	1,661,300	100,320	1,560,000	65,823	—	93.90

(12,13) were carried out on a microscale. Starting materials were the sodium salts of 1-C^{14}-n-pentanoic acid (Amersham-Searle) and 1-^{14}C-n-hexanoic acid (New England Nuclear). Purification of intermediates was avoided in the microsynthesis. Micro-Grignard reactions were started and kept going by the addition of ethyl bromide along with the labeled bromide. Final purification of the labeled compounds was achieved by gas liquid chromatography (GLC) on 20% butanediol succinate columns 3/8 in. in diameter and 2 m long operated at 145 C. A thermal conductivity detector was used, and the proper retention time for collection of the labeled material was determined by chromatography of unlabeled reference compounds under identical conditions. The purity of the labeled compounds was established by reinjection on GLC to show that all the label had the proper retention time. The aldehydes and ketones were converted to 2,4-dinitrophenylhydrazones (DNPH's), the alcohols to N,N-dimethyl-*p*-aminobeneneazobenzoyl esters (DABAB's), and all were shown to be homogeneous by thin layer chromatography (TLC). Because of the instability of 1-octen-3-one and 2,4-decadienal, these compounds were collected directly from the gas chromagraph into the oil in which they were to be oxidized. Analyses for purity were carried out immediately as described in the analyses for products in oil.

The pure, labeled carbonyl compounds were placed in soybean oil that had been deodorized for 6 hr at 190 C (14). The oil was oxidized in the apparatus shown in Figure 1. Peroxide values were determined by the method of Hamm et al. (15).

For carbonyls other than 1-octen-3-one that react normally with 2,4-dinitrophenylhydrazine, DNPH's were prepared and isolated from the fat by the method of Schwartz et al. (16). 1-Octen-3-one, 1-octen-3-ol and pentanol and their products were isolated from oil samples by passage three times through a micromolecular still (Kontes Glass Co.) operated at 2 x 10^{-3} torr. The oil samples were introduced at a rate of 8 drops per second. The first pass was at room temperature, but subsequent passes were 80 C. Volatiles were collected in a liquid-nitrogen-cooled trap. The volatiles from the first distillation were divided into three parts: the first 40% was converted to DNPH's by the method of Henick et al. (17); the second 40% was converted to DABAB's by the method of Churacek and coworkers (18,19); the remaining 20% was examined by GLC on butanediol succinate as described in the section on synthesis.

TABLE II

Labeled Carbonyls Produced by the Oxidation of *Trans,trans*-5-^{14}C-2,4-Decadienal

Compound	Sampling Time, cpm	
	25 days	38 days
2,4-Decadienal	860,000	70,200
2-Nonenal	2560	870
2-Octenal	760	330
Heptanal	15,520	14,685
Glyoxal	—	20
Malonaldehyde	—	69

DNPH's were separated by TLC according to Urbach (20) and Cobb (21). Sequential separations involving adsorption, partition and silver ion TLC were used where this was appropriate to insure separation of components containing label into pure compounds. DABAB's were separated by TLC on alumina plates developed with hexane-diethyl ether 85:15 v/v and on Silica Gel G plates impregnated with 10% kerosene (bp 221-230 C) in hexane and developed with dimethylformamide-methanol-water 4:1:1 v/v.

Acids formed during the oxidation were retained by the magnesia-Celite column used in the Schwartz procedure. After the DNPH's were eluted, the column packing was dumped out, treated with concentrated hydrochloric acid and extracted three times with hexane. The hexane layer was shaken with 5 N sodium hydroxide to extract the acids from the hexane. The sodium hydroxide layer was evaporated and reacted with N,N-dimethyl-*p*-aminobenzolazophenacyl chloride (DABAP) according to Churacek and coworkers (18,22). The DABAP's were separated by TLC on alumina plates developed with hexane-diethyl ether 85:15 v/v.

Malonaldehyde was isolated as a 2-thiobarbaturic acid derivative by the method of Dunkley and Jennings (23).

Fractions were analyzed for radioactivity in glass scintillation vials. The counting solution consisted of 5 g of 2,5-diphenyloxazol and 0.1 g of 2,4-bis(4-methyl-5-phenyloxazolyl)-benzene in 1 liter of toluene. The counter was a Packard 3320. GLC fractions to be counted were bubbled directly into counting solution. When fractions from TLC plates were counted, a counting solution containing 40 g Cabosil was used to suspend the particles of adsorbant and increase counting efficiency. Color quenching by the highly colored derivatives used for TLC was corrected by addition of an internal standard of ^{14}C-toluene. The degree of quenching of the known number of toluene counts was used to correct the counts in the sample.

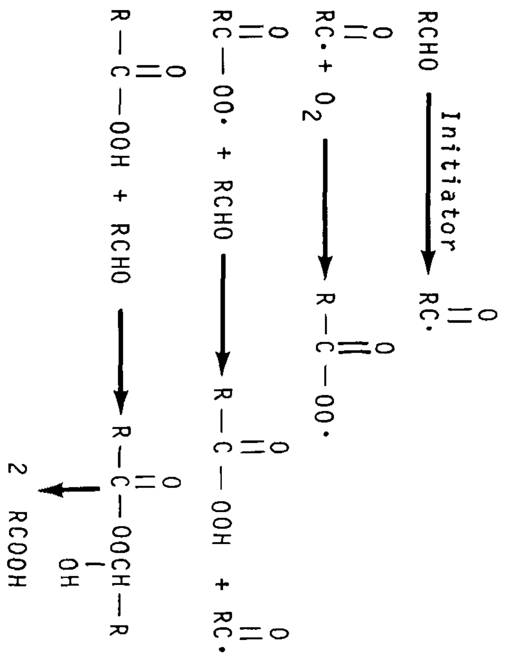
RESULTS AND DISCUSSION

Hexanal

2-^{14}C-Hexanal was placed in soybean oil at a concentration of 15 ppm, and the oil was allowed to oxidize. During oxidation at 25 C, most of the counts were recovered as 2,4-DNPH's. TLC of the 2,4-DNPH's revealed that only the hexanal derivative was labeled. At 25 C, the number of counts recovered as acid rose very slowly to ca. 1% at the end of 45 days. At higher temperatures (Table I), the conversion to acids is much more striking. TLC of the

DABAP's revealed that the only acid containing label was hexanoic. Even at 50 C, hexanal was the only carbonyl labeled. In all instances a certain number of counts were retained in the fat. These may be hexanal or hexanoic acid not retained by the procedure (16), or they may represent polymerized material or other products that will not react. The amount recovered in the fat increased with time.

These results agree with those of Lillard and Day (5) who oxidized pure nonanal and analyzed the products. A mechanism for the oxidation of aldehydes to acids has been proposed (24-26).



Degradation to shorter chain aldehydes proposed by Loury and Forney (6,7) evidently did not occur under our conditions.

2,4-Decadienal

Trans,trans-5-^{14}C-2,4-decadienal was placed in soybean oil at a concentration of 125 ppm and oxidized at 25 C. Results are given in Tables I and II. As the oil oxidized, the 2,4-decadienal was almost quantitatively converted into an acid. TLC of the DABAP derivative indicated that the isolated acid had an R$_f$ slightly greater than that of decanoic acid. *Trans,cis*-2,4-decadienoic acid was isolated from stillingia oil, and its DABAP derivative migrated slightly further than that isolated from the oxidized oil. We believe that the acid isolated is *trans,trans*-2,4-decadienoic acid, as this would account for the TLC results and would be the expected product from *trans,trans*-2,4-decadienal.

Of the material isolated as DNPH, only 2,4-decadienal was labeled at zero time, but as oxidation progressed TLC revealed other labeled components (Table II). Some label also was detected in the red pigment isolated in the TBA reaction.

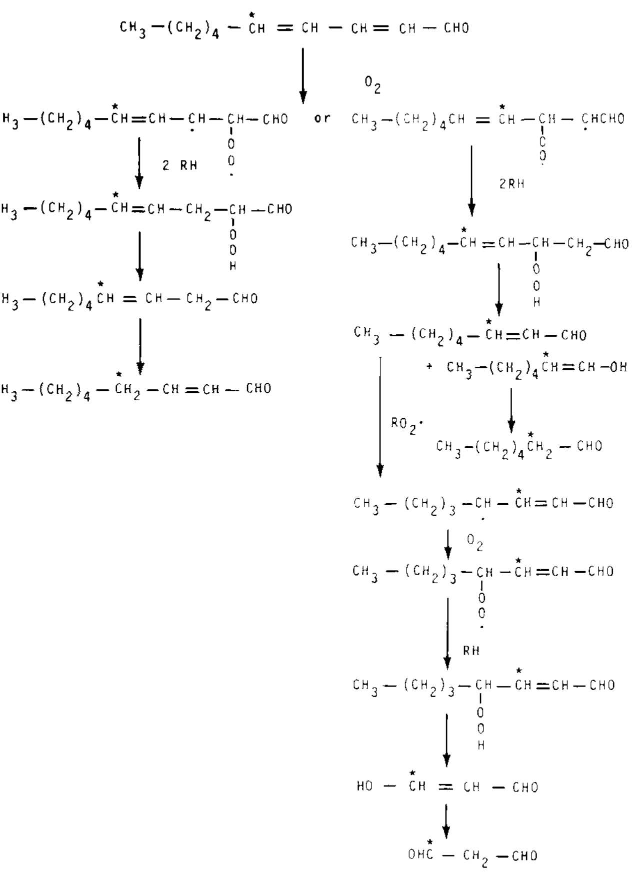
The amount of label recovered in the fat increased as oxidation proceeded. This increase probably represents polymer or other products incapable of being removed by the Schwartz column.

These results agree partly with those of Lillard and Day (5) who studied the autoxidation of pure 2,4-heptadienal at 45 C. They reported formation of small amounts of an unspecified acid, but polymerization was the main reaction pathway under their conditions. They did note the production of ethanal, propanal, butanal, glyoxal, 2-ketopentanal, 2-ketohexanal, 2-ketoheptanal, *cis*-but-2-en-2,4-dial and malonaldehyde. The major products were ethanal and propanal. The heptanal found in our experiments would correspond to the butanal Lillard and Day reported as a minor component. The reaction products can be accounted for by the mechanism postulated by Lillard (27).

TABLE III

Oxidation of 4-^{14}C-1-Octen-3-ol, 4-^{14}C-1-Octen-3-one and 1 ^{14}C-1-Pentanol

Temperature, C	Days	P.V.	Counts per minute				Per cent conversion to acid	Per cent conversion to ketone by	
			In 10 ml sample	Recovered as carbonyl	Recovered as acid	Fat		Thin layer chromatography	Gas liquid chromatography
1-Octen-3-ol									
25	7	4.05	115,171	—	—	9610	—	4.29	4.4
25	16	14.2	116,103	—	—	9595	—	10.19	10.0
25	22	33.0	114,479	—	—	12,200	—	25.1	26.6
50	32	89.0	84,529	—	—	7176	—	73.75	76.0
1-Octen-3-one									
22	5	9.38	145,313	—	—	13,223	—	—	—
22	9	23.3	145,395	—	—	14,238	—	—	—
22	20	49.5	122,723	—	—	12,912	—	—	—
50	33	114.7	122,638	—	—	10,380	—	—	—
1-Pentanol									
25	0	0.38	431,500	None	—	16,400	—	—	—
25	12	5.73	429,900	None	—	17,198	—	—	—
25	20	16.2	434,100	None	None	4005	None	—	—
50	31	86.5	428,500	None	15,274	5178	3.56	—	—
100	43	113.1	417,980	None	414,200	5460	99.09	—	—



1-Octen-3-ol

4-^{14}C-1-Octen-3-ol was placed in soybean oil at a concentration of 7 ppm. As the soybean oil oxidized, there was conversion of the 1-octen-3-ol to 1-octen-3-one (Table III). 1-Octen-3-one was the only carbonyl found labeled by GLC or by TLC of the DNPH's. No alcohol with label other than 1-octen-3-ol was discovered by GLC or TLC of the DABAB's.

These results agree with the mechanism postulated by Wilkinson (2). Although there is good conversion of the vinyl alcohols to the ketone, it should not be assumed that all the vinyl ketone in oxidized fats and oils comes from oxidation of the vinyl alcohols, especially since the mechanism of vinyl alcohol formation is far from clear.

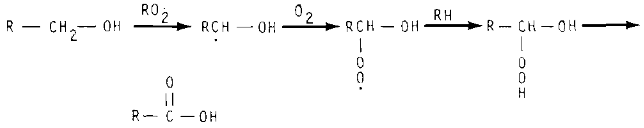
1-Octen-3-one

4-^{14}C-1-Octen-3-one was placed in soybean oil at a concentration of 9 ppm. The results of the oxidation are presented in Table III. The only compound found labeled by TLC of the DNPH's was 1-octen-3-one. No labeled acids could be found by reaction with DABAP. GLC of portions of the distillate showed a labeled compound with a retention time similar to that of 1-octen-3-one plus label which came out with a longer retention time and tailed out for a long period. This is evidently caused by polymerization of the 1-octen-3-one. The same polymer was encountered during the purification of the synthetic 1-octen-3-one by GLC.

These results are in agreement with those of Lillard and Day (5) who were not able to show any oxygen uptake when pure 1-octen-3-one was oxidized at 45 C. They reported only polymer formation.

1-Pentanol

Because 1-octen-3-ol oxidized readily in oxidizing soybean oil, 1-^{14}C-1-pentanol was prepared and placed in soybean oil at 19 ppm. The results of its oxidation are shown in Table III. In the first two samples molecular distillation was used to isolate the labeled compounds, but in the last three Schwartz's column was used to facilitate the search for labeled carbonyls and acids. The results indicate that alcohols do not oxidize appreciably at room temperature, but that at 50 C or higher there is good conversion into acids. The acids recovered from the Schwartz column were converted to DABAP's, and TLC revealed that only one component that migrated with pentanoic acid was labeled. No intermediate pentanal could be found. The alcohol may oxidize by the mechanism below.



ACKNOWLEDGMENTS

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