

Alteration of some long-chain esters during gas-liquid chromatography*

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SUMMARY

It has been found that the methyl esters of some naturally occurring fatty acids or their autoxidation products are altered during gas-liquid chromatography. Conjugated trienoates undergo *cis-trans* isomerization. The esters of vicinally unsaturated hydroxy derivatives, with either ethylenic or acetylenic bonds, are dehydrated. Acetylation of the hydroxy group provides little or no protection against such changes. Unsaturated hydroperoxides, which are primary products of autoxidation, are similarly altered to more highly unsaturated derivatives. Conjugated dienoates and hydroxy esters which are not vicinally unsaturated are stable under the same conditions. It is considered that these changes are caused primarily in the flash heater because of high temperature but that metal catalysis, by components of the flash heater, promotes alterations involving dehydration and deacetylation.

Examination of long-chain fatty materials by gas-liquid chromatography (GLC) requires high temperatures, and thermal alteration of some components may be expected. Stoffel *et al.* (1) have shown that the common methylene-interrupted polyunsaturated esters may be safely analyzed by GLC. Since these esters, and those of the saturated and monounsaturated acids, are the only known esters derived from most animal lipid fractions, GLC presumably may be applied to fresh ester samples from these sources without fear of ambiguous results.

Many vegetable oils, however, contain acids with conjugated unsaturation, acetylenic bonds, or functional groups containing oxygen. Until it is shown that these components are unaffected, or until the products of their alteration are known, GLC cannot be used indiscriminately for the analysis of vegetable oils of unknown composition. This paper describes alterations during GLC of the esters of several acids which occur naturally and in autoxidized oils.

MATERIALS AND METHODS

Methyl α - and β -elaeostearates and *cis,trans*- and *trans,trans*-conjugated linoleates were prepared in this

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Institute. Ultraviolet and infrared spectral studies indicated that the trienoates were essentially pure isomers but that the dienoates were each contaminated with some of the other isomer.

Methyl oleate hydroperoxides and methyl linoleate hydroperoxides and their hydroxyl analogues, formed by stannous chloride reduction, were supplied by O. S. Privett of this Institute and were more than 95% pure.

Methyl 9-hydroxy-*trans,trans*-10,12-octadecadienoate (methyl dimorphecolate) derived from *Dimorphothea aurantiaca* seed oil, which contains about 60% of the acid (2), was isolated by thin-layer silicic acid chromatography according to the method of Malins and Mangold (3).

The hydroxy ester fraction (4) of *Onguekoa Gore* (Boleka) seed oil was obtained by countercurrent partition between hexane and 80% methanol (5). The purity of all oxygenated esters was checked by thin-layer chromatography and, where necessary, purification was effected by column chromatography on silica gel.

Acetylation of reduced oleate hydroperoxides and methyl dimorphecolate was accomplished by heating with a mixture of acetic anhydride-pyridine 1/3 (v/v). The completeness of the reaction was checked by thin-layer chromatography (6).

Gas-liquid chromatographic studies were carried out with three separate columns. Two of these were copper columns, each having a separate brass flash heater.

The third was a glass column, the top 7 cm of which was packed with uncoated Celite®, and separately heated. This portion is subsequently referred to as the flash heater. The column conditions were:

(a) A 180 cm \times 3.5 mm I.D. copper column having as its stationary phase 15% of LAC 2-R446¹ polyester resin coated on acid- and alkali-washed Celite®, 80 to 100 mesh. The column was held at 196°, and the flash heater and the β -ionization detector at 250°. Argon at 25 psi flowed at a rate of 54 ml per minute (S.T.P.). The detector (Research Specialties) was operated at 800 volts. Under these conditions methyl palmitate had a retention time of 5.8 minutes.

(b) A 60 cm \times 3.5 mm I.D. copper tube packed with 20% Apiezon M² on acid- and alkali-washed Celite®, 80 to 100 mesh. The column was held at 204° and the flash heater and detector at 240°. Argon, as carrier gas, flowed at 54 ml per minute (S.T.P.) at a head pressure of 9 psi. Under these conditions methyl palmitate had a retention time of 7.8 minutes.

(c) A 90 cm \times 6 mm I.D. glass tube packed with acid- and alkali-washed Celite® (80 to 100 mesh) coated with 15% LAC 2-R446 polyester. The polyester used for this column was first purified from acetone solution by the method of Craig and Murty (7) and was thus not identical to that used in the copper column. The column was maintained at 190° and the β -ionization detector (Barber-Coleman Model 10) at 240°. The flash heater was held at temperatures of 300°, 250°, 225°, and 200°. Argon, under a head pressure of 24 psi, flowed at 40 ml per minute (S.T.P.). Under these conditions methyl palmitate had a retention time of 2.1 minutes. The brass detector was connected to the column by a length of Teflon® tube so that the detector could be bypassed in the collection of effluent fractions.

The acid- and alkali-washed Celite® support was coated by making a slurry with an acetone or hexane solution of the stationary phase and removing the solvent under vacuum in a rotary evaporator. Columns were conditioned at 200° for 3 to 5 days before use and thereafter no evidence of bleeding of stationary phase was obtained. The life of the columns was found to be at least two to three months. Samples were generally injected, through a silicone rubber cap, as 1 to 10 λ amounts of 1% to 2% solutions in acetone or

methanol. Fractions were collected after GLC by passing the effluent gas through a short Teflon® tube into methanol or hexane maintained at -70°. After collection of each fraction, the tube was removed and rinsed with the same solvent.

Ultraviolet spectra were measured with a Beckman DK2 Recording Spectrophotometer in conventional 1 cm fused silica cells. Infrared analyses on a micro-scale were obtained by P. R. Edmondson and H. Dinsmore of the Department of Medicine of this university, with a Perkin-Elmer 12C Spectrophotometer with Model 81 microscope and a Reeder thermocouple.

The initial study of all the esters described was carried out on the polyester-packed copper column and a few of the findings were checked on the Apiezon-packed copper column. Since it has been suggested that metal columns are likely to cause changes in lipid esters during GLC, whereas glass columns are unlikely to do so, five key compounds were closely examined on the polyester-packed glass column.

RESULTS

The results obtained on the three gas-liquid chromatographic columns used are summarized in Table 1, where they appear in the same order as described in the text. The carbon-number system proposed by Böttcher *et al.* (8) has been used to present gas-chromatographic data, rather than absolute or relative retention times and volumes which give a less clear over-all picture.

Conjugated Dienoates and Trienoates. Although the samples of *cis,trans* and *trans,trans* dienoates used were each contaminated with the other, giving two peaks, collecting and rechromatographing the separate components resulted in single peaks from all columns. Under the various conditions used, therefore, thermal *cis-trans* isomerization of these conjugated dienoates did not take place (Table 1). This conclusion is supported by the work of Beerthuis *et al.* (9), who separated the esters of the conjugated products of alkali isomerization of linoleic acid, presumably on a glass column, and obtained a far higher proportion of the *cis,trans* isomers than would have resulted if significant isomerization during GLC had occurred.

With conjugated trienoates, however, isomerization did occur. On the metal columns, α - and β -elaeostearate gave identical patterns, a composite first peak (carbon number 22.3), due to mixed *cis*- and *trans*-conjugated trienoates, and a sharp second peak (carbon number 22.7), due to the all *trans* isomer (Table 1). The chromatograms were identical to that illustrated in Figure

¹This material may be obtained from Cambridge Industries Company, Cambridge, Mass.

²Registered: Metropolitan-Vickers Electrical Co., Ltd., England.

1. Reinjection of the single-peak components showed that each isomer gave the same twin peak pattern. Since this pattern was obtained from both α - and β -elaeostearates, it must represent the equilibrium of thermal isomerization. Moreover, the shape of the peaks and the symmetry of the peak due to the all *trans* component in particular indicate that this equilibrium was attained in the flash heater immediately after injection, and before the components entered the column. If the equilibrium were attained gradually during passage through the column, the all *trans* peak, for example, would have an asymmetrical leading edge.

On the glass column, *cis-trans* isomerization of conjugated trienoates occurred also, but to a lesser extent. Equilibrium was not attained, as it was with the metal columns. As the temperature of the flash heater was reduced, the proportion of isomerized product to original material was reduced. The curves obtained with β -elaeostearate are shown in Figure 2. α -Elaeostearate gave a similar small proportion of the β -isomer under the same conditions, with chromatograms the inverse of those shown in Figure 2. The latter part of the β -elaeostearate peak (uncontaminated by α -isomer) was collected directly from the column exit

TABLE 1. CARBON NUMBERS AND ULTRAVIOLET SPECTRAL DATA OF FRACTIONS OBTAINED BY GAS-LIQUID CHROMATOGRAPHY OF SOME HYDROXY AND CONJUGATED POLYENOIC ESTERS

Methyl Ester Injected	Products	Carbon Number			Wave Length of Principal Maxima* in Product
		LAC 2-R446		Apiezon M	
		Copper	Glass	Copper	
					<i>mμ</i>
<i>cis,trans</i> -conj. dienoate	I	19.7	19.8	18.2	233(1)
<i>trans,trans</i> -conj. dienoate	II	20.5	20.5	18.7	231(1)
<i>cis,trans,trans</i> -conj. trienoate (α -elaeostearate)	III	22.3	22.1	19.4	269(3)
	IV	22.7	22.5	19.7	267(3)
<i>trans,trans,trans</i> -conj. trienoate (β -elaeostearate)	III	22.3	22.1	19.4	269(3)
	IV	22.7	22.5	19.7	267(3)
12-hydroxyoctadecanoate		24.2	24.1		
12-hydroxyoctadec-9-enoate		24.6	24.4		
12-hydroxyoctadec-9-ynoate		25.0	24.8		
Reduced oleate hydroperoxides	I	19.7	19.8	18.2	232(1)
	II	20.5	20.5	18.7	230(1)
Reduced linoleate hydroperoxides	III	22.3	22.1	19.4	270(3)
	IV	22.7	22.5	19.7	268(3)
Dimorphecolate (9-hydroxy octadec-10,12-dienoate)	III	22.3	22.1		270(3)
	IV	22.7	22.5		267(3)
2-hydroxyoctadecanoate		22.1	22.0		
Boleka nonhydroxy esters	bolekie	24.1			267(5)
	isanic	24.9			226(5)
Boleka hydroxy esters	V	23.7			267(7)
	VI	24.5			267(5)
Acetylated dimorphecolate	III	22.3	22.1		
	IV	22.7	22.5 †		
Acetylated reduced oleate hydroperoxides	I	19.7	19.8		
	II	20.5	20.5 †		
Oleate hydroperoxides	I	19.7	19.8		233(1)
	II	20.5	20.5		231(1)
Linoleate hydroperoxides	III	22.3	22.1		269(3)
	IV	22.7	22.5		268(3)

I = *cis,trans*-conjugated diene esters; II = *trans,trans*-conjugated diene esters; III = mixed *cis,trans*-conjugated triene esters; IV = *trans,trans,trans*-conjugated triene esters; V = probably octadec-7,13-diene-9,11-diynoate; VI = probably octadec-7,17-diene-9,11-diynoate.

* Number of maxima indicated in parentheses.

† Also peak at 24.0 (see text).

‡ Also peaks at 22.9 and 23.4 (see text).

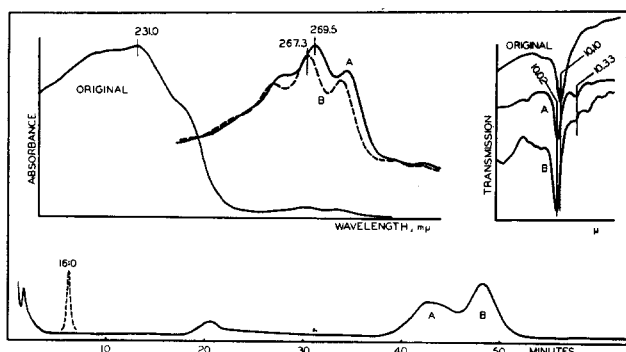


FIG. 1. Gas-liquid chromatograms of pure methyl dimorphocolate (9-hydroxyoctadec-*trans,trans*-10,12-dienoate) on 180 cm copper column of LAC 2-R446 at 196°, plus methyl palmitate dotted in as a reference. Inserts show ultraviolet and infrared spectra (10, 11) of the original ester and fractions A and B collected after GLC.

to avoid the influence of the metal of the detector; when rerun, the chromatogram was identical to that from which the collection had been made. Thus isomerization must have taken place in the glass system. The shapes of the curves (Fig. 2), and the fact that some isomerization occurs even when the flash heater (at 200°) is almost the same temperature as the column (190°), indicate that much of the isomerization is effected in the column. This is in contrast to the results obtained with the copper columns and possible reasons for this difference are discussed below.

According to Beerthuis *et al.* (9), GLC may be used to study the products of alkaline isomerization reactions. From our results, this is true only for reactions producing conjugated dienes, and not when conjugated trienes are formed. The gas-liquid chromatograms of these conjugated trienoates, and of all compounds mentioned hereafter which give rise to conjugated trienoates, show a peak with a carbon number of 20.0 on the polyester-packed copper column, or 19.8 on the glass column (see Figs. 1, 2, and 5). This peak trails into those due to conjugated trienoates, indicating continuous production along the length of the column in addition to the initial amount produced in the flash heater. Unless the probable occurrence of this peak in certain samples is recognized, there is a possibility of confusing it with methyl arachidate or methyl linolenate (carbon number 19.5). The structure of the component giving rise to this peak is unknown and not enough of it could be collected to obtain ultraviolet or infrared spectral characteristics. However, it seems possible that it may be a cyclic product similar to that formed on heat treatment of α -elaeostearate (12).

Hydroxy Compounds. Saturated hydroxy esters can be subjected to GLC without any alteration in struc-

ture. 12-Hydroxy stearate emerged with a carbon number of 24.2 from the polyester-packed copper column, and 24.1 from the glass column. Methyl ricinoleate and ricinostearate, their ethylenic and acetylenic bonds in the β -position to the hydroxy-substituted carbon, are similarly stable to GLC under the various conditions used. These esters emerge from the polyester-packed copper column with carbon numbers 24.6 and 25.0, respectively, and from the glass column with carbon numbers of 24.4 and 24.8, respectively. This stability is lost, however, when the hydroxyl carbon is adjacent to the center of unsaturation.

Reduced methyl oleate hydroperoxides (a mixture of octadec-*trans*-8-hydroxy-9-enoate, -9-hydroxy-10-enoate, -10-hydroxy-8-enoate, and -11-hydroxy-9-enoate) were completely dehydrated during GLC on both the polyester- and Apiezon-packed metal columns to give *cis,trans* and *trans,trans* conjugated dienoates (Fig. 3). These were formed in approximately equal proportions, showing that dehydration results equally in *cis* and *trans* double bonds. The carbon number and the ultraviolet spectrum of each component were identical to those of the corresponding standard dienoate.

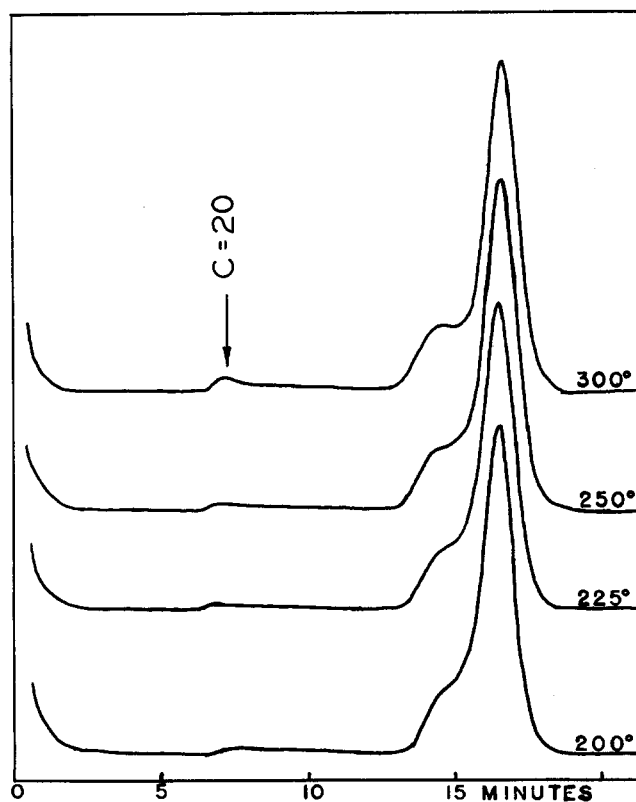


FIG. 2. Portions of gas-liquid chromatograms of pure ethyl β -elaeostearate on 90 cm glass column of LAC 2-R446 at 190°. Each curve was obtained with the flash heater at the temperature noted.

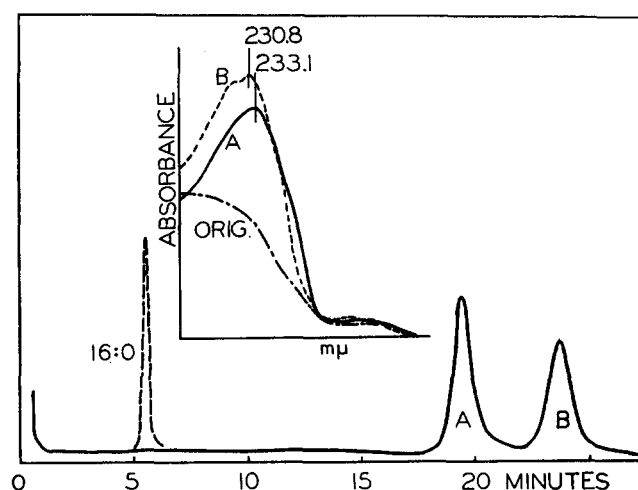


FIG. 3. Gas-liquid chromatography of reduced methyl oleate hydroperoxides on 180 cm copper column of LAC 2-R446 at 196°, plus methyl palmitate dotted in as a reference. Insert shows the ultraviolet spectra (10) of the original sample and fractions A and B collected after GLC.

On the glass column, dehydration of reduced oleate hydroperoxides was also complete at all flash heater temperatures. At a flash heater temperature of 300°, the two conjugated dienoate peaks were well separated and showed little tailing, suggesting that dehydration occurred almost exclusively in the flash heater (cf. Figs. 3 and 4). As the flash heater temperature was reduced, more and more of the dehydration occurred along the column giving patterns shown in Figure 4.

It should be noted that the reduced oleate hydroperoxide mixture may give rise to seven dehydration products, namely, *cis,trans* and *trans,trans* isomers of octadec-7,9-dienoate; the *cis,trans*, *trans,trans*, and *trans,cis* isomers of octadec-8,10-dienoate; and the *trans,cis* and *trans,trans* isomers of octadec-9,11-dienoate. Only the gross separation of *cis,trans* from *trans,trans* isomers was effected, the peaks representing mixtures of four components and three components, respectively. This is true also with the reduced linoleate hydroperoxides (*vide infra*), from which eight dehydration products may be formed.

Reduced methyl linoleate hydroperoxides (a mixture of *cis,trans*- and *trans,trans*-9-hydroxy-10,12- and 13-hydroxy-9,11-octadecadienoates), and methyl dimorphecolate (the *trans,trans* isomer of the former), are completely dehydrated during GLC on both polyester and Apiezon-packed copper columns to yield conjugated trienoates (Fig. 1). These chromatograms are almost identical to those of conjugated trienoates, having a composite first peak and a sharp second peak and the same carbon numbers (Table 1).

The U.V. curves of the constituents giving rise to these peaks (Fig. 1) correspond to *cis,trans,trans* and *trans,trans,trans* trienes, respectively, each contaminated with a small amount of the other because of overlapping. Infrared spectra of the separated fractions verify their *cis,trans,trans* and *trans,trans,trans* configurations (Fig. 1).

On the glass column, with the flash heater at 300°, methyl dimorphecolate (9-hydroxyoctadec-*trans,trans*-10,12-dienoate) was completely dehydrated to conjugated trienoates (Fig. 5). The chromatogram differed from that obtained with the copper column in that the first trienoate peak was not composite (cf. Figs. 1 and 5). This difference reflects the greater amount of subsequent isomerization obtained with the metal column, since apparently the initial products of dehydration emerged from the glass column. The two peaks have approximately equal areas, indicating that dehydration again results equally in *cis*- and *trans*-double bonds. As the flash heater temperature of the glass column was reduced, dehydration was still complete but was again obviously effected more and more

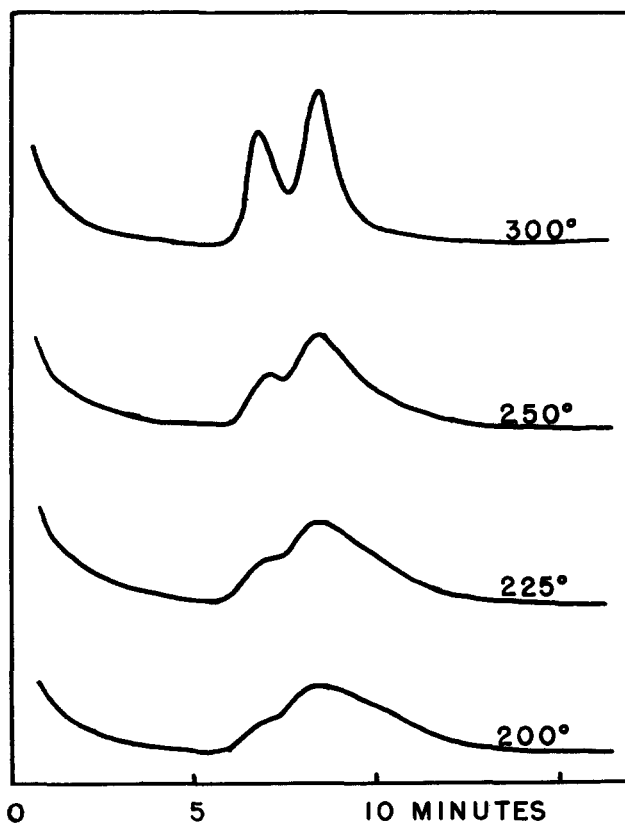


FIG. 4. Gas-liquid chromatograms of reduced methyl oleate hydroperoxides on 90 cm glass column of LAC 2-R446 at 190°. Each curve was obtained with the flash heater at the temperature noted.

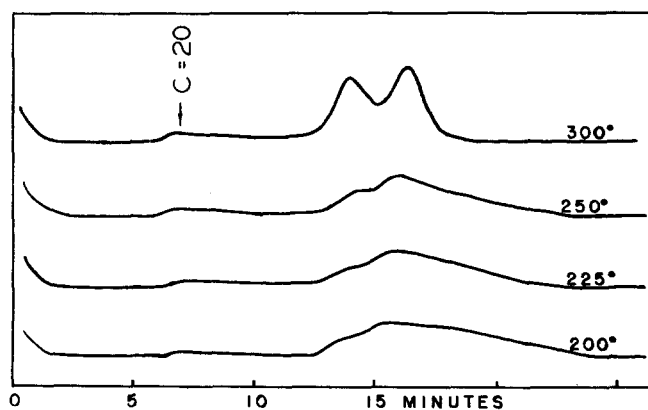


FIG. 5. Gas-liquid chromatograms of pure methyl dimorphocolate (9-hydroxyoctadec-*trans,trans*-10,12-dienoate) on 90 cm glass column of LAC 2-R446 at 190°. Each curve was obtained with the flash heater at the temperature noted.

during passage through the column rather than in the flash heater. This is obvious from a comparison of the curves illustrated in Figure 5.

By analogy with the unsaturated hydroxy derivatives, it seemed likely that a carboxyl group adjacent to a hydroxyl substituted carbon might provide sufficient activation for dehydration to occur under our experimental conditions. However, although methyl 2-hydroxy stearate emerged after a far shorter retention time than other hydroxy stearates (Table 1), the material collected from the effluent had the same adsorption characteristics on a thin-layer chromatogram as the original hydroxy ester. The retention time of 2-hydroxy stearate is about half that of other hydroxy stearates and this difference may be accounted for by strong hydrogen bonding between the hydroxyl group and the adjacent ester carbonyl group. The fact that this strong intramolecular hydrogen bonding is present in solution is shown by the near infrared spectrum of a dilute solution of methyl 2-hydroxy stearate in carbon tetrachloride. A strong sharp band occurs at 2.820μ , due to intramolecularly associated hydroxyl, but 6- or 12-hydroxy stearate have only a sharp unassociated hydroxyl band at 2.755μ . The greater speed of migration on a thin-layer chromatogram of the 2-hydroxy stearate compared to the 6- or 12-hydroxy stearate is also indicative of strong internal association of the 2-hydroxy group. The use of GLC retention volume data as evidence for intramolecular hydrogen bonding has recently been described (13), and the above results constitute another example of the use of GLC in studies of this phenomenon.

Boleka oil, besides containing a high proportion of conjugated diyne (isanic) and conjugated enediene (bolekiac) acids, contains hydroxy derivatives of these

acids with the hydroxyl group adjacent to acetylenic linkages (4). Although GLC of the mixed esters shows several minor unidentified components, the peaks due to bolekiac and isanic acids have been identified by ultraviolet spectra. GLC of the hydroxy ester fraction on the polyester-packed copper column gave two main peaks having smaller carbon numbers than the bolekiac and isanic esters, respectively. Since these peaks were missing from the curve for the nonhydroxy ester fraction, and since they emerged before rather than after the bolekiac and isanic esters, they have almost certainly resulted from the two hydroxy components by dehydration, and represent a conjugated enediene and a conjugated enediyne, respectively. Spectral evidence agrees with this supposition in the case of the hydroxy isanic acid, which, upon dehydration, gives rise to the same chromophore (an enediyne) as exists in bolekiac acid. Lack of an enediene standard prevented confirmation in the other instance. Emergence of the products of dehydration before their parent less-unsaturated esters may be due to conjugation of a double bond with an acetylenic system which lowers the melting and boiling points of the system (e.g., 17-octadecene-9,11-diynoic acid, m.p. 40°; 13-octadecene-9,11-diynoic acid, m.p. 17°). Although these esters were not studied on the glass column and many features of the GLC of the esters from boleka oil have still to be explained, it seems that the conjugated hydroxy acetylenic systems are dehydrated in the same way as are the conjugated ethylenic hydroxy derivatives.

Berthuis and coworkers (9) have published gas-liquid chromatograms of the methyl esters of *Ximenia caffra* seed oil obtained presumably on a glass column. This oil contains many acids which are not common constituents of seed oils, namely, homologous series of saturated and monoethenoid acids up to a chain length of twenty-eight carbons. The oil also contains ximenynic acid (octadec-*trans*-11-ene-9-ynoic acid) as a major component (about 25%) and the 8-hydroxy-derivative of this acid (3% to 4%). From retention volume considerations, the second of the two major peaks in their chromatograms, appearing near the retention volume of a C_{19} ester, must be that of methyl ximenynate. The small unidentified peak between the C_{20} and C_{22} esters is probably due to methyl octadec-7,11-diene-9-ynoate derived by dehydration during GLC from the hydroxyximenynate. The hydroxy ester, as such, would be expected to emerge from the column much later, and their chromatograms show no evidence of an unusual component after C_{22} .

Acetoxy Compounds. It was of interest to determine

whether conversion of hydroxy esters to acetoxy derivatives would prevent their alteration during GLC. Esters of dimorphecolic and reduced hydroperoxyoleic acids were acetylated and examined by GLC. On the copper column these vicinally unsaturated acetoxy esters were completely deacetylated to conjugated trienoates and dienoates, respectively, in the same way as their parent hydroxy esters were dehydrated.

On the glass column, acetylated methyl dimorphecolate (*trans,trans*-9-acetoxyoctadec-10,12-dienoate) gave conjugated trienoate patterns, at the various flash heater temperatures, almost identical to those illustrated in Figure 5. However, the all *trans* trienoate peak trailed just above the base line to a peak with carbon number 24.0, after which true base line was again attained. This peak amounted to about 10% of the total, and its proportion increased slightly as the flash heater temperature was lowered. The component giving rise to this peak was collected and when reinjected gave the same pattern. Thin-layer chromatography identified it as the original acetoxy ester.

The less strongly activated monounsaturated acetoxy esters derived from the reduced oleate hydroperoxides were more stable. Conjugated diene patterns similar to those shown in Figure 4 were obtained at the various flash heater temperatures, but these trailed obviously into two peaks with carbon numbers 22.9 and 23.4, which together amounted to about 60% of the sample. These peaks collected together were reinjected to give similar chromatograms and thin-layer chromatography again demonstrated their identity to the original sample components. On the glass columns all chromatograms of acetoxy esters demonstrated a small peak immediately after the solvent peak, at the retention volume of acetic acid.

Acetylation has been shown, therefore, to be no certain protection for unidentified hydroxy derivatives which are to be examined by GLC.

Hydroperoxy Compounds. In view of the results reported above, it seemed likely that hydroperoxides, formed during autoxidation of fats and present to some extent in all except completely fresh oil samples, would be altered during GLC to conjugated unsaturated esters. This was found to be true. Methyl oleate hydroperoxides and methyl linoleate hydroperoxides were completely altered on both metal and glass columns to conjugated dienoates and conjugated trienoates, respectively, in a manner analogous to that of their corresponding hydroxy derivatives.

Cause and Location of Alterations. It is believed that all the alterations which occurred during GLC on the metal columns were effected not on the column

but in the flash heater. If alteration took place on the column, the resulting peaks would be long and drawn out, as demonstrated with the glass column (Figs. 2, 4, and 5). The peaks obtained from the metal columns, however, were sharp and symmetrical. In addition, the same results were obtained with polar and nonpolar packings, suggesting that these took no part in the reaction. If these reactions were accomplished within the flash heater, either a thermal mechanism or a catalysis by the metal of the flash heater at high temperatures may be operative. Copper, especially as its oxide, has recognized catalytic activity for many reactions, and for this reason its use in GLC columns for lipid work has been avoided by some investigators.

To determine whether these alterations were caused by thermal or catalytic effects, and to test the possibility that the column packing might have some effect, experiments were performed with a simple all-glass flash heater. This was not connected to any GLC column, but consisted only of an electrically heated glass tube, packed with glass wool, and with an injection gasket of silicone rubber. The tube was heated to 250° with argon gas flowing through it. The sample was injected as a methanol solution, the tube was allowed to cool, the product was rinsed out with methanol, and its ultraviolet spectrum recorded. Methyl dimorphecolate and reduced linoleate hydroperoxides (the easiest samples to monitor) were completely dehydrated to conjugated trienoates, as evidenced by the replacement of their conjugated diene bands at 231 m μ by conjugated triene bands having maxima at 268 m μ . Lowering of the tube temperature resulted in progressively less dehydration, and at 170° very little dehydration occurred. Similar results were obtained for acetylated methyl dimorphecolate. Conjugated trienes, however, underwent little *cis-trans* isomerization on passage through this all-glass flash heater, as evidenced by ultraviolet spectra. These experiments demonstrated that a high temperature alone is sufficient to cause alteration of vicinally unsaturated hydroxy derivatives but that the extent of *cis-trans* isomerization of trienes is rather small under the same conditions. These results confirmed those already described, which were obtained with the polyester-packed glass column. Dehydration, deacetylation, etc., may therefore be accomplished purely by thermal effects. However, the results gained by metal columns suggested that metal catalysis, by components of the flash heater, might have some effect on *cis-trans* isomerization.

To test this possibility, the uncoated Celite® packing was removed from the flash heater of the polyester-

packed glass column and replaced with copper dust and glass wool impregnated with copper oxide. The column, after this modification, had the same retention characteristics as before. The five key esters (β -elaeostearate, free and acetylated dimorphecolate, and free and acetylated reduced oleate peroxides) were restudied in this column at flash heater temperatures of 250° and 200°, and column and detector temperatures the same as before. The dehydration of dimorphecolate and reduced oleate peroxides under these conditions was definitely catalyzed by the presence of the copper and copper oxide. The curves obtained when the flash heater temperature was 250° were almost identical with those obtained before at 300° (Figs. 4 and 5). Those obtained with a flash heater temperature of 200° were intermediate between the previous curves at 250° and 300°, and showed incomplete dehydration in the flash heater, the small residue of unchanged material then being dehydrated on the column.

The catalytic effect of the mixture of copper and copper oxide was even more pronounced with the acetoxy derivatives. The curves of acetylated methyl dimorphecolate on this column at flash heater temperature of 250° and 200° were almost identical to those obtained in the absence of metal at 300° and 250°, respectively. The acetylated, reduced oleate peroxide curves showed an even greater difference. At 250° flash heater temperature, in the presence of copper and copper oxide, about 80% of this sample was deacetylated in the flash heater. Most of the remainder was deacetylated in the column, leaving only 5% of the component with carbon number 23.4 unchanged. At 200°, more deacetylation occurred on the column and the proportions of sample deacetylated in the flash heater and emerging unchanged were about 60% and 10%, respectively. These results are very different from those obtained in the absence of catalyst.

The results of these experiments demonstrate that dehydration and deacetylation of vicinally unsaturated hydroxy or acetoxy esters may be caused by thermal effects alone but are also catalyzed by the copper and copper oxide mixture. This explains almost completely the differences in the results obtained with vicinally unsaturated oxy-derivatives on copper columns and on the metal-free glass column.

The greatest difference, however, between copper columns and the all-glass column was shown with the conjugated trienoates. The equilibrium attained from either α - or β -elaeostearate with the copper column (cf. Fig. 1) was not even approached with the glass column (Fig. 2). This suggests that metal cataly-

sis played a major role in *cis-trans* isomerization of trienoates. However, when β -elaeostearate was studied on the glass column, the curves obtained with or without copper and copper oxide in the flash heater were identical (Fig. 2). There was neither an increase in the proportion of α -elaeostearate formed, nor any indication that more of this had been formed in the flash heater rather than on the column. Thus, contrary to dehydration, etc., *cis-trans* isomerization of conjugated trienoates was not at all catalyzed by copper-copper oxide and is therefore purely a thermal reaction. The different results obtained with conjugated trienoates on the copper columns and on the glass column may follow from the much higher heat capacity and more efficient heat transfer of the brass flash heaters. This is probably also the reason why a small proportion of the acetylated samples emerged unchanged from the glass column even when the metal catalysts were included in the flash heater, whereas complete deacetylation resulted in the brass flash heater used with the copper column.

DISCUSSION

Some structures apparently change during GLC. Unsaturated compounds with more than two ethylenic bonds in conjugation show some *cis-trans* isomerization. Esters with a hydroxyl-substituted carbon adjacent to unsaturation, either ethylenic or acetylenic, decompose even if the hydroxy group is acetylated. Primary autoxidation products similarly change to more unsaturated compounds. This is somewhat analogous to the formation of conjugated ethylenic unsaturation observed in the bleaching of oxidized oils (14) and to the formation of conjugated trienes during alkaline isomerization of oils containing oxidized linoleate (15). Partial *cis-trans* isomerization of conjugated trienoates on a glass column with simultaneous formation of the earlier, possibly cyclized, component described above have been noted by Miwa³, who also observed partial dehydration of methyl dimorphecolate. On a similar column, Frankel⁴ obtained complete dehydration of methyl dimorphecolate to conjugated trienoates. The differences in the results of these workers may be due to differing efficiencies of their flash heaters.

The compounds described above probably do not

³T. K. Miwa, Northern Regional Laboratory, Peoria, Ill., personal communication.

⁴E. N. Frankel, Northern Regional Laboratory, Peoria, Ill., personal communication.

constitute a full list of esters of natural lipid components which may be expected to alter during GLC. We have noted also that the method of James and Martin (16) for identification of saturated components in a mixture by forming polybromides of the unsaturated components does not work when LAC 2-R446 polyester packing is used. In this case, the bromides are not held back by the column but apparently are altered by the stationary phase to a variety of compounds giving a very long, low band between C_{18} and C_{24} . This method has also been found not to work if the column or flash heater is of copper, even if the stationary phase is hydrocarbon.⁵

The use of copper or brass equipment for GLC of lipid components is condemned by some authorities on the assumption that the metal will catalyze alterations of some components. However, we have found that copper will not catalyze any alteration which would not otherwise have occurred to some extent due to thermal effects common to all columns. No experimental data to prove otherwise have been published as yet. On the other hand, a copper column fitted to an efficient brass flash heater unit gives complete alteration of all the compounds studied by us. The chromatograms obtained are at least reproducible, the number of products obtained is minimized, and smearing due to slow reaction on the column is avoided. For these reasons we suggest that this type of column is in many cases preferable to a glass column. The patterns due to alteration of certain components should be anticipated or recognized when they occur; then this phenomenon can be used to demonstrate the presence of specific groupings in naturally occurring acids (17).

⁵ N. Tuna, Medical School, University of Minnesota, Minneapolis 14, Minn., personal communication.

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