able for this as well as the ammonium hydroxide process by eliminating leakage of hazardous vapors.

A typical plant installation is shown in Figure 3, showing the compactness and neat housekeeping possible with the rotating contactor. These plants range in size from 4 to 10 tank cars per-day capacity.

Summary

New developments, using the rotating contactor, in the vegetable oil processing industry have been described and illustrated. Results from various refining operations on a number of oils have been presented. Economies from reduction of capital expenditures and operating costs are expected to accrue to the user. He can also choose the process to give those products best suited to the market. The improved rotating contactor offers to the vegetable oil processor a most versatile plant.

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Application of Gas-Liquid Partition Chromatography to the Quantitative Estimation of Monoglycerides [1,2]

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THE ISOLATION and fatty acid analysis of monoglycerides have recently received particular attention in regard to studies of the positional arrangement of fatty acids in natural triglycerides. Such studies have been made possible by the discovery that the hydrolysis of triglyceride by pancreatic lipase is a series of directed stepwise reactions from triglyceride to $\alpha\beta$-diglyceride to β-monoglyceride (8, 14). Thus the nature of the fatty acids present in the β-position of the original triglyceride is readily determined by isolation of the β-monoglycerides from the hydrolysate and analysis of their component fatty acids (9, 15).

Monoglycerides have been separated from mixtures of mono-, di-, and triglycerides by countercurrent distribution (4), silicic acid chromatography (3), reversed-phase chromatography on siliconized kieselguhr (13), or silica gel adsorption chromatography (12, 11). To obtain the β-monoglycerides free from the α-isomer, Borgström (3) proposed treating the isolated monoglycerides with periodic acid and then separating the β-monoglycerides from the products of oxidation of the α-isomers (glycoaldehyde esters) by silicic acid chromatography. Because of the ease of $\alpha \rightleftharpoons \beta$ isomerization of monoglycerides, Savary and Desnuelle (15) recently modified the procedure by treating the total glyceride mixture with periodic acid and then separating the resulting glycolaldehyde esters from the unreacted β-monoglycerides on a siliconized kieselguhr column; the di- and triglycerides remained on the column with the solvent system used.

The present investigation was aimed at developing a method for determining the fatty acid composition of monoglycerides in a mixture of glycerides, without first having to isolate the monoglycerides. The approach used was to convert only the monoglycerides to volatile derivatives, which could then be separated by gas-liquid partition chromatography (GLPC). Conversion of α-monoglycerides to their isopropylidene derivatives (1) appeared to be a promising method. However it was found that, although the isopropylidene α-monoglycerides containing C_2 to C_{18} fatty acids were readily separable by GLPC, the higher members of the series (C_{16}–C_{18}) had excessively high retention-times. Furthermore this method would be applicable only to the α-monoglycerides since the β-isomers do not form isopropylidene derivatives under the conditions used.

A more feasible method proved to be the conversion of both α- and β-isomers to allyl esters of the corresponding fatty acids, *via* the dimesyl derivatives, as outlined in Figure 1. Both α- and β-monoglycerides were found to be converted quantitatively to allyl

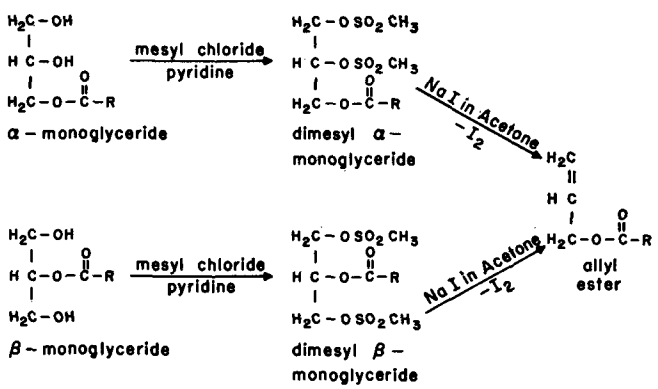

FIG. 1. Reaction scheme for the conversion of α- and β-monoglycerides to the allyl esters of the corresponding fatty acids.

esters, and the allyl esters of C_8 to C_{18} fatty acids proved to be readily separable by GLPC. Furthermore β-monoglycerides could be determined separately by the same method after removal of the α-isomers by periodate oxidation. Finally the analysis of monoglycerides could be carried out equally well

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[2] Presented at the 32nd fall meeting, American Oil Chemists' Society, Chicago, Ill., October 20–22, 1958.
[3] National Research Council Postdoctorate Fellow, 1956–1958.

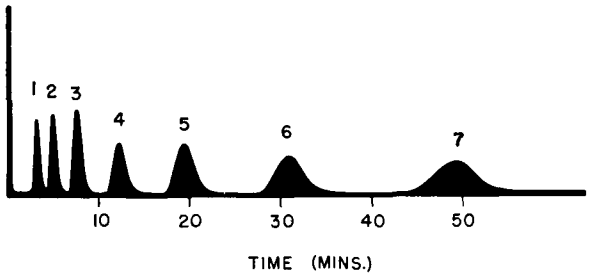

FIG. 2. Separation of isopropylidene monoglycerides of fatty acids from C_2 to C_8 inclusive on a 4-ft. column of Apiezon M–Celite at 198°C. Peak identification: (1) acetic, (2) propionic, (3) butyric, (4) valeric, (5) caproic, (6) heptanoic, (7) caprylic.

in the presence of fatty acids, diglycerides, and triglycerides, thus eliminating the necessity for isolating the monoglycerides.

Experimental

Analytical Methods

Iodine numbers were determined by the method of Yasuda (18), using a three-hour reaction time for the allyl esters.

Vicinal glycol titrations were carried out by the method of Martin (7).

All melting-points are uncorrected.

Gas-liquid chromatographic separations were carried out on a Podbielniak Chromacon (Series 9475-3V) with a thermal conductivity-detector. The apparatus was modified to take glass columns and to improve the pressure regulation, temperature control, collection and injection systems. The chromatographic columns (122 cm. long and 4 mm. inside diameter) were packed with 12 g. of Celite 545 impregnated with Apiezon M (4:1 w/w) (5). The samples were injected into the gas stream (helium), using 2–20 μl. pipettes. Peak heights were used to give a quantitative measure of allyl esters of fatty acids C_8–C_{12} whereas peak areas on the recorder were used for the C_{14}–C_{18} esters. Because of the difficulty of adding precise amounts of allyl esters to the chromatogram, a known weight of methyl laurate or methyl myristate was added to each sample to provide an internal standard.

Preparation of Isopropylidene Monoglycerides

Isopropylidene monoglycerides of fatty acids C_2–C_{18} were prepared by acylation of DL-acetone glycerol (10) with pure fatty acid chlorides by the method of Baer and Fischer (1).

Preparation of α-Monoglycerides

α-Monoglycerides were prepared by removal of the blocking group from the isopropylidene monoglycerides, as outlined by the above authors (1).

DL-α-Monolaurin

$C_{15}H_{30}O_4$ (M.W. 274.4),
Calculated: C,65.65; H,11.02
Found: C,65.43; H,11.15

M.P., 53.2–53.9°; reported for L-α-monolaurin, 54–55° (1).

Vicinal glycol content, 99.6% of theory.

DL-α-Monomyristin

$C_{17}H_{34}O_4$ (M.W. 302.4),
Calculated: C,67.5; H,11.33
Found: C,67.25; H,11.24

M.P., 62.7–63.3°; reported for L-α-monomyristin, 62–64° (1).

Vicinal glycol content, 99.8% of theory.

Preparation of β-Monoglycerides

β-Monoglycerides were synthesized *via* the 1,3-benzilidene derivatives, as described by Stimmel and King (16).

β-Monolaurin

$C_{15}H_{30}O_4$ (M.W. 274.4),
Calculated: C,65.65; H,11.02
Found: C,65.40; H,11.12

M.P., 49.5–50.5°; reported, 51.1° (16).

Vicinal glycol analysis showed the presence of 1.6% of the α-isomer.

Preparation of Dimesyl Monoglycerides

Dimesyl DL-α-monolaurin and dimesyl β-monolaurin were synthesized from α- and β-monolaurin, respectively, by the method of Baer and Newcombe (2). After crystallization from acetone-ethanol (2) the compounds gave the following analyses:

Dimesyl DL-α-Monolaurin

$C_{17}H_{34}O_8S_2$ (M.W. 430.4),
Calculated: C,47.42; H,7.96; S,14.89
Found: C,47.65; H,8.15; S,14.63

M.P., 53.2–54.2°.

The infrared spectrum showed the absence of free hydroxyl groups.

Yield from α-monolaurin, 99%.

Dimesyl β-Monolaurin

$C_{17}H_{34}O_8S_2$ (M.W. 430.4),
Calculated: C,47.42; H,7.96; S,14.89
Found: C,47.62; H,8.26; S,15.10

M.P., 55–56°; a mixed melting point of the α- and β-isomers was 48.7–49.3°. The infrared spectrum showed the absence of free hydroxyl groups.

Yield from β-monolaurin, 98.3%.

Preparation of Allyl Esters

Allyl esters of C_2–C_{18} fatty acids were synthesized from allyl alcohol and the free acids in the presence of naphthalene-2-sulfonic acid, by the method of Swern and Jordan (17).

Allyl laurate was also prepared from dimesyl α- and β-monolaurin as described in the section on Procedure for Analysis of Monoglycerides, in yields of 97.7% and 98.4%, respectively. Both allyl esters (from dimesyl α- and β-monolaurin) had an iodine value of 104, and their infrared spectra were identical with that of authentic allyl laurate.

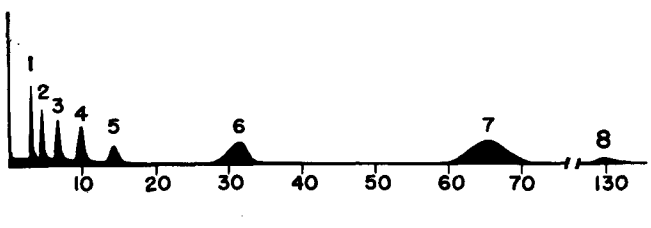

FIG. 3. Separation of isoproylidene monoglycerides of fatty acids from C_8 to C_{18} on a 4-ft. column of Apiezon M–Celite at 240°C. Peak identification: (1) caprylic, (2) pelargonic, (3) capric, (4) hendecanoic, (5) lauric, (6) myristic, (7) palmitic, (8) stearic.

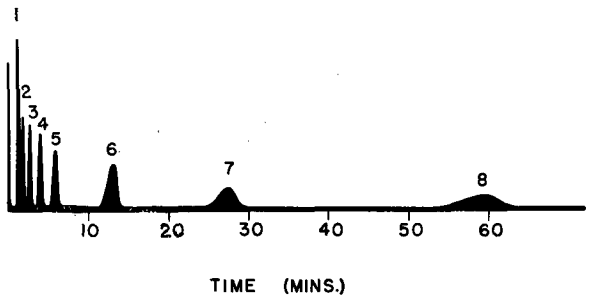

FIG. 4. Separation of synthetic allyl esters of straight chain fatty acids C_8 to C_{18} on a 4-ft. column of Apiezon–Celite at 240°C. Peak identification as in Figure 3.

Procedure for Analysis of Monoglycerides

a) *Total Monoglycerides.* One hundred milligrams of the monoglyceride or lipid mixture in a dry glass-stoppered test tube were dissolved in 1 ml. of alcohol-free anhydrous chloroform; 0.1 ml. of anhydrous pyridine was added, and the mixture was cooled to 0°C. in an ice bath. Redistilled methane-sulfonyl chloride (0.1 ml.) was added, and the contents were thoroughly mixed and allowed to stand at 0°C. overnight.

The mixture was quantitatively transferred into 100 ml. of ice-cold water, and the test tube was washed several times with chloroform. The aqueous-chloroform mixture was transferred to a 250-ml. separatory funnel, and the aqueous phase was extracted three times with 50-ml. portions of chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate and taken to dryness in a rotary evaporator at 35°C. The residue was dried in a vacuum desiccator over phosphorus pentoxide for 2 hrs., dissolved in anhydrous acetone, and transferred quantitatively to a Pyrex glass tube (22 cm. in length x 6 mm. inside diameter). The volume of the solution was adjusted to 1 ml. in a stream of nitrogen, and 1 ml. of a 25% solution of sodium iodide in anhydrous acetone was added. The tube was sealed and heated at 100°C. for 2 hrs. The contents of the tube were cooled and washed into a 250-ml. separatory funnel with several portions of water and ether. Free iodine was removed by addition of a 10% aqueous solution of sodium thiosulfate, and the aqueous phase was extracted with three 50-ml. portions of ethyl ether. The combined ether phases were dried over anhydrous sodium sulfate and taken to dryness in a rotary evaporator at 35°C. The residue was dissolved in cyclohexane, and aliquots of the solution were taken for analysis by GLPC.

b) *β-Monoglycerides.* A mixture of α- and β-monoglycerides containing not more than 100 mg. of the α-isomer was dissolved in 25 ml. of chloroform. The periodate-in-methanol reagent (25 ml.) of Kruty *et al.* (6) was added, and the sample was heated to boiling. After cooling for 30 min., the solution was quantitatively transferred to a separatory funnel containing 50 ml. of water. The chloroform phase was washed with three 50-ml. portions of water to remove iodic and periodic acids. The chloroform fraction was dried over anhydrous sodium sulfate and taken to dryness in a rotary evaporator at 35°C. The unchanged β-monoglycerides in the residue were converted to allyl esters, as described above. The periodate oxidation step could be carried out equally well by the method of Savary and Desnuelle (15).

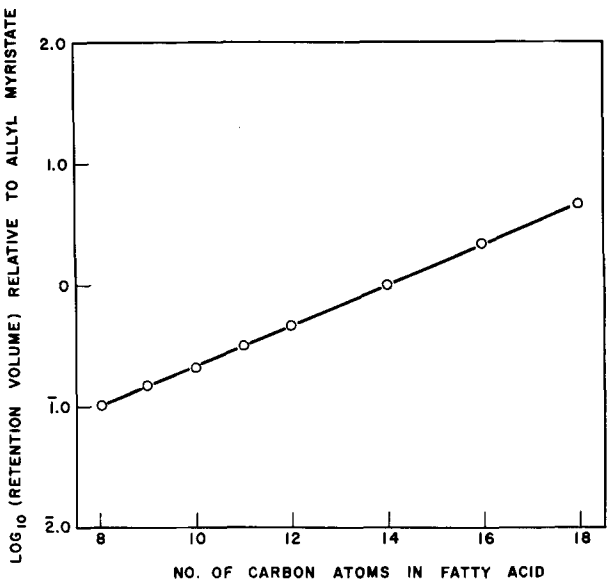

FIG. 5. Plot of log retention-time relative to allyl myristate *versus* number of carbon atoms in the constituent fatty acid of the allyl ester. Conditions as in Figure 4.

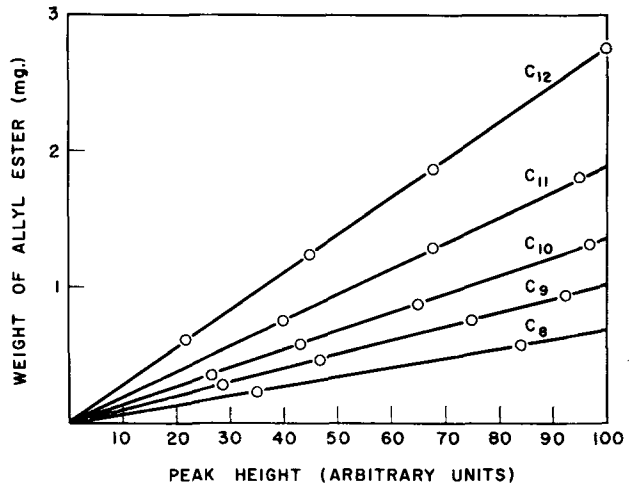

FIG. 6. Calibration curve for the estimation of allyl esters of fatty acids C_8 to C_{12}. Conditions as in Figure 4.

Results and Discussion

The isopropylidene monoglycerides of fatty acids C_2 to C_8 were readily separated by GLPC at 198°C. (Figure 2). The higher esters (C_8–C_{18}) required a higher temperature (240°C.), and even then the C_{18} derivative had a retention time of 130 min. (Figure 3). Nevertheless GLPC should prove useful for checking the purity of isopropylidene monoglycerides when these are used in the synthesis of α-monoglycerides.

Allyl esters of fatty acids C_8–C_{18} could be readily separated at 240°C.; the retention time for the C_{18} ester was 60 min. (Figure 4). The logarithms of the relative retention-times are a linear function of the number of carbon atoms in the fatty acid, as shown in Figure 5; the identification of an unknown allyl ester is thus easily made. When the instrument was calibrated with varying amounts of esters (Figure 6), the peak height of C_8–C_{12} esters increased linearly with the increasing weight of the ester injected over the range 0–3 mg.; with C_{14}–C_{18} esters the weight of esters was found to be proportional to the peak areas. To establish that no structural changes occurred dur-

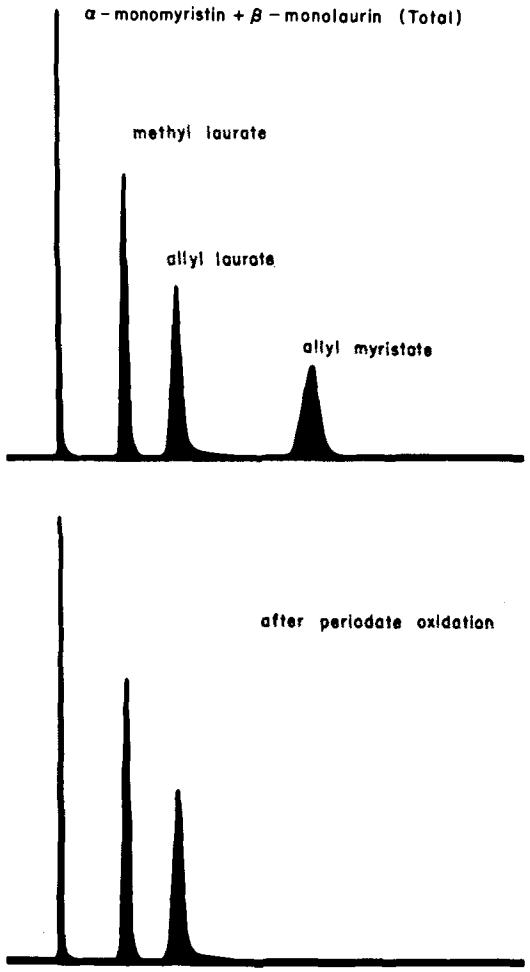

FIG. 7. Differentiation between α- and β-monoglycerides in a mixture of α- and β-isomers by the periodate oxidation technique. Methyl laurate added as internal standard for GLPC. Conditions as in Table I and Figure 4.

ing GLPC separation of the allyl esters, samples of synthetic allyl esters were collected from the effluent gas-stream. Iodine values and infrared spectra were found to be identical with those of the original esters.

The efficiency of each step in the analytical procedure was tested with α- and β-monolaurin as model substrates. Conversion of each isomer to the corresponding pure crystalline dimesyl derivative was achieved in yields of 98–99%, and each of the dimesyl derivatives was converted to allyl laurate in yields of approximately 98%. Since the dimesyl derivatives were not identical (as shown by depression of the mixed melting-point), conversion of the β-compound to allyl laurate must of necessity involve an intramolecular migration of the acyl group from β to α position (possibly *via* a cyclic ortho ester). The mechanism for this conversion was however not investigated.

The quantitative aspects of the over-all procedure (without isolation of intermediates) are shown in Table I and Figure 7. The over-all yield of allyl ester from both α- and β-isomers, determined either separately or together, was 96–98%; also differentiation between α- and β-isomers in a mixture was quantitative (Figure 7), and the presence of free fatty acids, di- and triglycerides, did not interfere with the reaction (Table I). As shown in Figure 7, free fatty acids (as the methyl esters) could also be determined by GLPC simultaneously with the allyl esters.

TABLE I

Gas Liquid Chromatographic Analysis of Monoglycerides

Lipid components	Weight of allyl ester estimated by GLPC [a]	Calculated wt. of allyl ester	Yield [b]
	mg.	*mg.*	%
α-Monolaurin..... (100 mg.)	85.0, 85.4, 85.9, 85.7, 86.0, 85.4 Ave., 85.6	87.6	97.7 ± 0.2
β-Monolaurin..... (100 mg.)	85.7, 85.9, 84.8, 85.1, 85.6, 85.2 Ave., 85.4	87.6	97.5 ± 0.2
α-Monomyristin.. (100 mg.)	86.0, 85.9, 86.2, 85.7, 85.0, 85.9 Ave., 85.8	88.7	96.7 ± 0.2
α-Monomyristin.. (50 mg.)	43.2, 42.9, 42.8, 42.7 Ave., 42.9	44.4	96.6 ± 0.3
+			
β-Monolaurin..... (50 mg.)	43.0, 42.8, 42.8, 43.0 Ave., 42.9	43.8	97.9 ± 0.1
Same as above after periodate oxidation.....	0	0	0
	42.0, 42.2, 43.1, 42.8 Ave., 42.5	43.8	97.1 ± 0.5
α-Monolaurin..... (100 mg.)	87.0, 86.8, 85.9, 86.0 Ave., 86.4	87.6	98.7 ± 0.3
α,β-Dimyristin.... (100 mg.)			
Trimyristin..... (100 mg.)			
Myristic acid..... (100 mg.)			

[a] Each value is the result of an independent determination.
[b] Values are averages with standard deviations.

The direct determination of α-monoglycerides by conversion to the glycolaldehyde esters, oxidation with potassium permanganate to the corresponding acids, and subsequent analysis of the methyl esters by GLPC was attempted, and preliminary experiments showed that these esters were separable. Further work to establish the quantitative aspect of this reaction is contemplated.

The high yields and reproducibility of the analyses for allyl esters given in Table I indicate that this method should prove valuable in the quantitative and qualitative estimation of α- and β-monoglycerides containing fatty acids of different chain lengths. In particular, the method should also be useful in studies of the positional arrangement of fatty acids in natural triglycerides.

Summary

A procedure has been developed for the quantitative estimation of monoglycerides in terms of their constituent fatty acids. The monoglyceride mixture is mesylated with mesyl chloride in the presence of pyridine, and the resulting dimesyl derivatives are converted to allyl esters of the constituent fatty acids by treatment with sodium iodide in anhydrous acetone at 100°C. The allyl esters are then analyzed quantitatively by gas-liquid partition chromatography at 240°C. on a column of Apiezon M—Celite. Both α- and β-monoglycerides are quantitatively converted to allyl esters by this procedure. β-Monoglycerides in a mixture of α- and β-isomers may be determined separately after removal of the α-isomers by oxidation with periodic acid. The analytical procedure is also applicable to monoglycerides in the presence of free fatty acids, diglycerides, and triglycerides.

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The Hydrogenation of Fatty Oils with Palladium Catalyst. III. Hydrogenation of Fatty Oils for Shortening Stock

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ALMOST ALL of the three billion pounds of hydrogenated edible oils produced annually in the United States are made with nickel. Although it has been known for a long time that palladium is an exceedingly active catalyst for carbon-carbon double bond hydrogenation, this catalyst has enjoyed no popularity in the edible oil field. The principal deterrent to the use of palladium has been economics, but, with the steadily decreasing ratio in the prices of palladium and nickel, palladium hydrogenations have become economically competitive. We set out therefore to find commercially attractive conditions by which palladium catalysts could give products of suitable stability and plasticity for use as shortening stocks.

Shortening stocks require a wide plastic range, which in turn demands a low amount of *trans* isomers. Usually palladium forms more *trans* isomers than nickel so an effort was made to ascertain how processing conditions could be adjusted to control the *trans* content within the desired limits. Proper control of selectivity was much less of a problem for in most experiments the selectivity was more than adequate. Although unsupported nickel is more selective than unsupported palladium, supported palladium has good selectivity with the conditions used in the present work. Palladium is more active and more selective than all other platinum group metal catalysts and was accordingly the platinum metal of choice for this study. Platinum, rhodium, and ruthenium were also examined, but each was inferior to palladium.

Experimental

Laboratory experiments were done in a stainless-steel hydrogenator of one-gallon capacity, provided with good mechanical agitation, a gas-dispersing system, a cooling coil, and an electrically-controlled heating system. Three pounds of oil were used in each experiment. The oils were soybean (iodine number = 127) and cottonseed and a 70/30 mixture (iodine number = 120) of the two. Processing was controlled by refractive index, and analyses of the oils were made by the official methods of the American Oil Chemists' Society. The *trans* content was calculated from the intensity of the 10.36 μ band, measured in a carbon disulfide solution on a Perkin-Elmer Model 21 infrared spectrophotometer, according to the procedure of Swern *et al.* (29), using the recommendations of the Spectroscopy Committee (30). The catalysts were all palladium on high-surface carbon. Catalysts A, F, G, and H were 1%, 5%, 2%, and 0.5% palladium on carbon powder, respectively. Catalysts B, C, D, and E were 1% palladium partially deactivated by silver and bismuth, containing 0.9%, 1.0%, 0.35%, and 0.5% silver and 0.6%, 0.35%, 0.25%, and 0.35% bismuth, respectively. All catalysts were prepared according to the directions given in the first paper (1) of this series except that the amounts of basic bismuth acetate, silver acetate, and palladous chloride were altered as needed to give these percentages.

Catalyst Activity

Palladium is an exceedingly active hydrogenation catalyst, and adequate hydrogen supply to the catalyst surface is a major problem. Some idea of how rapidly hydrogen is depleted from the catalyst surface may be gained from Figure 1. This is a semi-log

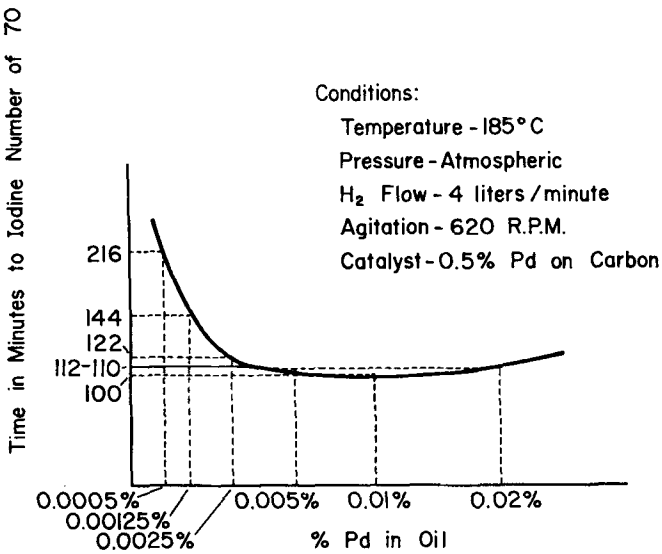

FIG. 1. Hydrogenation rate as a function of catalyst concentration.