Characterization of Some Heated Fat Components[1]

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Abstract

Substances produced at low levels in fat by heating were isolated and characterized. Partially hydrogenated soybean oil (Iodine Value 78) was heated at 182 C for 10, 8.5-hr, days with exposure to air. The oil was converted to ethyl esters, which were distilled and adducted with urea. The nonadductable fraction was subjected to chromatographic separations, and some of the components were purified sufficiently for chemical and spectroscopic characterization. Substances recognized include aromatic esters, saturated and unsaturated cyclic esters, ethoxyoctadecenoate, ethoxyhydroxyoctadecanoate, oxo-octadecanoate, oxo-octadecenoate, and cyclic hydroxy esters, all having 18 carbons in the acid chain.

Introduction

RECENT WORK (1) indicates that used frying fats do not adversely affect rats to which they are fed at high levels for long periods. To supplement that information we sought to find out what kinds of compounds are formed in fats during heating through temperature cycles comparable to those used for deep frying.

Many workers have heated fats under more or less severe conditions and carried out physical, chemical, or biological examinations on them, but relatively little progress has been made toward the actual identification of the substances present in fats after they have been heated at frying temperatures. The volatile materials which steam-distill out of fats during frying have been extensively characterized (2–8). The dimeric and polymeric materials which form in the fat appear to be so complex and intractable as to offer grave difficulties in their characterization (9–11), although some simple dimers have been identified (12,13) from abusively heated fatty materials. It has, of course, been widely recognized that cyclized fatty acids are present in severely heated fats (13–15).

Because of the likelihood that isolation of components from fat which had actually been used for frying would be complicated by the presence of substances derived from the food being fried, we chose, for the present work, to study fat heated in air at 182 C through cycles of heating and cooling not unlike those used for frying in some restaurants. From the heated fat we isolated the distillable nonurea-adductable fraction (16), separated this fraction into components, and characterized the components by chemical and spectroscopic methods. The components to be described are by no means all the components present in heated fat; they are the ones most amenable to the separation techniques employed, and many others, which could not be separated from each other, are not discussed here. The multistage separations used have not permitted us to make meaningful estimates of the levels at which the compounds to be described were present in the heated fat, although it is clear that they must have been quite low.

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Heating and Isolation Procedure

The fat used for this work was a soybean oil which had been refined, bleached, hydrogenated to iodine value (IV) 78, and deodorized under commercial conditions. Sixty kilograms of the fat were heated in a commercial gas-fired frying kettle at 182 C for 85 hr (10, 8.5-hr days, with cooling to room temperature overnight and on the weekend). By the end of this treatment the IV had fallen to 72, and the free fatty acid level had risen to 0.5%. Since this level of free fatty acid would have unduly complicated a base-catalyzed alcoholysis, the oil was saponified (in 23-kg portions) with alcoholic KOH. The fatty acids were extracted, dried, and esterified with absolute ethanol and H_2SO_4. The esters were then distilled under 5-torr pressure. Most of the material distilled quickly at head temperatures of 140–180 C. The pot temperature was briefly raised to 220 C to ensure nearly complete recovery of distillable materials; it is possible that some chemical changes may have occurred during this heating period. The distilled esters were adducted with urea by stirring at 50 C with 4 kg urea and 4 liters ethanol per liter of esters, cooling overnight to 25 C, and filtering. Two successive readductions with urea left about 135 g (0.6% of the fat) of distillable, nonurea-adductable material (DNUA) which was used for further work.

The first stage of separation was effected by stepwise gradient elution from a silica gel column. After application of 270 g DNUA to a column packed under hexane with 3 kg silica gel (Davison, 100–200 mesh, containing 5% added water), fractions were eluted with mixtures of hexane, benzene, and ether of gradually increasing polarity. The column was washed with each solvent until no more material was being eluted from it; then elution with a more polar solvent mixture was started.

The fractions eluted were further separated, either by repetition of the previous chromatographic procedure on a smaller scale, or by chromatography on silicic acid–silver nitrate (17). Stepwise gradient elution was employed for these chromatograms as well. The subfractions were examined by gas chromatography and, where it seemed applicable, preparative gas chromatography was employed for collection of individual components. Relatively nonpolar components were collected from 3/8-in. by 10-ft or 20-ft columns packed with 25% ethylene glycol adipate polyester (EGA) on Chromosorb W at 200 C with a He flow rate of 600 ml/min. After collection the components were freed of polyester decomposition products by chromatography on silica gel or by gas chromatography on silicone (SE-30). The more polar components were collected by preparative gas chromatography from a 3/8-in. by 10-ft column packed with 25% SE-30 on Chromosorb W. Adequate separations were maintained in most cases with sample injections of 100–200 μliter. Further details of the separations are given below for each component.

Table I summarizes the early stages of the separation scheme. It shows, for example, that the material eluted from silica gel with hexane:benzene 70:30 (vol/vol) was reapplied, after evaporation of sol-

TABLE I
Outline of Separation Scheme

DNUA adsorbed on silica gel eluted with	
Hexane	→ traces
Hexane:benzene 70:30	→ eluate divided into 2 portions, and readsorbed on
Silica gel eluted with	
Hexane	→ mixture
Hexane:benzene 90:10	→ linoleate
Hexane:benzene 80:20	→ mixture
Hexane:benzene 70:30	→ aromatic esters
	→ dioctyl phthalate
Hexane:benzene 60:40	→ ethoxyoctadecenoate
or Silicic acid-silver nitrate eluted with	
Hexane	→ hydrocarbons
	→ saturated cyclic esters
	→ branched long-chain esters
Hexane:benzene 90:10	→ unsaturated cyclic esters
Hexane:benzene 50:50	→ linoleate
Hexane:benzene 50:50	→ ethoxyoctadecenoate
Hexane:benzene 30:70	→ ethoxyoctadecenoate
Benzene	→ eluate readsorbed on
Silicic acid-silver nitrate eluted with	
Hexane:benzene 80:20	→ traces
Hexane:benzene 70:30	→ oxo-octadecanoate
Hexane:benzene 60:40	→ butyl carbobutoxymethyl phthalate
Hexane:benzene 50:50	→ mixtures
Hexane:benzene 40:60	→ mixtures
Hexane:benzene 20:80	→ oxo-octadecenoate
Benzene:ether 98:2	→ eluate readsorbed on
Silicic acid-silver nitrate eluted with	
Hexane:benzene 90:10	→ traces
Hexane:benzene 80:20	→ traces
Hexane:benzene 70:30	→ cyclic hydroxy esters
Hexane:benzene 60:40	→ ethoxyhydroxyoctadecanoate (liquid)
Benzene:ether 96:4	→ eluate readsorbed on
Silicic acid-silver nitrate eluted with	
Hexane:benzene 70:30	→ ethoxyhydroxyoctadecanoate (solid)

vents, to a silica gel column, and that elution with hexane:benzene (90:10) gave a concentrate from which linoleate was ultimately obtained. Designation of a product as a mixture indicates that no reasonably homogeneous substances could be concentrated from it by subsequent separation steps. Some solvents are shown to have eluted only traces of material; these elution steps are shown on the chart, because it was established that they were essential for development of the chromatogram and that omission of them gave inadequate separations with later solvent mixtures. Most of the substances shown as having been identified were accompanied in their respective eluate fractions by other materials from which they were separated by gas chromatography. Each of the columns indicated was washed with still more polar solvent mixtures, to achieve essentially complete recovery of the material applied; these more polar fractions are not shown in Table I since no components of them were identified.

Mass spectra of the purified components were obtained on an Atlas CH-4 spectrometer at 70 ev ionizing potential. Nmr spectra were run on a Varian instrument, either A-60 or HA-100, in CCl_4 with tetramethylsilane as reference. The shifts reported are accurate to at least $\pm$ 0.02 ppm.

Table II lists the nmr peaks observed for each of the components isolated, and shows the structural assignments made for them.

Hydrocarbons

A portion of the fraction of DNUA eluted from silica gel with hexane:benzene 70:30 was rechromatographed on silicic acid–silver nitrate. The first fraction eluted with hexane showed in its infrared spectrum no functional groups other than those attributable to saturated aliphatic hydrocarbons. It gave no response on the gas chromatograph under conditions suitable for fatty esters. It gave no recognizable parent peak on mass spectrometry, but its fragmentation pattern resembled that of hydrocarbons. The nmr spectrum showed only peaks characteristic of methyl and methylene protons, in a ratio of 1:3. It was concluded that the material was a hydrocarbon or mixture of hydrocarbons, of high molecular weight, and somewhat branched. Since such hydrocarbons cannot readily be visualized as having arisen from the familiar fatty acids or glycerides, we speculate that they might have been formed from the unsaponifiable portion of the fat, or might have been contaminants derived from solvents or stopcock grease.

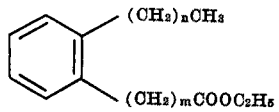
Ethyl Linoleate

Another portion of the fraction of DNUA eluted from silica gel with hexane:benzene 70:30 was rechromatographed on silica gel. The subfraction eluted from this second column with the same solvent mixture was subjected to preparative gas chromatography on EGA. One of the components collected was identified as ethyl linoleate by comparing its IR, nmr, and mass spectra with those of an authentic sample. The collected material differed from the reference in that the nmr triplet at 7.28 τ, attributed to the hydrogens on C_{11}, was only half as strong in the collected material as in the reference; this difference suggested that the collected sample contained substantial amounts of isomers in which the double bonds were not separated by a single methylene group. Such isomers are to be expected in hydrogenated fat. Other components of these fractions, which were not completely isolated, showed an infrared band at 10.3 μ, indicative of *trans* unsaturation. A variety of positionally and geometrically isomeric octadecadienoates are known to be components of hydrogenated soybean oil even before heating (18,19); they appeared in the DNUA because the urea adduction did not completely remove them from other nonadductable materials.

When the same fraction of DNUA was chromatographed on silicic acid–silver nitrate the octadecadienoates were partly eluted with hexane:benzene 50:50, and partly with more polar solvents.

Aromatic Esters

These compounds were eluted from two successive silica gel columns with hexane:benzene 70:30. The elution fraction in which they were found represented 0.3% of the DNUA. Its gas chromatogram showed a high proportion of a single peak. This material was collected from an SE-30 preparative column. Infrared absorption bands at 3.21, 6.22, 6.70, and 13.34 μ suggested that the material was an *ortho*-disubstituted aromatic. The mass spectrum of the isolated material showed a molecular weight of 304; its fragmentation pattern closely resembled that observed by Michael (20) for ethyl 8-(2-butylphenyl) octanoate. Detailed examination of the fragmentation pattern and of the nmr spectrum (Table II) led to the assignment of the following structure (where $n + m = 10$):



This corresponds to the structure established by Michael for material which he isolated from heated methyl linoleate. Our material, like his, apparently consists of several isomers which differ from each other in the lengths of the two side chains.

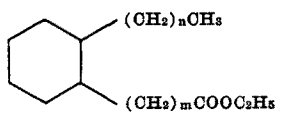
TABLE II
Nmr Spectra of DNUA Components

	Proton	Chemical[a] shift τ ppm
a c f h f c d e O g b $CH_3-(CH_2)_n-CH_2-C_6H_4-CH_2-(CH_2)_m-CH_2-CH_2-\overset{\overset{O}{\|}}{C}-O-CH_2-CH_3$ Aromatic ester $n+m=6$	a b c d e f g h	9.10 8.81 8.69 8.43 7.81 7.46 6.01 3.09
a c c c d e O f b $CH_3-(CH_2)_n-C_6H_{10}-(CH_2)_m-CH_2-CH_2-\overset{\overset{O}{\|}}{C}-O-CH_2-CH_3$ Saturated cyclic ester $n+m=8$	a b c d e f	9.10 8.76 8.72 8.47 7.80 5.96
a c c c e h h e c d f O g b $CH_3-(CH_2)_n-C_6H_{10}-(CH_2)_m-CH_2-CH=CH-CH_2-(CH_2)_j-CH_2-CH_2-\overset{\overset{O}{\|}}{C}-O-CH_2-CH_3$ Unsaturated cyclic ester $n+m+j=4$	a b c d e f g h	9.14 8.90 8.74 8.50 8.14 7.87 6.02 4.78
a H_3C a c c d e O f b $CH_3-(CH_2)_n-CH-(CH_2)_m-CH_2-CH_2-\overset{\overset{O}{\|}}{C}-O-CH_2-CH_3$ Branched chain ester $n+m=14, 15, 16, 17$	a b c d e f	9.10 8.76 8.74 8.44 7.84 5.99
a d i k k f d e g O j b $CH_3-(CH_2)_n-CH-CH=CH-CH_2-(CH_2)_m-CH_2-CH_2-\overset{\overset{O}{\|}}{C}-O-CH_2-CH_3$ $O-CH_2-CH_3$ (h, c) Ethoxyoctadecenoate $m+n=10$	a b c d e f g h i j k	9.10 8.89 8.76 8.67 8.45 8.00 7.83 6.83 6.60 5.98 4.70
a c d f O f d c d e O g b $CH_3-(CH_2)_n-CH_2-CH_2-\overset{\overset{O}{\|}}{C}-CH_2-CH_2-(CH_2)_m-CH_2-CH_2-\overset{\overset{O}{\|}}{C}-O-CH_2-CH_3$ Oxo-octadecano te $n+m=9$	a b c d e f g	9.10 8.84 8.71 8.48 7.79 7.72 5.97
a c e i i e c d g O g d c d f O h b $CH_3-(CH_2)_n-CH_2-CH=CH-CH_2-(CH_2)_m-CH_2-CH_2-\overset{\overset{O}{\|}}{C}-CH_2-CH_2-(CH_2)_j-CH_2-CH_2-\overset{\overset{O}{\|}}{C}-O-CH_2-CH_3$ Oxo-octadecenoate $n+m+j=5$	a b c d e f g h i	9.10 8.98 8.90 8.47 8.05 7.80 7.73 5.98 4.71
d HO O a c c c f c e g b $CH_3-(CH_2)_n-C_6H_{10}-(CH_2)_m-CH-(CH_2)_j-CH_2-\overset{\overset{O}{\|}}{C}-O-CH_2-CH_3$ Cyclic hydroxy ester $n+m+j=18$	a b c d e f g	9.10 8.76 8.69 8.50[b] 7.79 6.51 5.91
f i c HO O-CH_2-CH_3 O a d b h d e g j b $CH_3-(CH_2)_n-CH-CH-(CH_2)_m-CH_2-CH_2-\overset{\overset{O}{\|}}{C}-O-CH_2-CH_3$ Ethoxy-hydroxy-o tadecanoate $n+m=12$	a b c d e f g h i j	9.10 8.88 8.75 8.61 8.43 8.02[b] 7.80 6.96 6.50 5.90

[a] All shifts normalized on basis of terminal CH_3's at 9.10 τ.
[b] Disappears on shaking with D_2O.

Saturated Cyclic Esters

This component was also among the materials eluted from silica gel with 70:30 hexane:benzene. The fraction was rechromatographed on silicic acid–silver nitrate; one of the subfractions eluted from that column with hexane showed several peaks on gas chromatography. The component of lowest retention time was collected from an SE-30 column. The infrared spectrum of the isolated material was appropriate for a saturated fatty acid ester, except that the tetramethylene absorption at 13.9 μ was comparatively weak. The mass spectrum showed a parent peak at m/e 310, and a fragmentation pattern resembling those of the saturated monocyclic C_{18} acid esters prepared by Michael (20). The nmr spectrum showed peaks (Table II) consistent with the following structure (where $n+m=10$):



Unsaturated Cyclic Esters

Elution of the silicic acid–silver nitrate column referred to in the preceding paragraph with hexane:benzene 90:10 gave a concentrate from which a single peak was isolated by preparative gas chromatography on SE-30. The isolated material had an infrared spectrum appropriate for the ethyl ester of a fatty

acid, but also showed a band at 10.3 μ, indicating *trans* unsaturation. The mass spectrum showed a parent peak at m/e 308, corresponding to the ethyl ester of a C_{18} mono-unsaturated cyclic acid. Presence of a double bond was confirmed by the appearance in the nmr spectrum of a band at 4.78 τ. The substance was hydrogenated, and the hydrogenation product gave spectra closely resembling those of the saturated cyclic esters mentioned above.

The location of the double bond was not determined; the mass spectrum gave no clear indication of its position. It seems most likely that the material isolated consists of several isomers having the double bond in different positions in the two side-chains, and possibly in the ring as well. Thus the material resembles one of the components isolated by Michael (21) from heated methyl linoleate.

Branched Long-Chain Esters

The same chromatographic subfraction from which the saturated cyclic esters were isolated contained 4 additional compounds, of longer gas chromatographic retention times. They were collected as single peaks by preparative gas chromatography. The 4 materials had similar infrared spectra, appropriate for saturated fatty acid esters. The mass spectra showed parent peaks at m/e = 340, 354, 368, and 382, respectively. These molecular weights correspond to ethyl esters of saturated open-chained fatty acids having 20, 21, 22, and 23 carbon atoms.

The nmr spectra (Table II) showed the features expected for the ethyl esters of saturated fatty acids, except that in each spectrum the integrated areas indicated 6 terminal methyl protons, rather than 3 as in a normal ester. It was concluded that the 4 materials were a series of branched chain esters, having one branch per molecule.

The mass spectral fragmentation patterns of the 4 substances were examined in detail in an effort to establish the lengths and locations of the branches. Although the patterns suggested some possible locations for branch points, they were not conclusive, and the interpretation seems too equivocal to justify its being included here.

Ethoxyoctadecenoate

This, the most abundant single component of the DNUA, appeared in several fractions of the separation scheme (Table I). From those fractions which were richest in it, it was concentrated by preparative gas chromatography on EGA. Since preparative gas chromatography caused the material partly to decompose to conjugated diene, final purification was accomplished by column chromatography on silica gel. The infrared spectrum of the purified material showed, besides the usual fatty ester bands, absorptions at 9.15 and 10.28 μ, characteristic of an ether linkage and a *trans* double bond, respectively. Carbon and hydrogen analyses, IV, saponification value, and molecular weight (by mass spectrometry) closely fitted the values expected for ethyl ethoxyoctadecenoate.

The nmr spectrum showed bands appropriate for a molecule containing one double bond and an ethoxyl group in addition to the ester function. The vinyl protons showed a pattern indicative of the grouping $-CH-CH=CH-CH_2-$, since they appeared as an $ABXY_2$ multiplet with $J_{AB} \approx 16$Hz, $J_{AX} \approx 7$Hz, and $J_{BY} \approx 6$Hz. (J_{AY} and J_{BX} were too small to be measured in this spectrum.) This analysis of the nmr spectrum established that the ethoxyl group was attached to an allylic position.

It seemed likely that the ethoxyl group had been introduced adventitiously during the conversion of glyceride esters to ethyl esters. To verify this assumption, additional quantities of this and related compounds were prepared without acid-catalyzed esterification reactions. Pure methyl oleate was heated at 182 C for 50 hr with a slow stream of air bubbling through it. The dark-colored ester was distilled under vacuum; the distillate was adducted with urea, and the DNUA was chromatographed. The fraction corresponding to the heated fat fraction from which the component in question had been obtained yielded, by gas chromatography, a substance whose analytical values and spectra corresponded to those expected for methyl methoxyoctadecenoate. Treatment of this ester with ethanol and base afforded ethyl methoxyoctadecenoate. Treatment of the latter with ethanol and acid gave ethyl ethoxyoctadecenoate, which also was prepared de novo by heating ethyl oleate in air, followed by distillation, urea adduction, and chromatography as before. Finally the ethyl ethoxyoctadecenoate was converted to methyl ethoxyoctadecenoate by base-catalyzed transesterification with methanol. From these experiments it was clear that the alkyl group of the ester function could be exchanged by base-catalyzed transesterification without affecting the alkoxyl function, and that the latter could be exchanged by treatment with alcohol and acid. Such lability confirmed that the alkoxyl group was in an allylic position with respect to the double bond.

Interpretation of the mass spectra was facilitated by comparing the spectra of the various alkyl derivatives. Mass spectra of the substances derived from pure oleate showed only 4 peaks of significant size in the middle regions of the spectra. For the ethyl ethoxyoctadecenoate two of these were at m/e 197 and 241, attributed respectively to $H(CH_2)_8-CH=CH-CH(OR)^+$ and $RO_2C-(CH_2)_6-CH(OR)-CH=CH^+$, (where R = ethyl), formed by fragmentation of ethyl 8-ethoxyoleate. The other two were at m/e 183 and 255, attributed to $H(CH_2)_7-CH(OR)-CH=CH^+$ and $RO_2C-(CH_2)_7-CH=CH-CH(OR)^+$ from ethyl 11-ethoxyoleate. In the mass spectrum of methyl methoxyoctadecenoate, the corresponding fragments were seen at m/e 183, 213, 169, 227; they were attributed to the fragments shown above but with R = methyl. The presence of oxygen functions on the 8 and 11 carbon atoms of oxidized oleate is, of course, in agreement with the known mechanism of oleate autoxidation.

The methyl and ethyl derivatives prepared from heated triglyceride showed the same peaks in positions appropriate for the methyl and ethyl groups which they contained, but each of the peaks was in a series of peaks which differed from one another by 14 mass units. The range of these series showed that the material derived from heated fat was a mixture of substances having the double bond in various positions along the chain from the seventh to the fourteenth position, but predominantly at the ninth position. This is the normal distribution of double bonds in the mono-enoic acids of partially hydrogenated soybean oil.

Oxo-octadecanoates

The portion of the DNUA eluted from the silica gel column with benzene was rechromatographed

on silicic acid–silver nitrate. The middle portion of this eluate contained a major component, which was isolated by preparative gas chromatography on SE-30.

The infrared spectrum of the isolated material showed 2 peaks in the carbonyl region (5.7 and 5.8 μ). The mass spectral fragmentation pattern suggested the location of the keto group through comparisons with the fragmentation patterns established for oxo-octadecanoates by Ryhage and Stenhagen (22). There was a series of peaks having m/e = 14 n + 2, which could be attributed to ions of the type R–C(OH) = CH_2^+. The largest peak in this series was at m/e = 128, corresponding to C_6H_{13}–C(OH) = CH_2^+, an ion which would have arisen from 12-ketostearate. Ions derived from isomers having the keto group at other positions up and down the chain were also present in significant amounts. Another series of important peaks was the one having m/e = 14 n + 3, which could represent ions of the type $C_2H_5OOC-(CH_2)_m^+$. The most prominent ion of this series was at m/e = 199, which corresponds to the formula given when m = 9, and would have arisen from 11-ketostearate. Homologous ions were also present in significant amounts. Still another indicative series of ions had m/e = 14 n + 4, which could represent ions of the type $C_2H_5OOC-(CH_2)_m-C(OH) = CH_2^+$. The most prominent of these ions was at m/e = 256, corresponding to the formula shown with m = 10, and presumably arising from 12-ketostearate. The ion of m/e = 228, which would have come from 10-ketostearate, was also relatively abundant. These patterns make it seem likely that the keto group in this material is distributed up and down the chain, but is predominantly near the positions originally occupied by the double bonds of the fatty acid.

Epoxides have been reported (23,24) as components of oxidized fats. It is easy to formulate a rearrangement of epoxystearate to ketostearate. If the ketostearate does arise through such a pathway, then the keto group would be expected to occupy one of the carbon atoms originally linked to its neighbor by a double bond.

Oxo-octadecenoates

This material was eluted from the silica gel column with benzene, and from silicic acid–silver nitrate with 20:80 hexane:benzene. It was purified by preparative gas chromatography on SE-30.

The infrared spectrum showed 2 peaks in the carbonyl region and a band at 10.3 μ characteristic of *trans* unsaturation. Its nmr spectrum resembled that of oxo-octadecanoate, but also showed absorption at 4.71 τ due to vinyl protons and at 8.05 τ due to allylic protons. Its mass spectrum showed a parent peak at m/e = 324, appropriate for an unsaturated keto-ester. Hydrogenation of the material yielded a product whose spectra resembled very closely those of the oxo-octadecanoate described above. It is concluded that the substance was ethyl oxo-octadecenoate. No conclusions could be drawn from the mass spectrum regarding the location in the molecule of the double bond or the keto group.

Cyclic Hydroxy Esters

This component was found in the fraction of the DNUA which eluted from silica gel with benzene:ether 98:2 and, in a subsequent step, from silicic acid–silver nitrate with 70:30 hexane:benzene. Although it could be detected by analytical gas chromatography, it could not be collected by preparative gas chromatography without extensive decomposition. Final purification was accomplished by a repetition of the silicic acid–silver nitrate chromatography step.

The infrared spectrum of the material showed OH absorption at 2.8 μ, as well as the usual bands for ester and tetramethylene. The nmr spectrum also showed an OH band. The mass spectrum showed m/e = 308 as the heaviest ion. It was surmised that the compound was an alcohol which dehydrated in the heated inlet of the mass spectrometer. The compound was converted to the trimethylsiloxyl derivative by treatment with hexamethyldisilazane, and the derivative showed a parent peak at m/e = 398. This molecular weight is correct for the trimethylsiloxyl derivative of an alcohol having a molecular weight of 326. The molecular weight of 326 corresponds to the ethyl ester of a C_{18} acid having one hydroxyl group and one ring or one double bond. Since neither the IR nor nmr spectrum suggests the presence of a double bond, it is concluded that the material is a cyclic, hydroxyl-containing fatty ester. No further structural conclusions could be drawn with certainty from the spectra, and it is supposed that the material isolated is a mixture of isomers.

Ethoxy-hydroxy-octadecanoates

Components recognized as ethoxy-hydroxy-octadecanoates were found in 2 different fractions, as indicated in Table I. One of the materials, eluted from silica gel with benzene:ether 98:2, could not be solidified by cooling. The other, eluted from silica gel with benzene:ether 96:4, solidified on standing in the refrigerator. Each of the materials was examined separately. Infrared spectra indicated both of them to be long-chain esters containing hydroxyl and ether functions, and no double bonds. The nmr spectra showed one hydroxyl group and one ethoxyl group in each of them. Elemental analyses were consistent with this interpretation. Some key peaks from the mass spectra of the two compounds are listed in Table III. These spectra suggest that the solid material is largely ethyl 10-ethoxy-9-hydroxyoctadecanoate, while the liquid material is largely 9-ethoxy-10-hydroxyoctadecanoate, with each of these substances being accompanied by a range of positional isomers. Since it is not possible to distinguish between fragments containing hydroxyl groups and fragments containing ethoxyl groups on the basis of their masses alone, alternate explanations can be devised to account for the peaks listed in Table III, but the alternate explanations would require that the oxygen functions be predominantly at positions other than the ninth and tenth positions of the fatty

TABLE III
Mass Spectral Fragmentation Patterns for Alkoxy Hydroxy Stearates

		Solid isomers: $C_2H_5OOC-(CH_2)_n-CH(OH)-CH(OC_2H_5)-(CH_2)_mH$		Liquid isomers: $C_2H_5OOC-(CH_2)_n-CH(OC_2H_5)-CH(OH)-(CH_2)_mH$	
n	m	Fragment a	Fragment b	Fragment a	Fragment b
5	10	173 w	199 w	201 s	171 s
6	9	187 m	185 m	215 s	157 s
7	8	201 vs	171 vs	229 vs	143 vs
8	7	215 s	157 s	243 vs	129 vs
9	6	229 s	143 s	257 s	115 s
10	5	243 w	129 w	271 s	101 s
11	4	257 w	115 –	285 m	87 m

Letters indicate relative intensities of peaks observed in the spectrum: w = weak; m = medium; s = strong; vs = very strong; (–) = absent.

acid chains. Although other interpretations cannot be ruled out, they seem less likely than the one offered.

The presence of ethoxy-hydroxy-octadecanoates among the esters prepared from heated fats can be most readily accounted for by supposing them to have been formed when epoxyoctadecanoate, in the original fat, was treated with acidic ethanol for esterification.

Phthalate Esters

Phthalate esters were found in 2 of the fractions of the DNUA. They were easily recognized as phthalates by their characteristic infrared absorption bands at 6.2 and 6.4 μ. Comparisons of the nmr and mass spectra of the isolated materials with spectra of known compounds showed that the isolated materials were di-octyl phthalate and butyl butylphthalylglycolate, both of which are common plasticizers. The entire process of preparing and fractionating the DNUA was repeated, except that we used freshly distilled solvents and scrupulously avoided contact of the fatty material or solvent with polyvinyl tubing. In this experiment no phthalates were found, despite a deliberate search for them, and it was concluded that the phthalates observed earlier were contaminants (25).

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REFERENCES

1. Nolen, G. A., J. C. Alexander and N. R. Artman, J. Nutr. *93*, 337–348 (1967).
2. Kawada, T., R. G. Krishnamurthy, B. D. Mookherjee and S. S. Chang, JAOCS *44*, 131–135 (1967).
3. Krishnamurthy, R. G., and S. S. Chang, Ibid. *44*, 136–140 (1967).
4. Krishnamurthy, R. G., T. Kawada and S. S. Chang, Ibid. *42*, 878–882 (1965).
5. Ota, S., N. Iwata, A. Mukai and H. Enei, Yukagaku *12*, 403–409 (1963).
6. Wishner, L. A., and M. Keeney, JAOCS *42*, 776–778 (1965).
7. Akiya, T., Yukagaku *14*, 347-352 (1965).
8. Dornseifer, T. P., S. C. Kim. E. S. Keith and J. J. Powers, JAOCS *42*, 1073–1075 (1965).
9. Ota, S., N. Iwata and M. Morita, Yukagaku *13*, 210–217 (1964).
10. Ota, S., A. Mukai, N. Iwata, I. Yamamoto and M. Morita, Ibid. *13*, 471–477 (1964).
11. Sahasrabudhe, M. R., and I. G. Farn, JAOCS *41*, 264–267 (1964).
12. Paschke, R. F., L. E. Peterson and D. H. Wheeler, Ibid. *41*, 723–727 (1964).
13. Michael, W. R., J. C. Alexander and N. R. Artman, Lipids *1*, 353–358 (1966).
14. Crampton, E. W., R. H. Common, E. T. Pritchard and F. A. Farmer, J. Nutr. *60*, 13–24 (1956).
15. Matsuo, N., "Symposium on Foods: Lipids and Their Oxidation," H. W. Schultz, E. A. Day and R. O. Sinnhuber, ed, Avi Publishing Co., Westport, Conn., 1962, pp. 339ff.
16. Crampton, E. W., R. H. Common, F. A. Farmer, A. F. Wells and D. Crawford, J. Nutr. *49*, 333–346 (1953).
17. De Vries, B., JAOCS *40*, 184–186 (1963).
18. Kuemmel, D. F., and L. R. Chapman, Anal. Chem. *38*, 1611–1614 (1966).
19. Jones, E. P., C. R. Scholfield, V. L. Davison and H. J. Dutton, JAOCS *42*, 727–730 (1965).
20. Michael, W. R., Lipids *1*, 359–364 (1966).
21. Michael, W. R., Ibid. *1*, 365–368 (1966).
22. Ryhage, R., and E. Stenhagen, Arkiv Kemi *15*, 545–574 (1960).
23. Ellis, G. W., Biochem. J. *46*, 129–141 (1950).
24. Krull, L., Fette, Seifen, Anstrichmittel *61*, 223–227 (1959).
25. Artman, N. R., W. R. Michael and J. C. Alexander, JAOCS *44*, 372 (1967).

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