utilized for growth to some extent. Eventually it may be possible to calculate the percentage utilization of these products when the caloric availability of the component parts of the new-type fat is determined. Thus about 20% of the amylose stearate containing 18% amylose was digested. If amylose provides 4 calories per g and stearic acid 9 cal per g, then 20% of a 0.5-g supplement of amylose stearate could actually yield only 0.8 cal of energy to the animals which would be equivalent to 0.2 g of glucose.

The data emphasize the importance of the modification in the caloric availability assay that was introduced to detect false-positive weight gains for certain supplements. This was especially true for the 0.75-g adipostearin-supplemented group (−17) and the 0.75-g adipo-olein group (−12).

These data agree well with digestibility values reported by Shull et al. (6) for the diglyceride adipate, adipo-oleostearin.

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Cis-Trans Isomerization of Oleic, Linoleic and Linolenic Acids[1]

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Abstract

The equilibrium composition of *cis* and *trans* isomers obtained by isomerizing oleic, linoleic, and linolenic acids with selenium or nitrous acid has been studied using gas chromatography and infrared spectroscopy. The oleic/elaidic equilibrium mixture was found to contain 75–80% elaidic acid instead of the generally accepted 66% value. It is felt that the greater accuracy of gas chromatography and infrared analyses over older methods allows this equilibrium to be defined with greater precision.

Similar studies on the *cis-trans* isomerization of linoleic and linolenic acids indicated that their equilibrium mixtures also contained 75–80% *trans* double bonds. With linoleic acid, these *trans* bonds were shown to be randomly distributed among the double bonds present.

Cis-trans isomerization of linoleic or linolenic acids with selenium produced by-products having elution times equivalent to 18:2, 18:1, and 18:0 on a gas chromatograph. No such by-products were observed when oleic acid was isomerized. Apparently some type of hydrogen-transfer reaction accompanies the *cis-trans* isomerization of polyunsaturated acids with selenium.

Introduction

DURING RECENT WORK in this laboratory on the *cis-trans* isomerization of natural fats, it became necessary to know the maximum amount of *trans* bonds which such a process could introduce into the naturally-occurring *cis* fatty acids. It has long been recognized (1,2) that the *cis-trans* isomerization of unsaturated fatty acids is an equilibrium reaction, and that the complete conversion of all *cis* bonds to *trans* bonds in one reaction is impossible. Therefore, we set out to define the equilibrium concentration of geometric isomers for the three most common unsaturated fatty acids: oleic, linoleic, and linolenic.

Griffiths and Hilditch (1) studied the *cis-trans* isomerization of oleic, elaidic, petroselinic, and erucic acids using nitrous acid and sulfur as catalysts. They reported that the maximum amount of *trans* acid present at equilibrium was 66%. Bertram (2) reached similar conclusions after studying the isomerization of oleic and elaidic acids with selenium. It is now generally accepted that the equilibrium ratio of elaidic to oleic acid is 2:1, and that these equilibrium concentrations are independent of catalyst and processing conditions. However, the analytical techniques available to these workers 25–30 years ago were considerably less sophisticated than those available today; and, as Harwood (3) has recently pointed out, a reinvestigation of the oleic/elaidic equilibrium has been long overdue. Modern techniques such as gas-liquid chromatography (GLC) (4,5,6) and infrared spectroscopy (7) can now give a far more accurate picture of the geometric isomers present in *cis-trans* isomerization reaction mixtures.

The literature does not provide precise information as to the equilibrium ratio of *cis* and *trans* isomers for linoleic acid. MacGee, Mattson, and Beck (8) isomerized ethyl linoleate with SO_2 and were able to reduce the content of the 9-*cis*, 12-*cis* isomer to as low as 7%. They reported an 87% conversion of *cis* bonds to *trans* bonds based on infrared analysis of their reaction product. Subrahmanyam and Quackenbush (9) recently reported that approximately a 2:1 *trans* to *cis* ratio was achieved after the isomerization of ethyl linoleate with selenium. However, neither MacGee et al. (8) nor Subrahmanyam and Quackenbush (9) corrected their values for all the non-9, 12-octadecadienoate by-products present in their reaction mixtures, so that their equilibrium values are only approximate. *Cis-trans* isomerization is known to be accompanied by conjugation (8,9), polymerization (9), and catalyst addition products (1,10,19). On the basis of theoretical steric considerations, Blekkingh (11) predicted that the equilibrium ratio of *cis* and *trans* isomers for isomerized linoleic acid would be[3]: 17.6% 18:2—*9c,12c;* 17.6% 18:2—*9c,12t;* 17.6%

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[3] For brevity, the geometric isomers of oleic, linoleic, and linolenic acids are referred to in this paper by a shorthand designation. For example, 18:2—*9t,12c* refers to 9-*trans*, 12-*cis*-octadecadienoic acid. See Table I for a complete listing of such shorthand designations.

18:2—*9t,12c;* and 47.1% 18:2—*9t,12t*; but he reported no work to verify this prediction. Recent work on the equilibrium composition of *cis* and *trans* isomers for conjugated octadecadienoic acids (12,13) is not necessarily applicable to similar non-conjugated acids. Litchfield et al. (4) have recently reported a method for determining the four geometric isomers of methyl linoleate by gas chromatography, and their technique seems ideally suited for determing the *cis-trans* equilibrium point for linoleic acid.

No information concerning the equilibrium ratio of *cis* and *trans* isomers for linolenic acid was found in the literature.

This paper describes a study of the *cis-trans* isomerization of oleic, linoleic, and linolenic acids as well as their corresponding all-*trans* isomers (18:1—*9t;* 18:2—*9t,12t;* and 18:3—*9t,12t,15t*). The equilibrium concentration of *cis* and *trans* isomers was investigated for each acid using GLC and infrared spectroscopy.

Procedure

Materials

Oleic acid, 99+%, from Hormel Foundation, Austin, Minn., was used without further purification.

Elaidic acid was prepared from technical grade oleic acid using the procedure of Swern and Scanlan (16). The final product showed no impurities as determined by gas chromatography on a 200 ft Apiezon L capillary column, or by infrared absorption at 10.36 μ. Its melting point was 43.2–43.8C. Swern and Scanlan (16) report 44–45C.

Linoleic acid (18:2—*9c,12c*) was prepared essentially by the procedure of Keepler et al. (14). The final product showed no impurities as determined by gas chromatography on a 200 ft Apiezon L capillary column or by infrared absorption at 10.36 μ. The iodine value was 178.5 (theoretical: 181.0).

18:2—*9t,12t* was prepared by isomerizing 18:2—*9c, 12c* with selenium and then recrystallizing from methanol (15). The final product showed no impurities as determined by gas chromatography as above. Infrared absorption of its methyl ester at 10.36 μ was nearly twice that of methyl elaidate. The acid melting point was 27.8–28.4C. Kass and Burr (15) report 28–29C.

Linolenic acid (18:3—*9c,12c,15c*) was prepared from linseed oil essentially according to the bromination-debromination procedure of Rollett (17). The product analyzed 98% 18:3 by GLC on a diethylene glycol adipate polyester (DEGA) packed column. Infrared absorption at 10.36 μ indicated the presence of some *trans* bonds (4.7%) as is usual with fatty acids prepared by the bromination-debromination technique, but this impurity did not affect the usefulness of the material for our experiments.

18:3—*9t,12t,15t* was prepared from linseed oil using the procedure of Kass, Nichols, and Burr (18). The final product analyzed 99+% 18:3 by GLC on a packed DEGA polyester column. Infrared absorption at 10.36 μ indicated that 93% of the double bonds present had a *trans* configuration, but this small amount of *cis* impurity did not affect the usefulness of the material for our experiments. The melting point was 26–28C. Kass et al. (18) report 29–30C.

Methods

Gas Chromatography. A Barber-Colman Model 20 gas chromatograph with a capillary column and an argon-ionization detector was used for all high-resolution gas chromatography analyses. Operating conditions for analysis of the geometric isomers of oleic, linoleic, and linolenic acids on high-resolution capillary columns coated with Apiezon L, diethylene glycol succinate polyester, or nitrile silicone have been described (4,5,6,26). Where there was overlapping of 18:1, 18:2, and 18:3 isomers due to by-product formation (see Discussion), preparative gas chromatography was used to isolate relatively pure 18:2 and 18:3 fractions before analysis on the Apiezon L and nitrile silicone capillary columns. A 6 ft by $\frac{5}{8}$ in. packed column coated with 25% diethylene glycol succinate polyester (DEGS) and operated at 190C gave good preparative separations of 18:1 from 18:2 from 18:3 but did not distinguish between geometric isomers. Normal GLC analyses were run at 185C on 6 ft by $\frac{1}{4}$ in. packed columns coated with either 20% DEGS or DEGA polyester.

Infrared Spectroscopy. Infrared analyses to determine the amount of isolated *trans* bonds were run on Beckman IR-4 and IR-5 infrared spectrophotometers. The AOCS tentative method Cd 14–61 (7) was used with minor modifications. Pure 18:2—*9t,12t* methyl ester was used as a primary calibration standard instead of methyl elaidate when measuring the per cent *trans* bonds in isomerized polyunsaturated fatty acid methyl esters. Methyl elaidate was used as a primary calibration standard only with oleate/elaidate mixtures. Results were computed in terms of the per cent of double bonds present which had a *trans* configuration, rather than in terms of methyl elaidate. When gas chromatography showed that a sample contained small amounts of contaminating material (such as conjugated dienes), infrared results were corrected to give only the per cent *trans* bonds in the material being analyzed for.

Experimental

The following fatty acids were each isomerized to equilibrium using the two isomerization procedures described below: oleic acid; elaidic acid; 18:2—*9c,12c;* 18:2—*9t,12t;* 18:3—*9c,12c,15c*; and 18:3—*9t,12t,15t.*

CIS-TRANS *Isomerization with Selenium.* Five to ten g of fatty acid and 0.1–2.0% gray powdered selenium (depending on the amount of isomerization required) were placed in a magnetically-stirred round bottom flask with nitrogen blanketing. The flask was then placed in an oil bath preheated to 210C and maintained at this temperature until all the gray selenium disappeared and the fatty acid turned red. The reaction was performed in a fume hood to avoid harmful effects from the selenium vapors carried out by the nitrogen. The oil bath was not allowed to reach 216C, since selenium melts at this temperature, and in the liquid state its catalytic efficiency is greatly reduced. At the end of the reaction, the flask was removed from the oil bath and quickly cooled. No attempt was made to remove the selenium from the isomerized fatty acids, since it would not interfere with the analyses which followed. The reaction products were then converted to methyl esters by diazomethane, H_2SO_4-catalyzed esterification with methanol, or BF_3-catalyzed esterification with methanol.

Reactions with 18:2—*9t,12t* and 18:3—*9t,12,15t* were carried out in a 10% methyl laurate solution to conserve these synthesized isomers. Methyl laurate was chosen as a diluent since it would not interefere with the reaction nor the subsequent analyses.

It was presumed that equilibrium had been reached when repeating the selenium isomerization produced no further changes in the relative heights of the *cis-*

TABLE I
Per cent *Trans* Bonds After Equilibrium *Cis-Trans* Isomerization of C_{18} Unsaturated Fatty Acids

Fatty acid			% *Trans* bonds[a, b]			
			Se catalyst		HNO_2 catalyst	
Systematic name	Trivial name	Shorthand designation	By GLC	By I.R.	By GLC	By I.R.
9-*cis*-octadecenoic acid	Oleic	18:1—*9c*	75.0	74.0	79.6	74.6
9-*trans*-octadecenoic acid	Elaidic	18:1—*9t*	79.5	75.4	79.0	72.2
9-*cis*,12-*cis*-octadecadienoic acid	Linoleic	18:2—*9c,12c*	76.0	.....	75.4	75.8
9-*trans*,12-*trans*-octadecadienoic acid	Linolelaidic	18:2—*9t,12t*	76.3	.....	76.2	.....
9-*cis*,12-*cis*,15-*cis*-octadecatrienoic acid	Linolenic	18:3—*9c,12c,15c*	.....	.....	82.5	80.0
9-*trans*,12-*trans*,15-*trans*-octadecatrienoic acid	Linolenelaidic	18:3—*9t,12t,15t*	75.8	81.5	80.2	73.7

[a] Computed as the per cent of isolated double bonds present which have a *trans* configuration.
[b] The *cis-trans* isomerization of polyunsaturated fatty acids with either selenium or nitrous acid leads to the production of numerous by-products. These by-products were separated before analysis. Therefore, the per cent *trans* bonds reported here refers only to the non-conjugated 18:2 or 18:3 acids found in the reaction products.

trans isomer peaks on a gas chromatogram. Another check on equilibrium composition was when the pattern of GLC peaks for the all-*cis* isomer reaction appeared identical with those for the corresponding all-*trans* isomer reaction. It was usually necessary to perform several isomerizations on polyunsaturated fatty acids in order to reach equilibrium.

CIS-TRANS *Isomerization with Nitrous Acid.* Five to ten g of fatty acid and 3-15 ml (depending on the amount of isomerization required) of freshly prepared 2M $NaNO_2$ were placed in a round bottom flask equipped with a magnetic stirrer, a nitrogen purging system, and a dropping funnel. A flask having minimum volume was used to avoid loss of the gaseous catalyst. The system was purged with nitrogen and placed in a 60C oil bath. Nitrogen flow was stopped and the system closed except for exhaust through a bubble counter. 2-10 ml (⅔ of the vol of 2M $NaNO_2$) of 6M HNO_3 was then added dropwise with vigorous stirring to ensure mixing of the fatty and aqueous layers. After 3 hr, the flask was again purged with nitrogen and quickly cooled to room temperature. Distilled water was added and the isomerized fatty acid was extracted with Skellysolve F in a separatory funnel. The extract was washed repeatedly with distilled water to remove the yellow-brown catalyst addition products, dried over anhydrous Na_2SO_4, filtered, and the solvent evaporated. The reaction products were then converted to their methyl esters as described above. The presence of equilibrium was determined in the same manner previously described for selenium isomerized acids.

Analysis of Reaction Products. Each reaction mixture was analyzed for the geometric isomers present by GLC on high-resolution capillary columns. GLC data are shown in Table I. Results from oleic and elaidic acids are expressed as the per cent *trans* bonds in the octadecenoic acid from the reaction products. Results for 18:2 and 18:3 are expressed as the per cent *trans* bonds in the non-conjugated 18:2 or 18:3 acids found in the reaction products.

The oleic and elaidic reaction mixtures were analyzed directly for percent isolated *trans* bonds by measuring their infrared absorption at 10.36 μ (see Table I).

The *cis-trans* isomerization reaction mixtures of the polyunsaturated acids could not be analyzed directly by infrared for per cent *trans* bonds because of the presence of numerous reaction by-products (polymers, conjugated fatty acids, catalyst addition products, etc.). To eliminate these contaminants, relatively pure fractions were isolated by preparative gas chromatography on a packed DEGS polyester column. The fractions so isolated were analyzed for per cent isolated *trans* bonds using infrared. It has already been shown (4) that infrared and GLC analyses are equivalent for determining the per cent isolated *trans* bonds in a mixture of 9,12-octadecadienoate isomers. Therefore, infrared analysis was run only on the 18:2—*9c, 12c* nitrous acid reaction product to check the GLC results. As expected, the two methods agreed closely (75.4% *trans* by GLC, 75.8% *trans* by infrared). Three of the 18:3 acid reaction products were also isolated by preparative GLC and then analyzed by infrared. In the case of the selenium isomerized 18:3—*9c,12c,15c,* however, it was necessary to use so much selenium (4.5%) to reach equilibrium that a great many by-products were produced (see Discussion). Capillary column GLC showed that some of these unknown by-products eluted with 18:3 peaks and would be contaminants of unknown identity in any collected 18:3 fraction from preparative GLC. For this reason, it was not possible to determine the per cent isolated *trans* bonds in 18:3—*9c,12c,15c* isomerized to equilibrium with selenium.

Discussion

The data in Table I on oleic and elaidic acids indicate that the oleic/elaidic equilibrium mixture does not contain the expected 66% elaidic acid. GLC and infrared results on selenium isomerized material point to an equilibrium containing 74–80% elaidic acid. GLC results on nitrous acid isomerized material are in the same range, but infrared results are a few per cent lower. Since the monoene infrared samples were run without prior purification by preparative GLC, the presence of nitrogen-containing catalyst addition products (1,10) probably accounts for these lower infrared values. In any case, the amounts of elaidic acid found by infrared in the nitrous acid isomerized monoenes were still more than the expected 66%.

GLC results on both selenium and nitrous acid isomerized 18:2 acids indicate that about 75–77% *trans* bonds are present at equilibrium. The actual isomer content found at equilibrium was approximately: 6% 18:2—*9c,12c;* 19% 18:2—*9c,12t;* 19% 18:2—*9t,12c;* and 56% 18:2—*9t,12t.* Previous work (4) has shown that such GLC results are equivalent to those determined by infrared, but one reaction product was checked to verify this. After purifying the non-conjugated 18:2 material from the nitrous acid catalyzed reaction with 18:2—*9c,12c* using preparative GLC, the amount of isolated *trans* bonds measured 75.8% by infrared compared to 75.4% by GLC.

Infrared and GLC results on selenium and nitrous acid isomerized 18:3 acids were more scattered (possibly due to the presence of small amounts of non-18:3 by-products in the collected GLC fractions), but they generally point to an equilibrium composition containing between 74 and 82% *trans* bonds.

From the above results, it appears that the per cent

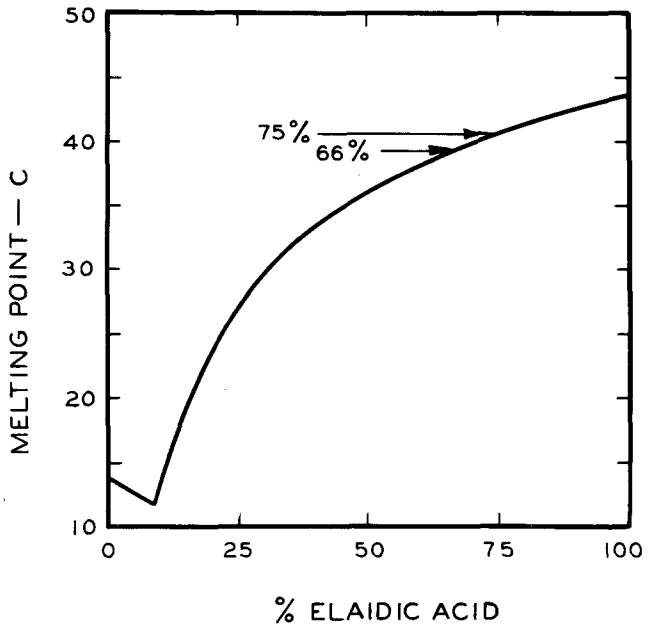

FIG. 1. Melting point diagram for mixtures of oleic and elaidic acids according to Griffiths and Hilditch (1).

trans bonds at equilibrium was about the same for all the fatty acids tested. The final product contained ca. 75–80% *trans* bonds whether the initial acid contained one, two, or three double bonds. This indicates that there is no interaction of one isolated (non-conjugated) double bond with another as far as *cis-trans* equilibration is concerned. (Double bonds close to the carboxyl or other substituent groups might possible deviate from this pattern, however.) Switching from selenium to nitrous acid as an isomerization catalyst did not appreciably alter the equilibrium compositions for any of the acids tested.

Because our results on the oleic/elaidic equilibrium differed appreciably from those previously reported by Griffiths and Hilditch (1) and Bertram (2), their data were re-examined in detail. Griffiths and Hilditch used a lead salt precipitation method for analyzing the amounts of oleic and elaidic acids in their reaction products. At the time of their research, they were hoping to develop an analytical procedure for determining oleic acid by converting it to elaidic acid, which could be much more easily precipitated for gravimetric analysis. To do this, they investigated the per cent conversion of oleic to elaidic acid. They concluded that: "The *trans* acid is formed to the extent of about 66% of the acid isomerized" (1). Therefore their 66% elaidic acid was a *yield* figure, and did not necessarily represent the equilibrium between oleic and elaidic acids. They also reported appreciable percentages of nitrogen-containing catalyst addition products in their reaction mixtures. If one calculates the oleic/elaidic equilibrium from their data using only the amounts of oleic and elaidic acids found in the reaction products, values of 75–87% elaidic acid are obtained. On this basis, the work of Griffiths and Hilditch (1) does not disagree greatly with the work reported here.

The oleic/elaidic equilibrium data of Bertram (2) was obtained using the iodine equilibrium constant method of van der Steur (27). It is not known why Bertram's results differ from those presented here.

Some workers (1,19) have used the melting point of oleic/elaidic acid mixtures to determine their composition. Such a melting point vs. composition curve is shown in Figure 1. This analytical method has two weaknesses: a) in the 60–80% elaidic acid range, the melting point is fairly insensitive to changes in composition; b) any catalyst addition products present could artificially lower the melting point and thus indicate a fictitiously low elaidic acid content. The melting point data of Bertram (2) is markedly different than that shown in Figure 1.

In view of the above, we feel that the greater accuracy of GLC and infrared analyses over older methods of measuring *trans* fatty acids now allows *cis-trans* equilibria to be defined with greater precision than before.

McCutchon et al. (10) have studied the elaidinization of methyl ricinoleate using nitrous acid as a catalyst. Using infrared analyses, they found that the maximum *cis* to *trans* conversion of the ethylenic bond was about 76%. This value agrees with our equilibrium determinations. In their article, McCutchon et al. attributed this high equilibrium value to the influence of the nearby hydroxyl group in methyl ricinoleate. However, in light of the work reported here, the double bond in methyl ricinoleate may have acted independently of the hydroxyl group and have merely reached the same equilibrium point as we have shown for oleic acid.

Similar *cis-trans* equilibrium concentrations have been shown for the polymer of 1,3-butadiene, which contains a 1,4-diene system similar to linoleic and linolenic acids. According to Berger and Buckley (25) and Golub (25), an equilibrium mixture of 75–80% *trans* and 20–25% *cis* double bonds results when this polymer is isomerized with ultraviolet irradiation in the presence of phenyl disulfide. These equilibrium values (obtained by infrared analysis) agree closely with our results with fatty acids.

High-resolution gas chromatography can now be used to follow the *cis-trans* isomerization of linoleic acid (4) and determine the content of the four geometric isomers at any point in the reaction. During the course of the experiments described here, a considerable amount of such data was obtained. We decided to use this information to determine the randomness of the *cis-trans* isomerization reaction with linoleic acid, i. e., if the *trans* bonds produced were randomly distributed among those double bonds present.

If one assumes that during *cis-trans* isomerization of linoleic acid *cis* bonds are converted into *trans* bonds (and vice versa) in a random manner, then the isomer content of the product can be computed at any given content of *trans* bonds by using the laws of probability. The curves in Figure 2 show the percentages of 18:2—*9c,12c*, 18:2—*9c,12t* plus 18:2—*9t, 12c*, and 18:2—*9t,12t* isomers in such randomly isomerized linoleic acid. Experimentally determined values using both selenium and nitrous acid catalysts are also shown, and they agree closely with the calculated curves. The amount of each isomer appears to be just about that which would be predicted by a random reaction mechanism. Peak separation in the GLC analysis (4) is such that the 18:2—*9c,12c* and 18:2—*9t,12t* isomers are determined most accurately. Because of peak overlapping at certain isomer ratios, the mono-*trans* isomer (18:2—*9c,12t* and 18:2—*9t,12c*) determinations are somewhat less accurate. Therefore, the curve in Figure 3 shows the combined amount of these two isomers. However, in those reaction products where accurate analyses could be obtained, the two mono-*trans* isomers were found to be present in

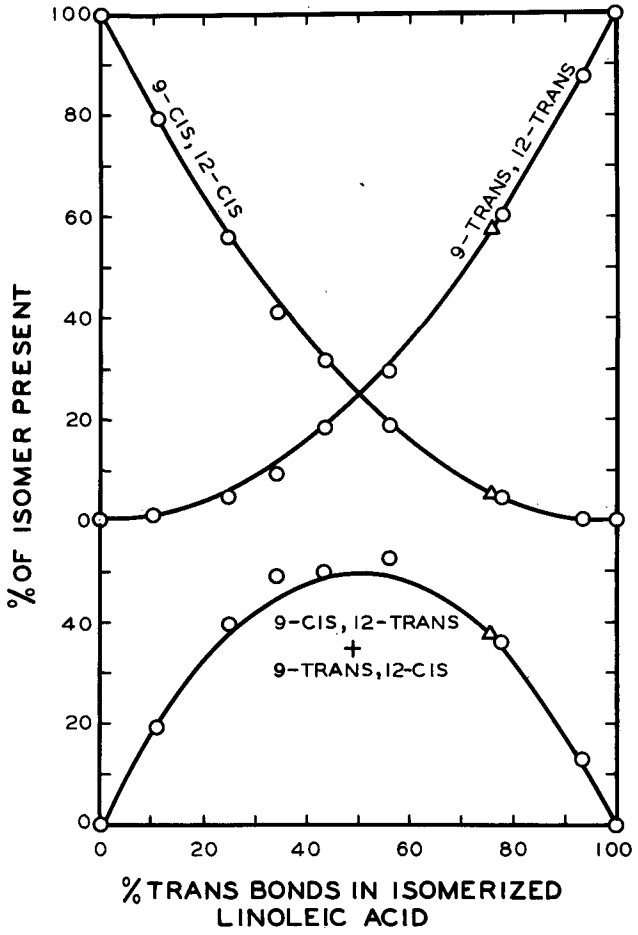

FIG. 2. Isomer composition of randomly *cis-trans* isomerized linoleic acid ——— calculated curve for random isomerization reaction. ○ experimental values using selenium catalyst. Δ experimental values using nitrous acid catalyst.

approximately equal amounts. It appears quite likely, therefore, that the *cis-trans* isomerization of linoleic acid with selenium or nitrous acid proceeds in a random manner. If this is true, then the isomers produced by *cis-trans* isomerization of linoleic acid can be computed from the per cent *trans* bonds (by infrared spectroscopy) found in the 9,12-octadecadienoic fraction of the products.

While running GLC analyses on selenium isomerized linoleic and linolenic acids, we noted with surprise that the isomerization reaction produced by-products with GLC elution times equivalent to 18:2, 18:1, and 18:0. Figure 3 shows a typical example. Before isomerization, the methyl linolenate eluted as one peak. After isomerization, there were also 18:0, 18:1, and 18:2 peaks present. The same by-products had similar elution characteristics on Apiezon L columns. No such by-products were observed when oleic or elaidic acids were isomerized. Apparently some type of hydrogen-transfer reaction accompanies the *cis-trans* isomerization of polyunsaturated fatty acids with selenium. No similar by-products were found when nitrous acid was used as a catalyst.

Teeter et al. (20) have reported a reaction which may offer a possible explanation for these by-products. These authors reported that at 250C selenium can cause the cyclization of conjugated octadecadienoic acid into a six membered ring by abstracting hydrogens from the methylene groups adjacent to the double bonds. The hydrogen thus removed is transferred to another unsaturated fatty acid, resulting in

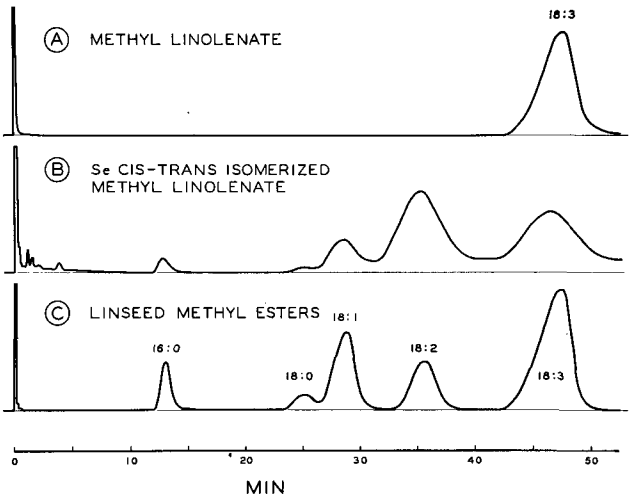

FIG. 3. Gas chromatograms showing the appearance of more saturated by-products during *cis-trans* isomerization with Se. (A) Linolenic acid before isomerization. (B) Linolenic acid after isomerization with 4.5% Se. (C) Linseed methyl esters used to identify peaks.

hydrogenation. Since conjugated fatty acids are known to be by-products in the selenium catalyzed isomerization of polyunsaturated fatty acids (8,9,11), and since no hydrogen-transfer occurred with oleic or elaidic acids, this proposed mechanism fits the observed facts. Similar hydrogen-transfer reactions between selenium and an olefin have also been observed with guaiene by House and Orchin (21), with cholesterol by Doree and Petrow (22), with several unsaturated fatty acids by Yohoyama and Kotake (23), and with various indenes by Ruzicka and Peyer (24).

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