Fatty Acids of Lard. A. Identification by Gas-Liquid Chromatography[1]

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Abstract

A fresh commercially rendered lard was separated into its component fatty acids by subjecting the methyl esters to gas-liquid chromatography on nonpolar and polar columns. The lard methyl esters were first chromatographed on a silicone column. This column achieved separation of the component esters principally on the basis of chain length, and fractions which were represented by a single peak or series of peaks were collected. The collected fractions were then rechromatographed on an ethylene glycol succinate polyester column to separate and identify the saturated and the unsaturated esters. Qualitative evidence was obtained for the presence of 29 fatty acids ranging in chain length from 10 to 20 carbon atoms. Included were the esters of the following: Saturated acids (10,12,14,15,16,17,18,19,20, and 22 C-atoms), monounsaturated acids (14,16,17,18,19, 20, and 22 C-atoms), and polyunsaturated acids (18:2, 20:2, 22:2, 18:3, 20:3, 22:3, 20:4, 22:4, 20:5, and 22:5).[3] Peaks for several additional trace components were also observed.

Introduction

THE IDENTITY of the principal and some of the minor fatty acids of lard has been known for at least two decades. They include lauric, myristic, myristoleic, palmitic, pamitoleic, stearic, oleic, linoleic, linolenic, arachidic, and arachidonic acids. Later pentaenoic acid of undetermined chain length (4) was added to the list.

In previous work from this laboratory (3), gas-liquid chromatography (GLC) was applied directly to the total methyl esters of lard. Evidence for presence of arachidonate or pentaenoate could not be seen on the chromatogram, although ultraviolet spectrophotometric analysis showed they were present in concentrations of 0.3–0.5 and 0.1–0.2%, respectively. The chromatogram, however, suggested the presence of small amounts of esters of lower molecular weight than myristate and also an unidentified ester having greater retention than linolenate but which did not agree with the retention of a tetraenoic or pentaenoic ester. Craig and Murty (1) have indicated that even-numbered carbon acids from C_{10} to C_{20} were present in lard along with a number of unsaturated acids in each group.

In the present work, principal emphasis was given to identification of the minor and trace fatty acids of lard. A GLC column with a nonpolar stationary phase (silicone) was employed first to separate lard methyl esters primarily on the basis of chain length. Fractions collected from this operation were then chromatographed on a polar polyester (EGS) column in order to separate saturated and unsaturated esters. This procedure enabled identification of minor and trace fatty acids to be made with greater certainty. A number of fatty acids were identified which hitherto had escaped detection. Normal saturated and monoethenoid acids having 15, 17, and 19 carbon atoms were found but none with branched chains. A series of C_{20} and C_{22} acids having from 0–5 double bonds was detected. Some of these have not been reported previously.

Experimental

A sample of commercial steam rendered lard, obtained on the same day it was produced, was promptly converted to methyl esters by methanolysis (6) and separated by GLC on a nonpolar column. Six fractions were collected and each rechromatographed on a polar (EGS) column, before and after hydrogenation.

Gas-Liquid Chromatography. The apparatus was a conventional type with thermal conductivity detector and was described previously (3). The separation on nonpolar stationary phase was performed with a stainless steel coiled column, 4 ft × ¼ in. OD (0.180 in. ID), packed with 42–60 mesh chromosorb "W" coated with 20% Dow Silicone Fluid 710. The polar column was 8 ft × 3/16 in. OD (0.118 in. ID) and was packed with 42–60 mesh Chromosorb "W" coated with 25% ethylene glycol succinate polyester. The washing of the Chromosorb with acid and base, the preparation of the polyester, and the method of collecting components from the GLC operations are described elsewhere (2,7).

Results and Discussion

The chromatogram of methyl esters of lard fatty acids separated according to chain length by gas-liquid chromatography on a nonpolar (silicone) column is shown in Figure 1. Six fractions from the column were collected at points indicated by arrows on the chromatogram. Fraction 1 included esters of 10, 12, and 14 carbon acids. A small peak for C_8 is shown but the presence of this acid was not con-

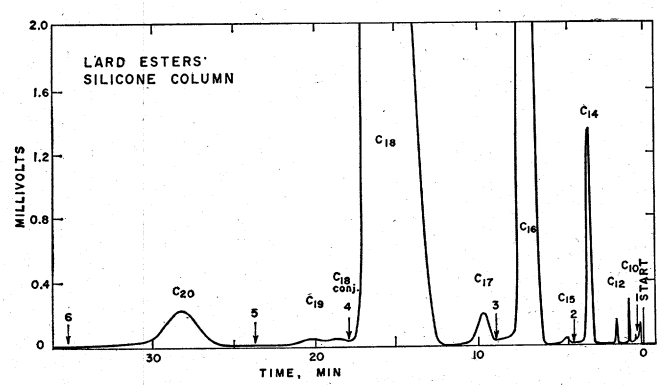

FIG. 1. Chromatogram of lard methyl esters. Column 4 ft × ¼ in., temp 210C, 20% silicone 710. Arrows indicate starting point of fraction collection.

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[3] A "shorthand" designation of structure of each fatty acid is used throughout this paper: 18:0 = saturated C_{18} acid; 18:2 = C_{18} with 2 double bonds; 18:0 br = branched chain C_{18} acid, etc.

firmed. Fractions 2, 3, and 4 included C_{15} and C_{16}, C_{17} and C_{18}, and conjugated C_{18} diene and C_{19}, respectively. Fraction 5 included only C_{20} esters. Fraction 6 was collected through the C_{22} region which is not on the figure because the peak was barely discernible. In order to obtain sufficient amounts of some of these fractions for further examination, it was necessary to repeat the collections from successive injections of 10-microliter portions of the methyl esters. On rechromatographing these fractions it was observed that components having shorter retention times were present as contaminants. This was always observed when silicone columns were employed but when components were collected from polyester columns in the same apparatus under the same conditions, the contamination occurred to only a minor extent. This fact would eliminate defects in the collection system as a possible cause. Therefore, it would seem likely that minor amounts of each component were retained in the silicone stationary phase after its peak was recorded probably owing to solubility and related molecular forces. Hence, it was necessary to rechromatograph and recollect these fractions to reduce this contamination to a negligible extent. Raising the column temperature from 210C to 225C also reduced this solubility "carry-over."

Each of these fractions (1–6) as obtained by recollection from the silicone column was then chromatographed on a polar column (EGS) in order to separate saturated from unsaturated esters. A portion of each fraction was hydrogenated and rechromatographed to give confirmation of chain length and to aid in establishing identity of some of the unsaturated components. Table I gives a summary of the fatty

TABLE I

The Fatty Acid Esters Identified in Each Lard Fraction Before and After Hydrogenation

Fraction 1	Hyd.	Fraction 2	Hyd.	Fraction 3	Hyd.	Fraction 4	Hyd.	Fraction 5	Hyd.	Fraction 6	Hyd.
10:0	10:0	15:0	15:0	17:0	17:0	18:0	18:0	20:0	20:0	22:0	22:0
12:0	12:0	16:0	16:0	17:1		19:0	19:0	20:1		22:1	
14:0	14:0	16:1		18:0	18:0	19:1		20:2		22:2	
14:1				18:1		conj. 18:2 *c,t*[a]		20:3		22:3	
				18:2		conj. 18:2 *t,t*[a]		20:4		22:4	
				18:3				20:5		22:5	

[a] *c,t* and *t,t* = *cis,trans* and *trans,trans*.

acid esters identified in each of the fractions before and after hydrogenation. Fractions 1, 2, and 3 were relatively simple and offered no difficulty in establishing the identity of the components from their relative retentions on the EGS column compared to known references. The identification of the esters in Fraction 4 required some supplemental examination. Since this fraction was small and was eluted from the silicone column (Fig. 1) immediately after the very large amount of C_{18} esters, it is not surprising that some of the latter would be present as shown on the EGS chromatogram (Fig. 2). Tentative assignments of identity were made on the basis of relative retention in the usual manner. The presence of conjugated *cis,trans* (or *trans,cis*) and conjugated *trans, trans* dienoic esters was confirmed by infrared spectrophotometry. The identity of the minor component whose retention falls between the conjugated isomers was not established. The largest peak on the chromatogram (Fig. 2) had a retention time corresponding to 19:1[3] and represented 34% of the total area

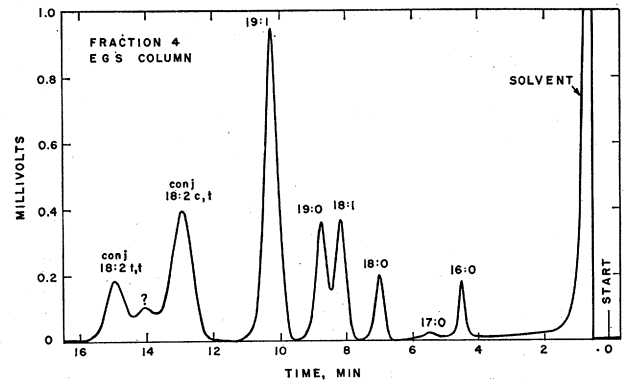

FIG. 2. Chromatogram of Fraction 4 collected from the silicone column. Column 8 ft × 3/16 in., temp 214C, 25% EGS polyester; *c,t* and *t,t* = *cis,trans* and *trans,trans*.

of the chromatogram, while that of 19:0 represented about 10%. After hydrogenation of a portion of this Fraction 4 and rechromatographing, the area represented by 19:0 (Fig. 3) amounted to 46.8% of the total area, a value in agreement with that expected (34.0 + 10.0).

Fraction 5, the C_{20} esters, which was collected from a single peak from the silicone column (Fig. 1) was rechromatographed on the EGS column (Fig. 4).

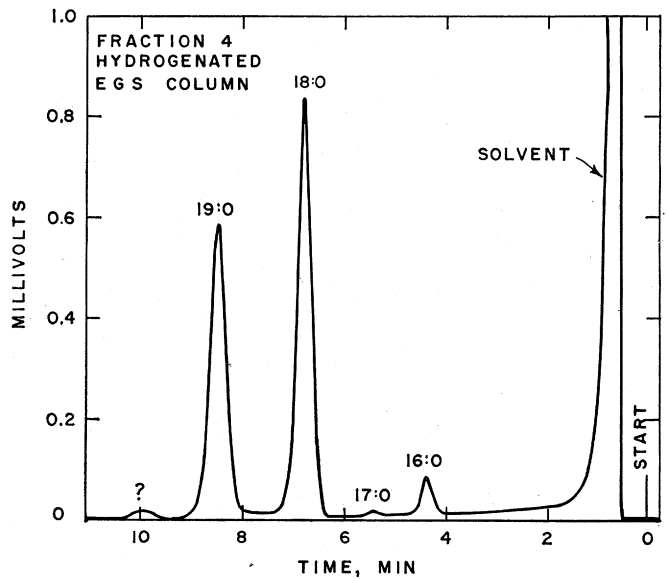

FIG. 3. Chromatogram of Fraction 4 (Fig. 2) after hydrogenation. Column same as in Fig. 2.

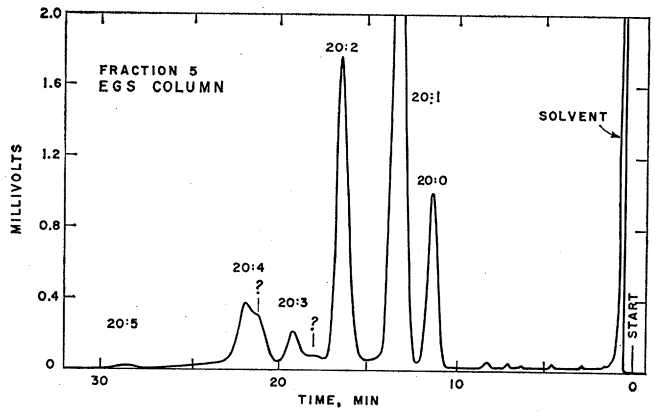

FIG. 4. Chromatogram of Fraction 5 collected from the silicone column. Column same as Fig. 2.

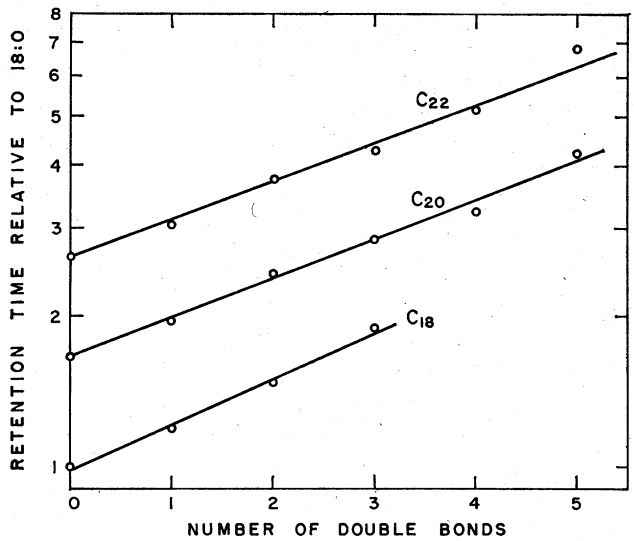

FIG. 5. Plot of log relative retention time vs. number of double bonds for 18, 20, and 22 carbon chain length methyl esters of lard.

Except for traces of shorter chain esters, the components were C_{20} esters. The peaks representing 20:0, 20:1, 20:2, 20:3, 20:4, and 20:5 are quite definite. The shoulder on the 20:4 peak and the small amount of material shown between 20:2 and 20:3 are believed to be isomeric 20:4 and 20:3 esters. The 20:0, 20:1, 20:4, and 20:5 esters were identified by comparison of retentions with those of reference compounds; the 20:2 and 20:3 esters by interpolation of the plot of log relative retention time against number of double bonds as shown in Fig. 5. This plot is reported to produce a straight line (5,8) with C_{18} esters. However, this can only be an approximation since position and geometrical isomers are known to have detectable differences in retention times. The presence of essentially only C_{20} esters in Fraction 5 was confirmed by chromatographing a hydrogenated portion (Fig. 6). The unidentified ester of lard mentioned in the previous publication (3) was undoubtedly 20:1. This ester accounts for about 50% of the C_{20} esters present in lard.

The small amount of material collected as Fraction 6, the C_{22} esters, was treated in the same way as Fraction 5; the 22:0, 22:1, and 22:5 esters were identified by comparison of retention with that of known references. The 22:2, 22:3, and 22:4 esters

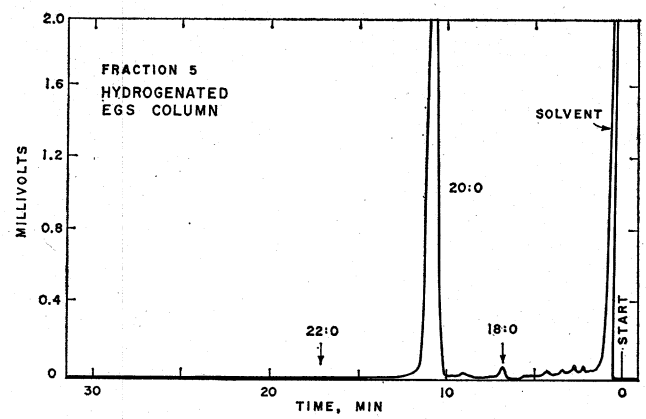

FIG. 6. Chromatogram of Fraction 5 (Fig. 4) after hydrogenation. Column same as Fig. 2.

were identified from a plot of log of relative retention time against number of double bonds. The principal C_{22} esters were 22:4 and 22:5.

The presence of tetraenoic and pentaenoic acids in the original lard was shown by the ultraviolet spectrophotometric method. However, this method does not differentiate by chain length. Owing the uncertainty as to quantitation of collection of the fractions from the silicone column, no attempt was made to estimate percentage distribution of the acids. However, by inspection of the area relations in all the figures shown, many of the components obviously were present only in trace amounts.

Further work in which the esters were fractionated by silicic acid column chromatography and the percentage distribution of esters in the fractions determined by GLC will be reported separately.

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