J. Lipid Research, April, 1961
Volume 2, Number 2

# Composition of aldehydes derived from some bovine lipids

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[Received for publication July 29, 1960]

## SUMMARY

The compositions of the aldehydes derived from phosphorus-free lipids of milk fat and ox heart, as well as from phosphatides of butter and ox heart, were investigated. In addition to normal aldehydes, considerable amounts of branched aldehydes were found, in which the branching occurred at carbon atoms situated $\alpha$, $\beta$, and, in a few cases, $\gamma$ to the terminal carbon.

In the literature a number of data can be found concerning the nature of the aldehydes derived from plasmalogens from various sources. Leupold (1) found octadecanal, hexadecanal, tetradecanal (probably), *cis*-octadecen-9-al and *cis*-octadecen-11-al released by plasmalogens from horse brain. The same aldehydes, with the exception of *cis*-octadecen-11-al, were obtained by Klenk and Friedrichs (2) from plasmalogens of horse carcass and heart muscle. The presence of octadecanal, hexadecanal, and an octadecenal in aldehydes liberated from horse brain has also been demonstrated by Kaufmann and Kirschnek (3). Gray (4) subjected the aldehydes from ox heart plasmalogens, in the form of their dimethyl acetals, to gas-liquid chromatography and was able to detect 18 different components, of which two could be identified as octadecanal and hexadecanal, respectively. In a recent publication Gray (5) describes the gas-liquid chromatographic analysis of the dimethyl acetals of aldehydes derived from ox-spleen choline plasmalogens. He based a tentative identification on the retention volumes of these acetals. In addition to normal aldehydes (dodecanal up to and including octadecanal), ten probably branched aldehydes were shown to be present. Finally, Van Duin (6) analyzed the aldehydes from butter plasmalogens in the form of their dinitrophenylhydrazones and established the presence of octadecanal and hexadecanal.

In the present work the aldehydes are liberated from the phosphorus-free lipids (7) of milk fat and ox heart. After conversion of the aldehydes, via the corresponding acids, into the methyl esters, the latter are separated by gas-liquid chromatography. This separation enables tentative identification of the fractions collected. The aldehydes generated from phosphatides of butter and ox heart are similarly analyzed and compared.

## METHODS AND RESULTS

(*a*) *Aldehydes from Milk Fat.* Milk fat (1050 g) containing no detectable amounts of phosphorus (less than 0.0001% according to the method of Zilversmit and Davis [8]) was obtained from 4 liters of cream. In order to remove volatile aldehydes, if present, the fat was heated in 150 g portions, as described by De Bruyn and Schogt (9), for 5 hours at $10^{-6}$ mm and 120°. In model experiments it was ascertained that free aldehydes up to and including pentadecanal are removed. To liberate bound aldehydes, the fat was stirred at 50° for 4 hours with 4 liters of a mixture of glacial acetic acid and 2 N hydrochloric acid 1/1(v/v), after which the fat was washed free of acid and dried over magnesium sulfate. The liberated aldehydes were subsequently separated from the fat by degassing for 5 hours at 120° and $10^{-6}$ mm, and collected in a cold trap. The aldehydes were taken up in petroleum ether (b.p. 40°–60°), purified according to Van der Ven and de Jonge (10). To convert them into their 2,4-dinitrophenylhydrazones, the solution was brought in portions onto eight 15 × 270 mm Celite® 2,4-dinitrophenylhydrazine-HCl columns (11).

Because traces of short-chain carbonyl compounds from the solvent also give dinitrophenylhydrazones, the mixture was subjected to partition chromatography according to Kramer and van Duin (12) on eight 15 × 210 mm $SiO_2$ columns. The $SiO_2$ was prepared ac-

cording to Gordon *et al.* (13). Dinitrophenylhydrazones of aldehydes with a chain length of more than 11 carbon atoms (retention volumes less than that of undecanal) were collected, and the solvent removed under vacuum at 40°. To remove traces of nitromethane and improve the stability of the preparation, the dinitrophenylhydrazones were purified over two 15 × 170 mm alumina columns. The alumina (Imperial Chemical Industries) had previously been dried at 160° and deactivated with 8% water. Petroleum ether plus 5% diethyl ether was used as eluting solvent. Ninety-three mg of dinitrophenylhydrazones was obtained.

The aldehydes were regenerated by a modification of the method of Keeney (14): 47 mg of dinitrophenylhydrazones was heated under reflux with 45 ml of glacial acetic acid and 5 ml of a levulinic acid-water mixture 9/1 (v/v) for 30 minutes at 120°, and then for 15 minutes at 130°. The mixture was taken up in petroleum ether and washed with water. It was found that 6 mg of dinitrophenylhydrazones had not decomposed. The mixture was separated on a 10 × 170 mm alumina column. The aldehydes were eluted with petroleum ether containing 5% diethyl ether immediately before the undecomposed dinitrophenylhydrazones. After removing the solvent, the free aldehydes could be separated by gas-liquid chromatography. However, if it is desired to investigate the individual fractions further, difficulties arise due to partial oxidation of the aldehydes. Therefore, in all subsequent experiments, aldehydes were oxidized to acids and then converted into their methyl esters. The aldehydes liberated from 46 mg of dinitrophenylhydrazones were oxidized by the method of Mitchell and Smith (15). Freshly precipitated silver oxide was prepared from 1.4 g of silver nitrate and 8.3 ml of 1 N alkali, washed with water and then with dioxane, and transferred to a 25 ml Erlenmeyer flask with ground glass joint. To this were added the aldehydes in 9 ml dioxane and 2 ml water. The mixture was magnetically stirred and refluxed for 1 hour at 60° to 65°. After diluting with 50 ml of water, 4 ml of 1 N alkali was added and the excess silver oxide was filtered off. The alkaline filtrate was extracted with 25 ml of diethyl ether to remove any unoxidized aldehyde, and then acidified with 5 ml of 2 N $H_2SO_4$. Nineteen mg of acid was obtained,[1] which was taken up in a few milliliters of absolute diethyl ether and esterified with etherial diazomethane. The solution of esters was divided into two equal portions. Analysis of the first portion by gas-liquid chromatography is shown in Figure 1. The other half was dissolved in 3 ml of methanol and hydrogenated at room temperature, using 50 mg Adams' platinum oxide catalyst. The hydrogenated esters were also subjected to gas-liquid chromatography. Peaks *2*, *4*, *6*, and *8* correspond to the esters of normal tri-, tetra-, penta-, and hexadecanoic acids. Only peak *7* disappeared after hydrogenation and was therefore attributed to the methyl ester of a hexadecenoic acid. The increase after hydrogenation of peak *8* confirms this. As it is known that branched-chain methyl esters emerge from the column before the corresponding straight-chain esters (16), it was assumed that peaks *1*, *3*, and *5* were due to branched-chain esters.

Fractions representing peaks *3* and *5* were collected separately and saponified (steam bath, 15 minutes, 0.5 ml of 0.5 N methanolic KOH), yielding 2.7 mg of acid (m.p., 45°–47°) and 3.2 mg of acid (m.p., 20°–23°), respectively. To prove that these acids were branched, they were oxidized with chromic acid according to Archer and Hickinbottom (17). Parallel oxidations were carried out with the model branched-chain acids, while *n*-tetradecanoic acid (myristic acid) was run as a control. All the oxidations were carried out as described for the acid from the fraction in peak *3*. One and eight-tenths mg of this acid was mixed in a 10-ml flask with 0.2 ml of glacial acetic acid (Analar) and 0.1 ml of pure acetic anhydride. Fifty mg of powdered chromic acid was added to the cooled solution, which was subsequently stirred for 20 hours at 0°. Large, colorless crystals, presumably acetic acid, formed. After stirring for another 4 hours at room temperature, the reaction product was transferred with 12 ml of water into a 30-ml flask. The mixture was acidified with 2 ml of 0.1 N $H_2SO_4$ and sufficient dilute sodium bisulfite solution was added to decompose the unreacted chromic acid, just causing the color of the solution to change to green, taking care that no excess bisulfite

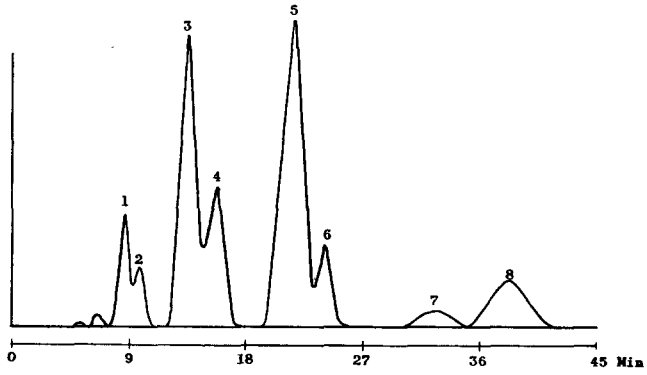


FIG. 1. Methyl esters corresponding to the aldehydes, obtained from milk fat. (Nitrogen flow 43 ml/minute, temperature 197°, pressure 38 cm Hg, Celite®/Apiezon L 4:1.)

[1] In a model experiment 12.7 mg of tetradecanal dinitrophenylhydrazone submitted to the same sequence of reactions yielded 4.8 mg of tetradecanoic acid.

was present. Subsequently, 12 ml of liquid was distilled off. The distillate was mixed with a solution of 0.06 g dinitrophenylhydrazine in 9 ml of 2 N hydrochloric acid. This reagent solution has previously been vigorously shaken with two 10-ml portions of petroleum ether to remove impurities. After allowing to stand for 16 hours at room temperature, the dinitrophenylhydrazones were extracted from the mixture with two portions of petroleum ether, and subsequently analyzed on the standard nitromethane column (12). Two bands, having retention volumes of 450 and 870 ml, respectively, were obtained. Band *1* was isolated, purified over an alumina column, and dissolved in 80 mg of chloroform. The ultraviolet absorption spectrum gave a maximum at 362 mμ, E = 0.444 cm²/mg. The retention volume and the ultraviolet maximum corresponded with those of acetone dinitrophenylhydrazone. From the extinction, 92 μg was present. Band *2* was probably acetaldehyde dinitrophenylhydrazone. This band was observed in all experiments, even in the oxidation of myristic acid, and might have originated from the solvents. The results of the oxidations are recorded in Table 1.

TABLE 1. OXIDATION OF BRANCHED ACIDS WITH CHROMIC ACID

| Acids | | μg Carbonyl Compounds Found After Oxidation | | | Yield of Theoretical |
|---|---|---|---|---|---|
| | | Acetone | Butanone | Pentanone | |
| *mg* | | | | | *per cent* |
| 2.0 | 11-Me-dodecanoic acid | 195; 150 | 0 | 0 | 30 |
| 2.0 | 11-Me-tridecanoic acid | 13 | 17 | 0 | 3 |
| 2.0 | 10-Me-tridecanoic acid | 7 | 0 | 9 | 1 |
| 2.0 | *n*-tetradecanoic acid | 9; 9; 7 | 0 | 0 | |
| 1.8 | acid from peak 3 | 92; 50 | 0 | 0 | |
| 1.7 | acid from peak 5 | 29 | 14 | 0 | |

The acid from peak *3* gave only acetone, and in view of the retention time of its methyl ester, it is therefore 12-methyltridecanoic acid. Its melting point of 45°–47° is compatible with data from the literature: 53.3°, Weitkamp (18), 53.3°–53.6° Arosenius *et al.* (19).

The acid from peak *5*, which on oxidation gave acetone and butanone, is apparently a mixture consisting largely of 12-methyltetradecanoic acid. Its melting point of 20°–23° is in fair agreement with that of the optically active 12-methyltetradecanoic acid (m.p., 23.0) from lanolin (18). From the amount of acetone formed, a small percentage of 13-methyltetradecanoic acid must also be present.

From the results it follows that, apart from normal aldehydes, the aldehydogenic lipids in butterfat also yield 12-methyltridecanal and 12- and 13-methyltetradecanal.

(*b*) *The Aldehydes from Phosphorus-free Ox Heart Lipids.* From 12 kg of ox heart muscle, 92 g of phosphorus-free lipids was obtained, using the procedures previously described (7). The lipids were saponified, and the unsaponifiable fraction isolated by repeated extraction. Since the lipids had not previously been heated in high vacuum, the unsaponifiable fraction might contain free aldehydes in addition to the aldehydes bound as enol ethers (7). The unsaponifiable fraction was treated with silver oxide, and any acid formed was removed by alkali extraction. The aldehydogenic lipids are not affected by this treatment. The unsaponifiable fraction, free of aldehyde, was dissolved in 15 ml of petroleum ether and passed through three 33 × 270 mm Celite®-dinitrophenylhydrazine-hydrochloric acid columns (11), thus splitting off the aldehydes from α,β-unsaturated ethers and converting them into the dinitrophenylhydrazones. The latter were freed from sterols on an alumina column in which sterols are retained. Subsequently, the dinitrophenylhydrazones of aldehydes with more than 11 carbon atoms were isolated on the nitromethane column.

The purified dinitrophenylhydrazones, corresponding to about 58 mg of aldehydes, were investigated via the corresponding methyl esters as described under (*a*). In the gas-liquid chromatogram (Fig. 2) peaks *2*, *4*, *6*, *8*, and *10* correspond to the normal saturated esters ($C_{14}$ to $C_{18}$). After hydrogenation peak *9* disappeared and was therefore probably due to the methyl ester of an octadecenoic acid. Fractions from peaks *1*, *3*, and *7* were collected separately and, after saponification, oxidized with chromic acid. The results are shown in Table 2. Of fraction 5, insufficient material was obtained for an oxidation test.

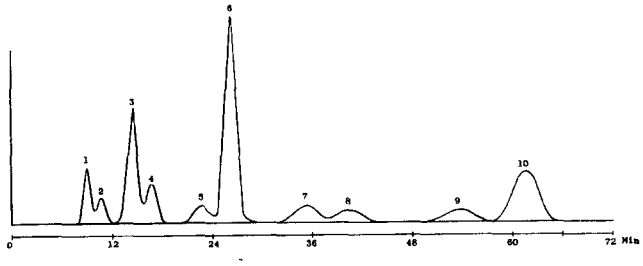


FIG. 2. Methyl esters corresponding to the aldehydes from non-phosphatide lipids in ox heart. (Nitrogen flow 30 ml/minute, temperature 197°, pressure 34 cm Hg, Celite®/Apiezon L 4:1.)

TABLE 2. CHROMIC ACID OXIDATION OF ACIDS OBTAINED BY OXIDATION OF ALDEHYDES OBTAINED FROM PHOSPHORUS-FREE OX HEART LIPIDS

| Acid from Peak | μg Carbonyl Compounds Found After Oxidation | | |
|---|---|---|---|
| | Acetone | Butanone | Pentanone |
| 1 | 21 | 0 | 0 |
| 3 | 18 | 6 | 3* |
| 7 | 16 | 3 | 0 |

* Paper chromatography of the dinitrophenylhydrazone indicated that this was probably pentanone-2.

In the mass spectrum of the acids from peaks *3* and *5*, the patterns of the hydrocarbon chains prove the acids to have a preference for splitting off $C_3$ and $C_5$ fragments. This behavior is to be expected for anteanteiso compounds[2] ($CH_3—CH_2—CH_2—C(CH_3)H—$), since splitting of the bonds on either side of a secondary carbon atom occurs easily. Experimentally, the validity of this determination of the position of branching was checked on a series of acids in the $C_{14}$ group, including the anteiso- and anteanteiso isomers.

From these results it follows that, apart from the normal aldehydes $C_{14}$ to $C_{18}$, the aldehydes derived from phosphorus-free ox heart lipids also contain 12-methyltridecanal, 11-, 12-, and 13-methyltetradecanal, 12-methylpentadecanal, and 14-, and possibly 15-methylhexadecanal.

(*c*) *The Aldehydes from the Phosphatides of Butter.* Two and seven-tenths g of phosphatide was isolated from 3 kg of butter, using the method of Koops (20). To liberate the aldehydes, the phosphatide, suspended in 25 ml of petroleum ether, was brought onto a 35 × 270 mm Celite®-dinitrophenylhydrazine-hydrochloric acid column. The dinitrophenylhydrazones (corresponding to 16 mg of hexadecanal) were purified and converted into methyl esters as described under (*a*). Because the amount of material was small, the total mixture was hydrogenated prior to gas-liquid chromatography (Fig. 3). Peaks *2*, *4*, *6*, *8*, and *9* correspond to the normal $C_{14}$ to $C_{18}$ esters. It is clear that branched chains are present in aldehydes obtained from butter phosphatides.

(*d*) *Aldehydes from Ox Heart Phosphatides.* As described (7), the phosphatides from ox heart were separated from the total lipids by means of dialysis, according to Van Beers *et al.* (21). Two g of this lipid mixture was investigated as described under (*c*). Three hundred and sixty mg of dinitrophenylhydrazones was obtained, corresponding to 206 mg of hexadecanal or to 613 mg of phosphatide, assuming choline as base and palmitic acid as fatty acid. The corresponding esters were subjected, before and after hydrogenation, to gas-liquid chromatography (Fig. 4). Only peak *9* (methyl ester of an octadecenoic acid) disappeared after hydrogenation. Peaks *2*, *4*, *6*, *8*, and *10* were in the positions of the normal fatty methyl esters $C_{14}$ to $C_{18}$.

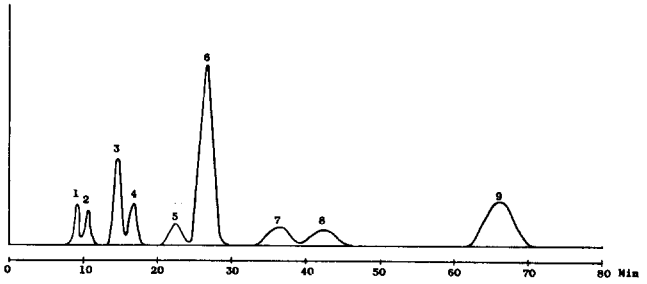


FIG. 3. Methyl esters corresponding to the aldehydes from butter phospholipids. Hydrogenated. (Nitrogen flow 100 ml/minute, temperature 200°, pressure 53 cm Hg, Celite®/Apiezon L 4:1.)

It might be expected that fractions representing peaks *1*, *3*, *5*, and *7* contained branched esters. This was confirmed for peaks *3* and *7* after saponification and oxidation with chromic acid (Table 3).

It may be concluded that peaks *3* and *7* contain both iso acids and anteiso acids. Thus the aldehydes derivable from ox heart phosphatides contain 13-methyltetradecanal, 12-methyltetradecanal, 15-methylhexadecanal, and 14-methylhexadecanal, in addition to normal chain compounds.

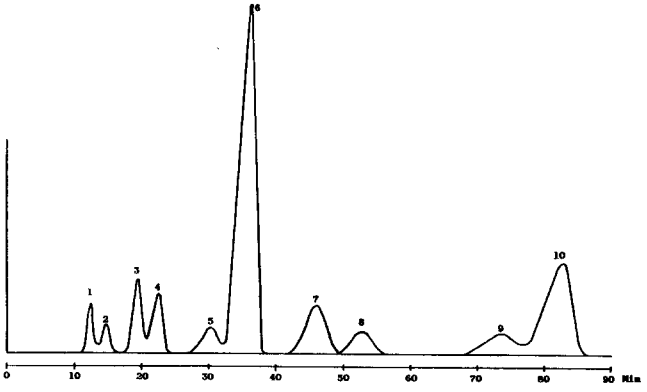


FIG. 4. Methyl esters corresponding to the aldehydes in ox heart phospholipids. (Nitrogen flow 30 ml/minute, temperature 197°, pressure 45 cm Hg, Celite®/Apiezon L 4:1.)

[2] The terms "anteiso-" and "anteanteiso-" mean that the branching occurs at carbon atoms situated β and γ to the terminal carbon, respectively.

TABLE 3. CHROMIC ACID OXIDATION OF ACIDS DERIVED FROM ALDEHYDES ISOLATED FROM OX HEART PHOSPHATIDES

| 2 mg Acid from Peak | μg Carbonyl Compounds Found After Oxidation | | |
|---|---|---|---|
| | Acetone | Butanone | Pentanone |
| 3 | 33 | 8 | 0 |
| 7 | 42 | 10 | 0 |

*Synthesis of Model Substances.* 11-Methyldodecanoic acid ethyl ester was prepared using the Kolbe synthesis, as described by Weedon (22) and Milburn and Truter (23), from ethyl hydrogen sebacate and 3-methylbutyric acid. The ester boiled over a range of 123°–135° at 3.5 mm. Yield based on sebacate, 51.4%. The free acid had a melting point of 40.6°–41.4°. Arosenius *et al.* (19) reported 40.6°–41.3°.

DL 11-Methyltridecanoic acid ethyl ester was prepared in a similar way from ethyl hydrogen sebacate and 3-methylpentanoic acid. The ester boiled at 153°–157° at 9 mm. Yield based on sebacate, 57%. The free acid melted at 14.5°–16.5°.

DL 10-Methyltridecanoic acid ethyl ester was prepared according to Cason and McLeod (24) by allowing propylmagnesium bromide to react with 10-keto-undecanoic acid ethyl ester (synthesized from dimethylcadmium and 9-carbethoxy-nonanoic acid chloride), dehydration of the 10-methyl 10-hydroxytridecanoic acid ethyl ester so formed and catalytic hydrogenation of the 10-methyltridecanoic acid ethyl ester. The yield of 10-methyltridecanoic acid ethyl ester (boiling range: 99°–106° at 3 mm) was 62% based on 10-keto-undecanoic acid ester.

The ethyl ester was converted via the free acid into the methyl ester. Gas-liquid chromatographic analysis showed that the final product was not pure. By fractionation over a preparative 1.5 × 200 cm gas-liquid chromatographic column (Celite® 150–300; 30% Silicone oil; 360 ml nitrogen/minute; temperature, 185°) the pure ester was obtained.

## DISCUSSION

The complicated nature of the bound aldehydes from the various sources is bewildering at first sight. When we compare what is known about fatty acids, however, some analogies are clearly apparent. Shorland (25) demonstrated the presence of both iso acids and anteiso acids in various animal fats. The percentages of branched-chain acids are, according to Shorland, very small, in total never exceeding 1%. Table 4 records the composition of the aldehydes found in our experiments. It is surprising that the percentage of branched-chain compounds is much higher, viz., about 30%.

TABLE 4. PERCENTAGE COMPOSITION OF ALDEHYDES DERIVED FROM VARIOUS LIPID FRACTIONS UP TO AND INCLUDING $C_{18}$

| Aldehydes | Butter-fat* | Non-phosphorus Ox Heart Lipids | Butter Phospha-tides | Ox Heart Phospha-tides |
|---|---|---|---|---|
| Branched $C_{13}$ | 4.5 | — | — | — |
| *n*-$C_{13}$ | 3.5 | — | — | — |
| Branched $C_{14}$ | 22.5 | 9 | 4.5 | 2.5 |
| *n*-$C_{14}$ | 11 | 4 | 4 | 1.5 |
| Branched $C_{15}$ | 39 | 19 | 13.5 | 5.5 |
| *n*-$C_{15}$ | 7 | 4.5 | 6.5 | 5 |
| Branched $C_{16}$ | | 2 | 3.5 | 3.5 |
| *n*-Hexadecenal | 3.5 | | } 35 | |
| *n*-$C_{16}$ | 8.5 | 36 | | 46.5 |
| Branched $C_{17}$ | | 4.5 | 7 | 7.5 |
| *n*-$C_{17}$ | | 1.5 | 5 | 4 |
| *n*-Octadecenal | | 4 | } 21 | 4.5 |
| *n*-$C_{18}$ | | 15.5 | | 19.5 |
| Per cent branched | | 34 | 28 | 23 |

* The values in this column are too high. The aldehydes were, in this case, obtained by degassing at 120° from the fat after this had been treated with acid. During this procedure neither octadecanal nor hexadecanal was recovered quantitatively. The values given are of importance, therefore, only insofar as they reflect the ratio of the branched and nonbranched $C_{13}$, $C_{14}$, and $C_{15}$ aldehydes.

According to Hartman (26), the branched acids can be related to the amino acids valine, leucine, and isoleucine. By decarboxylation and deamination of valine and leucine, branched $C_4$- and $C_5$-fragments are formed, which, on being built up with $C_2$-units, may give the even- and odd-numbered iso acids. Isoleucine could lead in the same way to the odd-numbered anteiso acids. This theory, when applied to the bound aldehydes, seems to fit fairly well.

The branched-chain aldehydes with an even number of carbon atoms were generally found to be of the iso type, while those containing an odd number of carbon atoms were apparently often a mixture of iso- and anteiso isomers. However, from the experiments with phosphorus-free ox heart lipids, it appeared that, among other aldehydes, 11-methyltetradecanal and 12-methylpentadecanal occur. These two aldehydes cannot be derived from amino acids in an analogous way.

An explanation for the facts observed can be given only when the role of the aldehydes in lipid metabolism is more clearly understood.

The authors wish to thank Mr. W. Sonneveld for his mass-spectrographical information, and Miss T. A. Vos and Mrs. J. de Waard for the synthesis of model substances. They are also indebted to the management of Unilever N.V., Rotterdam, for permission to publish this work.

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