The addition of 10% of either highly hydrogenated rapeseed or cottonseed oils to nonhydrogenated rapeseed oil gives a product with harder consistency than when the corresponding hydrogenated oils are added to nonhydrogenated cottonseed oil. Linteris and Thompson (12) in experiments with somewhat similar mixtures came to the conclusion that the presence of long-chain fatty acids and a suitable diversity of fatty acids in the constitutent triglycerides promoted the precipitation of fine crystals and helped subsequently in aeration during the mixing of cake batters.

Highly hydrogenated rapeseed oil is probably the highest melting and most viscous fat which may be incorporated in food products. The Swedish oil when hydrogenated to an I.V. of 1.5 had the following viscosity:

Temp, °C	Viscosity, centistokes
65	30.8
70	26.5
75	23.0
80	20.2
85	17.9
95	14.2

The fat had a melting range of 69.3–70.6C, but it solidified at 57–58C when cooled rapidly.

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Gas Liquid Chromatographic Analysis of Hydroxy Fatty Acids, as Their Trimethylsilyl Ether Derivatives[1]

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Abstract

A quantitative method is described for the rapid gas liquid chromatographic analysis of mono- and polyhydroxystearates as their trimethylsilyl (TMS) ether derivatives. The TMS derivatives of the hydroxy esters are formed rapidly and quantitatively at room temp. The retention times of ricinoleic acid as the methyl ester, acetylated derivative and the TMS derivative are compared. Purified diastereoisomers of di-, tri- and tetrahydroxystearates and their mixtures were analyzed on three different columns. Gas liquid chromatographic analysis of methyl mono-, di-, tri- and tetrahydroxysteartes were shown to give quantitative results as TMS derivatives. Analyses were carried out on a diethylene glycol succinate polyester (DEGS) packed column and on large-bore capillary columns of DEGS and Apiezon L. The elution order of the TMS derivatives of the hydroxy esters from the DEGS large-bore capillary column was not the same as that obtained from the DEGS packed column. The unusual elution pattern of the large-bore capillary column is discussed.

Introduction

THE QUANTITATIVE DETERMINATION of long-chain mono- and polyhydroxy fatty acids by gas liquid chromatography (GLC) is presently unsatisfactory. Their high polarity and low volatility gives rise to undesirably long retention times.

Methyl ether derivatives of hydroxy acids have been used bv Kishimoto and Radin (1) to increase their volatility. Downing and co-workers (2,3) reported that α-hydroxy acids can be reduced to their corresponding 1,2-diols from which a more volatile isopropylidene derivative may be prepared and analyzed by gas chromatography. The former method is nonquantitative and laborious, while the latter can only be used for vicinal dihydroxy or α-hydroxy acids. Acetylated hydroxy acids have been used by Kishimoto and Radin (4), and more recently by O'Brien and Rouser (5), with some success, but still have undesirably long retention times, especially on polar liquid phases. The gas chromatographic analysis of long-chain dihydroxy acids is nonquantitative and limited (6–8), while the analysis of long-chain tri- and tetrahydroxy acids by this method has not been reported, to the authors' knowledge.

The use of the TMS ether derivatives of hydroxy compounds has opened new areas in which GLC can be used for their analysis. Some of the areas where the use of the TMS derivative has been proven to be successful have been reviewed recently (9).

This report describes the quantitative analysis of mono- and polyhydroxy long-chain fatty acids using this technique.

TABLE I

Quantitative Determination of Hydroxy Acids as Their TMS Derivatives by GLC Analysis[a]

Hydroxy acid	Actual[b]	Found[c]
	%	%
12-hydroxystearic acid	14.07	14.06
Threo-9,10-dihydroxystearic acid	21.87	22.47
Threo-9,10-12-trihydroxystearic acid	27.91	27.97
Erythro-9,10-*erythro*-12,13-tetrahydroxystearic acid	36.14	35.50

[a] Analysis was carried out on 3 ft x ¼ in. column packed with 20% DEGS on 80–100 mesh Chromosorb W at 215C. A typical chromatogram of this mixture is shown in Figure 4 (middle).
[b] Prepared by weighing out purified acids.
[c] Values derived by measuring the area under the peaks with a planimeter.

[1]Presented at the AOCS Meeting in New Orleans, 1964.

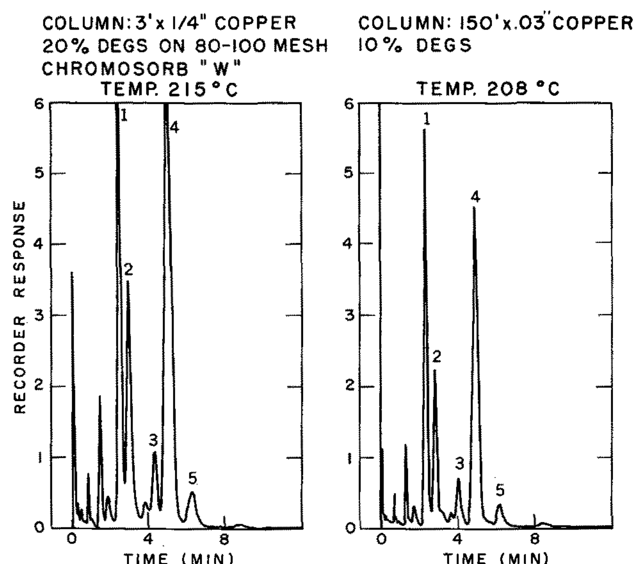

FIG. 1. Analysis of methyl esters from rat adipose tissue on the two different columns used to analyze mono- and polyhydroxy esters of long-chain fatty acids. The major peaks are: 1) methyl palmitate; 2) methyl palmitoleate; 3) methyl stearate; 4) methyl oleate; and 5) methyl linoleate.

Experimental

Gas Chromatography. A Research Specialties Co. Model 600 gas chromatograph equipped with a hydrogen flame ionization detector and an on-column injection system was used throughout this study. A high resolution 3 ft x ¼ in. copper column, packed with 20% DEGS on 80–100 mesh Chromosorb W, was used. The column packing procedure has recently been described (9). Two large-bore capillary columns (100 and 150 ft x 0.0625 in OD, 0.030 in ID) coated with Apiezon L and DEGS, respectively, were also used. The columns were coated with a 15% carbon tetrachloride solution of Apiezon L and a 10% chloroform solution of DEGS and conditioned according to the procedure described by Litchfield et al. (10). An inlet pressure of 10 psi was used to maintain a helium flow of 60–70 ml/min through the columns. The hydrogen and air flow through the detector were 30 and 300 ml/min, respectively. The temp of the detector was maintained a few degrees above the maximum temp of the column.

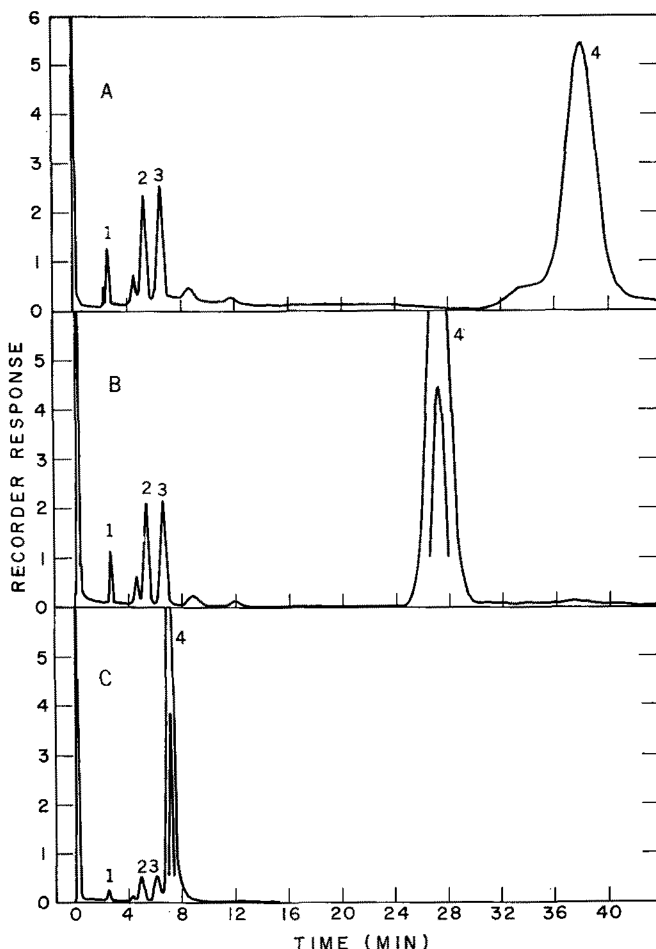

FIG. 2. Analysis of ricinoleic acid in castor oil fatty acids as: A) methyl ester, B) acetylated derivative, and C) TMS derivative. The major peaks are: 1) methyl palmitate, 2) methyl oleate, 3) methyl linoleate, and 4) methyl ricinoleate.

Thin-Layer Chromatography. Commercially available silica gel G was applied in 1-mm thickness to 8 x 8 in. glass plates. The plates were used to isolate methyl ricinoleate from castor oil methyl esters. The chromatoplates were developed with hexane-diethyl ether-acetic acid 60:40:1 (v/v/v), in a saturated chamber. The desired region of the heavily loaded plates was located visually and scraped into a fritted glass funnel. The methyl esters were then eluted from the silica gel with diethyl ether.

Balance and Melting Point Apparatus. A Mettler Model M5 micro balance was used to weigh accurately the purified hydroxy acids to the fifth decimal place to prepare the standard mixture. A Thomas Hoover capillary melting point apparatus was used to determine melting points.

Materials. The fatty acids used in the synthesis of the hydroxy acids were either previously prepared in this laboratory or obtained from reliable commercial sources. The castor oil used in this study was packaged by Welton Laboratories, Inc., New York. The methyl 12-hydroxystearate was obtained from Applied Science Laboratories. Thin-layer chromatography was used to isolate methyl ricinoleate from castor oil methyl esters. The polyhydroxy acids were prepared by alkaline permanganate oxidation of the corresponding unsaturated acids according to the procedure of Wiberg and Saegebarth (11). Purification of the polyhydroxy acids was achieved by crystallization at zero degrees from a saturated solution of diethyl ether. *Threo-* and *erythro*-9,10-dihydroxystearic acids were prepared from elaidic and oleic acids, respectively. The melting point of the *threo* acid was 93.6–94.5C [94C, according to (12)], and that of the *erythro* acid was 129.5–130.5C [130–131C, according to (11)]. Ricinelaidic and ricinoleic acids were oxidized to yield *threo-* and *erythro*-9,10-12-trihydroxystearic acids, respectively. The diastereoisomeric trihydroxy mixture obtained from ricinelaidic acid melted at 97.2–100.5C, while the mixture obtained from ricinoleic acid melted at 108.6–127.3C. Permanganate oxidation of linoleic acid yielded a diastereoisomeric mixture of *erythro*-9,10-*erythro*-12,13-tetrahydroxystearic acids. This tetrahydroxy mixture melted at 154–165C [154–163C reported by (13)]. Eleven, 12-dihydroxystearic acid was prepared from a vaccinic acid sample of unknown purity and configuration which had mp 75.2–78.7 (after two crystallizations). Methyl esters were prepared by treating the acids with an ethereal solution of diazomethane. Acetylated methyl ricinoleate was prepared according to the procedure of O'Brien and Rouser (5). Hexamethyldisilazane was obtained from Peninsular Chemresearch, Gainesville, Fla.; trimethylchlorosilane was obtained from K & K Laboratories, Plainview, N.Y. Solvents and other reagents were reagent grade and used without further purification.

Preparation of TMS Derivatives. The procedure by Wood et al. (9) for the formation of the TMS derivatives of monoglycerides was used to prepare the TMS derivatives of the hydroxy acids. The slight exothermic reaction appeared to proceed equally fast at room temp for the long-chain hydroxy esters as previously noted for the monoglycerides.

Purity of Materials. All of the fatty acids used in the preparation of the hydroxy acids were more than 98% pure as determined by GLC. Methyl ricinoleate and methyl 12-hydroxystearate were 98% pure as determined by GLC analysis of their methyl esters and TMS derivatives of their methyl esters. GLC analysis of the TMS derivatives of each of the dihydroxy acids revealed only one component. Melting points of the acid were in close agreement with those previously found. Each of the diastereoisomeric trihydroxy mixtures were more than 98% trihydroxy as determined by GLC analysis of their TMS derivatives. GLC analysis of the TMS derivatives of the diastereoisomeric tetrahydroxy mixture indicated only traces of impurities.

Results and Discussion

Gas Chromatography Columns. Low pressure drop, high resolution columns are required to separate, without peak asymmetry, mono- and polyhydroxy esters by GLC as their TMS derivatives. This requisite can be achieved with short, high-resolution packed columns or large-bore capillary columns. The latter type is the more desirable of the two, since its preparation is more reproducible. Figure 1 shows the effectiveness and rapidity of the separation of methyl esters of rat adipose tissue fatty acids on two of the columns used to separate TMS derivatives of hydroxy esters. A complete chromatogram was obtained in approx 8 min with good resolution of monoenes from their corresponding saturated acids. The large-bore Apiezon L capillary column used was equally fast but, of course, the resolution pattern was different.

Monohydroxy Acids. The GLC analyses of ricinoleic acid from castor oil as the methyl ester, acetylated and TMS derivatives are shown in Figure 2. The TMS derivative of methyl ricinoleate eluted approx four times faster than the acetylated derivative, and five times faster than the methyl ester. Ricinoleic acid represented 89, 86 and 85% of the total acids as determined by the TMS derivative, acetylated derivative and methyl ester, respectively, based on peak area. Twelve-hydroxystearic acid, the corresponding saturated acid of ricinoleic acid, behaved in a similar manner to that shown in Figure 2 for ricinoleic acid with respect to all three derivatives.

Figure 3 shows the analysis of a mixture of TMS derivatives of methyl ricinoleate and methyl 12-hydroxystearate on two different DEGS columns. The expected elution pattern was obtained in each case. The saturated acid was eluted from the columns more quickly than the corresponding unsaturated acid. Enough resolution to permit quantitative estimation was not achieved on a packed column, but it was achieved on the large-bore DEGS capillary column.

Dihydroxy Acids. The TMS derivatives of racemic methyl *threo*- and *erythro*-9,10-dihydroxystearate were analyzed on all three columns. Each was eluted from the columns without peak asymmetry. However, a mixture of the two diastereoisomers was not resolved sufficiently for quantitative estimation on any of the columns. The large-bore Apiezon L capillary column operating at a temp of 212C gave the best results, but only partial separation. Should a longer column of this type or a more effective stationary liquid phase be found which could make such separation, it would then be possible to determine the percentage of *cis* and *trans* forms in a monoene mixture since permanganate

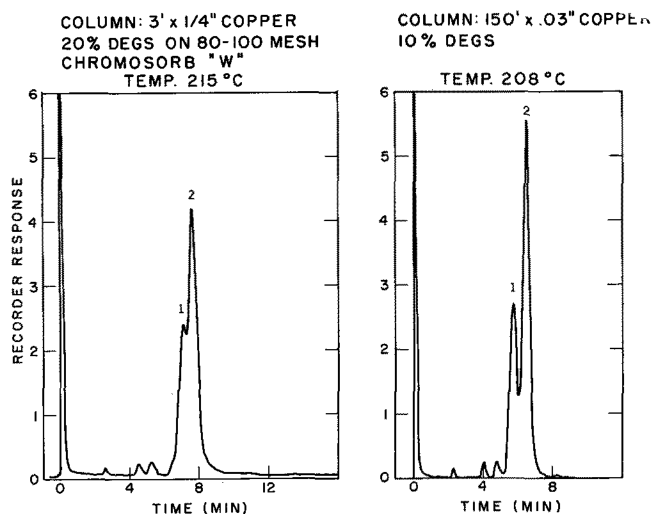

FIG. 3. Analysis of a mixture of 1) methyl 12-hydroxystearate and 2) methyl ricinoleate as their TMS derivative on packed and large-bore capillary DEGS columns.

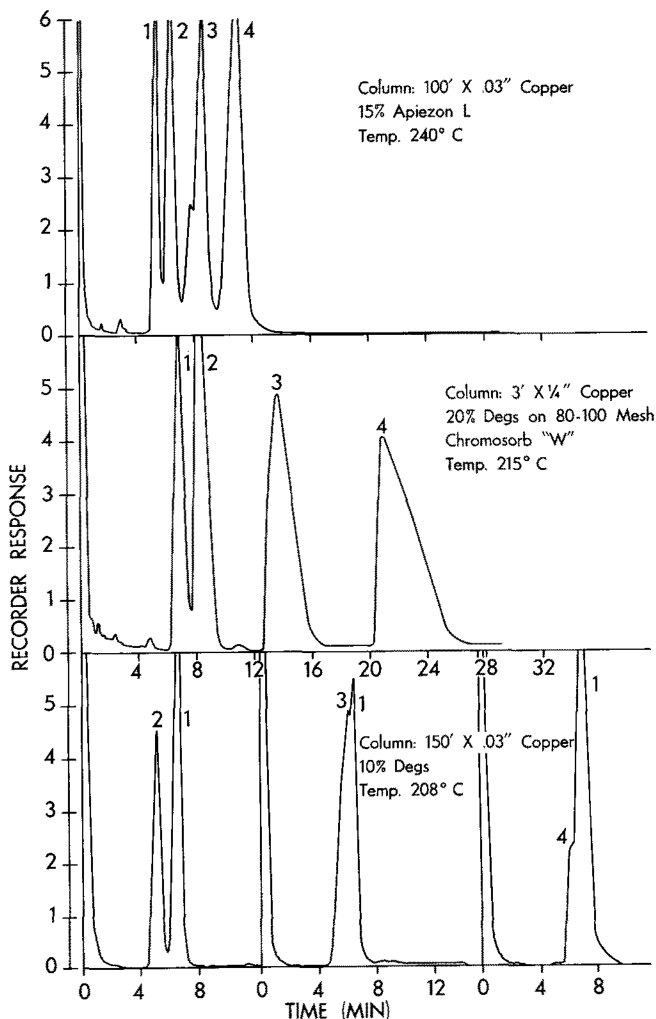

FIG. 4. GLC analysis of mono- and polyhydroxy TMS derivatives of long-chain fatty acids on three different columns. The major peaks are: 1) methyl 12-hydroxystearate, 2) methyl *threo*-9,10-12-trihydroxystearate, and 4) methyl *erythro*-9,10-*erythro*-12,13-tetrahydroxystearate. Analysis on the DEGS capillary column was carried out in pairs to show the complex elution pattern.

oxidation of each geometric isomer yields a distinct diastereoisomer.

The TMS derivatives of methyl 11,12-dihydroxystearate were prepared and their elution times compared with those of the methyl 9,10-dihydroxystearates in hope that the method could be used to determine positional dihydroxy acid isomers prepared from positional monoene isomers. Neither of the diastereoisomeric methyl 9,10-dihydroxystearates were resolved from the methyl 11,12-dihydroxystearate isomer sufficiently for quantitative analysis. However, the Apiezon L column again gave partial separation. Possibly, the conditions necessary for the separation of the TMS derivatives of methyl *threo-* and *erythro-* 9,10-dihydroxystearates will also allow the resolution of the positional dihydroxy acid isomers.

Trihydroxy Acids. Alkaline permanganate oxidation of ricinoleic and ricinelaidic acids yields two racemic diasteroisomers for each acid. The ratio for each diastereoisomeric pair is not necessarily one, due to their differences in solubility and the steric effect of the hydroxy group on carbon 12 during their formation. These four distinct diastereoisomeric trihydroxy acids have been prepared and characterized by Kass and Radlove (14).

The TMS derivatives of methyl *threo-* and *erythro-*9,10-12-trihydroxystearate were subjected to GLC analysis on each of the three columns. The *erythro* diastereoisomeric mixture was eluted from each column as one peak with the indications of two components present. The *threo* diastereoisomeric mixture was only partially resolved on the DEGS column, while the Apiezon L column operating at a temp of 222C achieved separation sufficient for quantitative estimation. The predominant and presumably least polar of the two isomers eluted last on this column and represented 72% of the mixture. A mixture of the 4 isomeric trihydroxy TMS derivatives was not separated on any of the columns, but the Apiezon L column gave partial separation.

Tetrahydroxy Acids. Alkaline permanganate oxidation of the possible *cis-trans* isomers of linoleic acid can yield 16 possible tetrahydroxystearic acid isomers or eight racemic pairs. These eight racemic diastereoisomers have been prepared and characterized by McKay and Bader (15). Unfortunately, their proposed structural formulas have been shown to be incorrect as they made the false assumption that the hydroxy derivatives obtained from alkaline permanganate oxidation of a *cis* double bond are *threo.* They also falsely assumed the peracetic acid oxidation and bromination of a *cis* double bond yields the *erythro* forms. However, correct formulas can be obtained by correction of the invalid assumptions as to the products obtained from the permanganate and peracetic acid oxidation and bromination of a *cis* double bond. A mixture of the two diastereoisomers is obtained by alkaline permanganate oxidation of linoleic acid (*cis*-9, *cis*-12-octadecadienoic acid). Riemenschneider et al. (13) have shown the diastereoisomers to be a eutectic mixture consisting of 30% of one isomer (mp 164C) and 70% of the other isomer (mp 174C) which melted at 154–163C. The melting point of our preparation was found to be 154–165C.

The diastereoisomers of methyl *erythro*-9,10-*erythro*-12,13-tetrahydroxystearate TMS derivatives were eluted as one peak on each of the three columns used. The peaks were asymmetric and skewed to the right in the case of the DEGS columns and to the left in the case of the Apiezon L columns. This asymmetry was probably due to the lack of sufficient temp for volatilization of these high mol wt molecules, since the Apiezon L column operating at 240C gave more symmetrical peaks. Changes in solubility of the TMS derivatives as the number of trimethylsilyl ether groups increase could also cause peak asymmetry as well as partial resolution of the diastereoisomers.

Mixture of Mono- and Polyhydroxystearic Acids. Figure 4 shows the analysis of a mixture of methyl 12-hydroxystearate, methyl *threo*-9,10-dihydroxystearate, methyl *threo*-9,10-12-trihydroxystearate and methyl *erythro*-9,10-*erythro*-12,13-tetrahydroxystearate as their TMS derivatives on three different columns. A mixture was eluted from the packed DEGS and Apiezon L large-bore capillary columns in the expected order of increasing mol wt and polarity, mono-, di-, tri- and tetrahydroxy, in that order. Surprisingly enough, the elution pattern from the DEGS large-bore capillary was different from that obtained on the DEGS packed column. The mixture was eluted from the DEGS large bore-capillary column as one large distorted peak. Comparison of each of the polyhydroxy with the mono-TMS derivative revealed the elution pattern as di-, tri-, tetra- and monohydroxy, in that order, which is also shown in Figure 4. The order of elution is contrary to theoretical considerations and is apparently the first phenomena of its nature reported. Methyl ester analyses, already shown in Figure 1, are similar for the two columns, yet these high mol wt TMS derivatives behave differently. The major differences between the two columns are the amt of exposed copper surface in the capillary column and the acid base washed Chromosorb W in the packed column. The latter appears to be a contributing factor, since the Apiezon L capillary column had a similar exposed copper surface and gave the expected elution order. It may well be that increasing the number of trimethylsilyl ether groups on a long-chain fatty acid decreases its solubility in DEGS and increases its mol wt and size sufficiently to allow the Chromosorb W to act as a molecular sieve or have adsorption properties which would account for the different elution pattern from the packed and large-bore capillary columns containing the same liquid phase.

Quantitation. It has been shown in the previous paper (9) that monoglycerides may be quantitatively determined as their TMS derivatives. Table I shows the quantitative analysis of a mixture of mono- and polyhydroxystearates as their TMS derivative. The percentage composition as determined by planimetric measurement of peak area agreed well with the actual values obtained from wt measurements of the acids in the synthetic mixture. Although the method has only been demonstrated to be quantitative for the analysis of a mixture of hydroxystearates of increasing number of hydroxyl groups, it appears possible that the method could also be used for the quantitative analysis of a homologous series of mono- or polyhydroxy acids. From these results it can be seen that the method is quantitative for hydroxy acids as well as for monoglycerides.

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Elaidic and ricinelaidic acids from Carter Litchfield.

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Chemical Reactions Involved in the Catalytic Hydrogenation of Oils. II. Identification of Some Volatile By-Products[1,2]

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Abstract

Gas chromatography of the volatile by-products produced during the catalytic hydrogenation of an autoxidized soybean oil with a peroxide number of 11.2 meq/kg yielded approx 41 peaks. Twenty-one of the gas chromatographic fractions were collected and rechromatographed, and their chemical identity studied with micro-IR and mass spectrophotometry. These volatile by-products, according to peak area of gas chromatogram, showed a predominance of hydrocarbons and alcohols.

The fractions which have been identified are *n*-octane, *n*-nonane, *n*-decane, *n*-heptadecane, *n*-hexanol, *n*-heptanol, *n*-decanol, *n*-hexanal, *n*-decanal and 3-nonanone. Among the gas chromatographic fractions collected, *n*-decanol has an odor most reminiscent of that of catalytic hydrogenation.

Introduction

THE IMPORTANCE OF THE chemical characterization of the volatile by-products of hydrogenation and the scarcity of literature in this field were discussed previously (1). The present paper reports an investigation of the isolation, fractionation and chemical identification of some volatile by-products which are produced during catalytic hydrogenation of a soybean oil with a peroxide value of 11.2 meq/kg.

Experimental

Volatile By-Products of Hydrogenation. The volatile by-products isolated from 9.5 gal of hydrogenated soybean oil as previously described (1), were used for the present investigation.

Gas Chromatography. The ether solution of the volatile by-products was fractionated with a Beckman GC-2A gas chromatograph. The temp was linearly programmed with a Thermotrac Temperature Programmer from 45–200C in 30 min and then maintained at 200C for the remainder of the chromatography. An 8-ft aluminum column, ¼ in. I.D. packed with 15% Ucon Polar 50HB 280X on 80/100 mesh Chromosorb W (Acid washed), was used at a helium flow rate of 80 ml/min.

Collection of Gas Chromatographic Fractions. The gas chromatographic fractions were collected with a fraction collector designed and built in our laboratories (2). This fraction collector can be used to collect 18 fractions from one run. The gas chromatography was repeated 10 times. Each fraction was accumulatively collected in one cold trap. In this investigation, two runs of collection were made. In the first, 10 fractions were collected; in the second, 18 fractions. Since some of the fractions collected were duplicate peaks, a total of only 21 different gas chromatographic fractions were collected and studied.

Identification of Gas Chromatographic Fractions. The gas chromatographic fractions were identified by their IR spectra with the use of Sadtler standard spectra. The identifications were then confirmed by comparison of retention times with known compounds. The IR spectra of the gas chromatographic fractions in carbon tetrachloride solution were determined with a Beckman IR-8 spectrophotometer using ultra-micro cavity cells of 0.1-mm path length and beam condenser (Connecticut Instrument Corp.). An attenuated grid and variable wedge cell (W-1, Connecticut Instrument Corp.) were used in the reference beam to compensate for the adsorption due to solvent. By this technique, an IR spectrum could be obtained from 0.2 mg of sample.

When IR spectrum alone was insufficient for the chemical characterization of a gas chromatographic fraction, its mass spectrum was determined with a Bendix time-of-flight mass spectrometer.

Results and Discussion

Gas chromatography of the volatile compounds which were produced from soybean oil (PV, 11.2 meq/kg) during catalytic hydrogenation at 180C with 0.125% nickel catalyst (1) yielded approx 41 peaks (Fig. 1). The gas chromatogram is considerably different from the one published in our previous paper because of temp programming and the use of a more concd solution. Carbowax 20M and Carbowax 1000 were also tried as the stationary phase but did not give as good resolution as Ucon Polar 50 HB 280X. As shown by the Arabic number on the chromatogram, 21 of these gas chromatographic fractions were collected. Each of the collected fractions was then rechromatographed under the original conditions to insure that it was not contaminated by its neighboring peaks. Each fraction yielded only one symmetrical peak.

The volatile by-products of hydrogenation according to peak area of gas chromatogram, showed a predominance of hydrocarbons and alcohols. As determined by the IR spectra of the 21 collected gas chromatographic fractions (Fig. 1), 10 were hydrocarbons (fractions 2,3,4,6,7,8,14,16,17 and 20), three were alcohols (fractions 10,13 and 18), two were aldehydes (fractions 5 and 15), one was a ketone (fraction 12), three were other carbonyl compounds (fractions 1,9

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