by water from a constant temperature source. Total oil extracted was determined at the end of 10-min. intervals but only the results for 60-min. extractions are shown (Table I). Two other series in which the grits were preheated did not produce significantly different data and are not included.

As would be expected greater extraction was secured with a decrease in grit size and an increase in temperature. Absolute ethanol was a better solvent than 95% ethanol. This slower extraction probably resulted in part from the relatively low solubility of the oil. Rao, *et al.* (4), have shown that while peanut oil is completely miscible at 72C in absolute alcohol its solubility is less than 20% in 95% ethanol at this temperature. Solubility in 95% ethanol at 72C, and absolute alcohol at 43C, are not greatly different. At the higher temperature the coagulating effect on the protein of the absolute alcohol may also be a factor.

TABLE I
Residual Extractables in Solvent Extracted Peanut Grits
(Extraction time: 60 min)

Solvent	Temperature °C	Residual extractables, % of total	
		Grit size	
		+14 −20	+20 −30
Hexane	26	35.2	16.3
	43	31.0	14.6
95% Ethanol	43	77.5	63.8
	72	34.4	13.0
Absolute ethanol	43	31.0	10.8
	72	16.5	1.3

TABLE II
Peanut Oil Quality *

Sample No.	Mesh size	Extraction Solvent temp °C	Neutral oil, %	Phospholipids, %	Free fatty acid, %	Iodine value	Color	Sap. value
1	−14 +20	Hexane at 44C	91.8	6.8	0.6	84.9	1.58	192.0
2	−20 +30	Hexane at 44C	92.7	5.9	0.8	84.7	1.67	194.0
3	−14 +20	95% Alcohol at 72C	92.4	6.5	1.7	82.5	2.92	192.0
4	−20 +30	95% Alcohol at 72C	94.0	5.5	1.3	87.8	2.53	190.5
5	−14 +20	Abs. alcohol at 72C	94.9	4.9	1.4	83.9	2.41	193.8
6	−20 +30	Abs. alcohol at 72C	94.5	4.8	1.4	84.9	3.41	191.8
7	−14 +20	Hydraulic pressed	92.4	6.5	1.0	86.3	0.33	193.1
8	−14 +20	Abs. alcohol at 72C followed by ether	92.0	6.9	1.2	84.8	2.50	192.1

* Neutral oil and phospholipids were determined by the method of Choudhury and Arnold (2). Other determinations by standard A.O.C.S. Methods. + Extraction time 2 hr. Rate, 10 ml per min.

It was noted that part of the extracted material was a solid. This was separated by filtering and extracting with ethyl ether to remove the lipids. It was found that the solid non-lipids extracted by the 95% ethanol at 72C amounted on the average to 17.4% of the total extracted material and 20.9% of the lipids extracted. The corresponding values for the non-lipids extracted by absolute alcohol were 7.2% and 7.7%, respectively. The protein content of the solids extracted by the 95% ethanol averaged 17.6% and those extracted by the absolute ethanol 6.9%. Coagulation of more protein by the absolute alcohol probably reduced the amount extracted.

The oil, filtered free of solids, was evaluated for quality. See Table II. The hexane oils are lighter in color and have a lower FFA content. The oil extracted with absolute alcohol has a slightly lower phospholipid content than the other oils.

In summary, these studies indicate the following general conclusions: A) absolute ethanol is definitely a better solvent for extracting peanut oil from grits than 95% ethanol; B) at 43C hexane is superior to 95% ethanol and slightly inferior to absolute ethanol as a solvent; C) more non-lipid solids are extracted at 73C by 95% ethanol than by absolute ethanol; and D) ethanol-extracted oils are slightly higher in color and FFA than hexane-extracted oils.

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Analyses of Lipids and Oxidation Products by Partition Chromatography: Hydroxy Fatty Acids and Esters[1]

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Abstract

A liquid-partition chromatographic procedure was used to separate hydroxy fatty acids, their methyl esters, and reduced fatty ester hydroperoxides. Mixtures of methyl stearate, mono- and dihydroxystearate, and mixtures of the corresponding free fatty acids were easily separated. Chromatographic determinations for ricinoleate in castor oils compared favorably with the chemical and infrared analyses.

The chromatographic procedure was used to separate hydroxy fatty acids in *Dimorphotheca* and *Strophanthus* seed oils. The methyl ester of dimorphecolic acid, the principal hydroxy fatty ester of *Dimorphotheca* oil, behaved like reduced methyl linoleate hydroperoxide and showed a polarity intermediate between methyl 12-hydroxystearate and methyl 9,10-dihydroxystearate. The 9-hydroxy-12-octadecenoic ester of *Strophanthus* oil had a larger retention volume than methyl

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ricinoleate and could be separated from it. The purity of reduced methyl linoleate hydroperoxides and methyl dimorphecolate, isolated chromatographically, was comparable to that reported in the literature.

ZILCH AND DUTTON (22) showed that oxygen-containing fatty esters can be easily separated from ordinary fatty esters by countercurrent distribution between 80% ethanol and hexane. This classical study led a number of workers to apply liquid-partition chromatography (LPC) to effect these separations. Reverse-partition chromatography based on Boldingh's rubber column (4) has been used to separate various oxygen-containing acids from total fatty acids (7) and to analyze the hydroxy acid content of castor oil (21). These techniques, like many in elution chromatography, require eluants of varying composition to gain the fractionation desired. Direct LPC for the analysis of methyl ricinoleate in methyl esters of castor oil was also reported by Bergier (2), who used aqueous methanol as the stationary phase and petroleum ether as the mobile phase.

Previous papers in this series described a liquid chromatographic method to determine dimeric and polymeric products in fats and fatty acid hydroperoxides (9) using 20% methanol as the stationary phase and 2% methanol in benzene as the mobile solvent. The present paper deals with the application of LPC to the separation of naturally occurring hydroxy fatty acids, their methyl esters, and reduced fatty ester hydroperoxides.

Experimental

The methyl esters were obtained from the hydroxy fatty acid-containing oils by either of two procedures: a) Saponification by the AOCS method (Ca6b-53) using absolute methanol instead of ethanol as solvent. The reaction mixture was cooled to room temperature and gaseous HCl bubbled into it to obtain a 10% excess of acid. The reaction mixture was then allowed to stand at room temperature for 1 hr. b) Transesterification with freshly prepared potassium methylate by a procedure based on that described by Luddy *et al.* (12). Free fatty acids of castor oil were prepared by a standard saponification procedure. Reduced fatty ester hydroperoxides were prepared by reacting chromatographically pure hydroperoxides with sodium borohydride by the procedure of Mattic and Sutton (13).

The chromatographic procedure was the same as that described for the determination of dimeric and polymeric acids (9). One modification was in the preparation of the chromatographic column based on

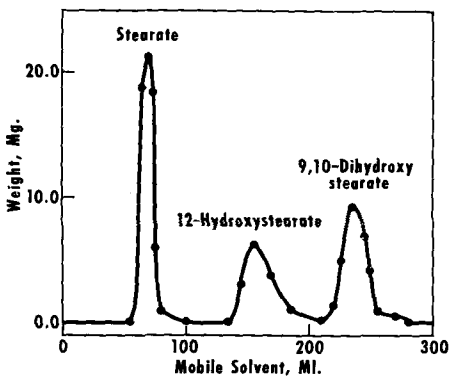

FIG. 1. Chromatographic separation of a mixture of methyl stearate, 12-hydroxystearate, and 9,10-dihydroxystearate.

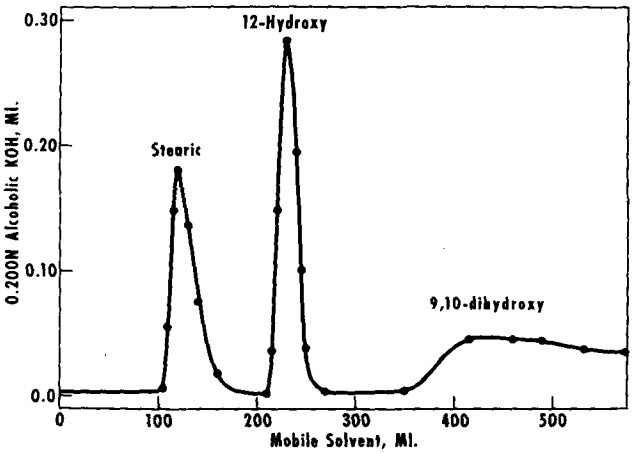

FIG. 2. Chromatographic separation of a mixture of stearic, 12-hydroxystearic and 9,10-dihydroxystearic acids.

a slurry procedure described by Jones and Stolp (11). The dry silicic acid (50 g) was slurried with benzene in a 250-ml Erlenmeyer flask. The stationary phase (20% methanol in benzene) was then added slowly from a burette with constant shaking by hand. This procedure is simpler and more reproducible than that which involved mixing the stationary phase with the dry silicic acid in a mortar. The fractionation of the acids or methyl esters was performed as described previously (9).

Ultraviolet absorption measurements were made in absolute methanol solution (Cary recording spectrophotometer). Infrared determinations of hydroxyl were made using pure methyl ricinoleate as a standard in carbon tetrachloride solutions (Baird-Atomic KM-1 instrument). The absorbance values at 2.8 μ followed Beer's law in the range of 1 to 5% methyl ricinoleate. Chemical analysis for hydroxyl was made by the method of Ogg *et al.* (15).

Results and Discussion

The chromatographic fractionations of methyl stearate, mono- and dihydroxystearate, and the corresponding free acids are shown in Figures 1 and 2, respectively. The retention volume of monohydroxystearate corresponds to that of fatty ester hydroperoxides (9). The monohydroxystearic acid and the methyl dihydroxystearate are eluted in positions where a sharp increase is observed in the methanol concentration of the effluent. The dihydroxystearic acid tailed considerably, but the peak could be sharpened by increasing the methanol concentration of the mobile solvent from 2 to 4% after elution of the monohydroxy acid.

Figures 3 and 4 show the chromatographic fractionation of methyl esters and free fatty acids of one sample of castor oil. Methyl ricinoleate and ricinoleic acid are eluted in the same position as the corresponding hydroxy stearate and hydroxy stearic acid. In the ester fractionation a third minor peak was identified tentatively as methyl dihydroxystearate from its relative retention volume. There is good agreement in the fatty acid composition when the castor oil sample is separated chromatographically either as methyl esters or as free acids.

In Table I the chromatographic analysis of a castor oil is compared with chemical and infrared hydroxyl determinations. There is fair agreement between the results obtained by the three methods. Triplicate analyses show deviations from the average up to 0.3 and 2.3% for the chromatographic separations of the acids and esters, respectively; about 2% for the chemi-

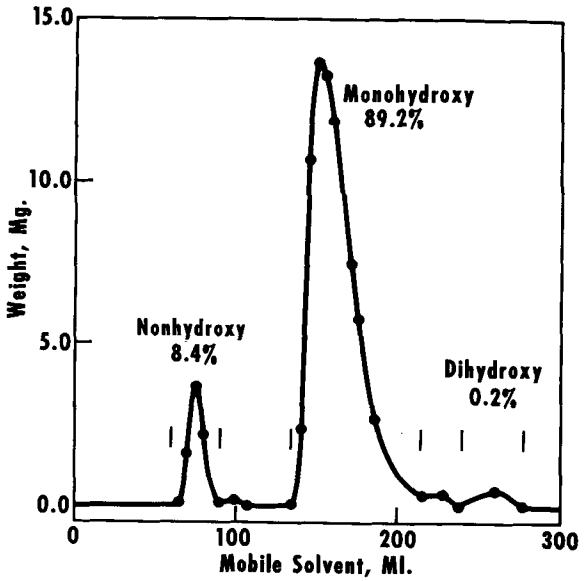

FIG. 3. Chromatographic fractionation of methyl esters of castor oil.

cal analyses; and up to 4% for the infrared analysis of the esters.

Reduced methyl linoleate hydroperoxide is reported to be a mixture of four isomeric conjugated dienoic hydroxy esters: 9-hydroxy-*cis-trans* and *trans-trans*-10,12-octadecadienoic, and 13-hydroxy-*cis-trans* and *trans-trans*-9,11-octadecadienoic esters (1,3,18). Chromatographic fractionation of a hydroxydienoate mixture (Fig. 5) shows that it has a larger retention volume than methyl ricinoleate or hydroxystearate. The greater polarity of the reduced hydroperoxides may be attributed to the presence of the conjugated diene system. The appearance of a double peak (Fig. 5A) is caused by the rise in methanol concentration of the eluate during elution of the reduced hydroperoxides. The double peak can be eliminated by decreasing the amount of immobile solvent incorporated in the silicic acid column (Fig. 5B). Because silicic acid in this column retains more methanol from the mobile solvent, a lower methanol concentration in the effluent solvent results for the first 260 ml. Under these conditions the reduced linoleate hydroperoxide is eluted as the concentration of methanol in the eluate increases sharply from 0.18 to 1.30%.

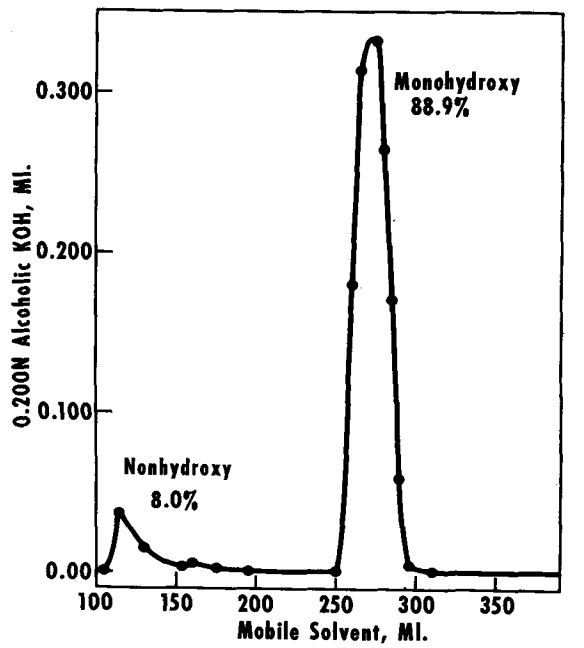

FIG. 4. Chromatographic fractionation of the fatty acids of castor oil.

TABLE I
Monohydroxy Acids in Castor Oil (%)

Sample	Chromatographic		Chemical	Infrared
	Acid	Methyl ester		
A	88.9	89.2	88.2	85.6
	89.4	85.0	91.9	92.8
	89.2	86.9	.....	.....
Average	89.2	87.0	90.1	89.2

LPC was applied to the fractionation of fatty esters of *Dimorphotheca* and *Strophanthus* seed oils, which contain unique hydroxy fatty acids. The oil of *Dimorphotheca aurantiaca* was shown by Smith *et al.* (20) to contain, as the chief constituent, a hydroxy fatty acid, which he named dimorphecolic acid, and which was characterized as 9-hydroxy-*trans,trans*-10,12-octadecadienoic acid. This acid is also one of the isomers obtained by reduction of methyl linoleate hydroperoxide. Figure 6A shows the chromatographic fractionation of the methyl esters of a sample of *Dimorphotheca* oil. Methyl dimorphecolate was eluted in a position similar to that of reduced methyl linoleate hydroperoxide but as a sharper peak. The position of this peak was intermediate between the mono- and dihydroxystearates. The greater polarity of methyl dimorphecolate than of methyl hydroxystearate enables partial separation of a mixture of these esters (Fig. 6B). This separation was further improved by decreasing the methanol concentration in the mobile solvent from 2 to 1% (Fig. 6C).

Strophanthus oil was reported by Gunstone to contain 6.6% of 9-hydroxy-12-octadecenoic acid (10). The chromatographic analysis of the fatty acids of this oil showed 7% hydroxy fatty acids. In the separation of the methyl esters (Fig. 7A), the hydroxy esters showed a double peak which can be attributed,

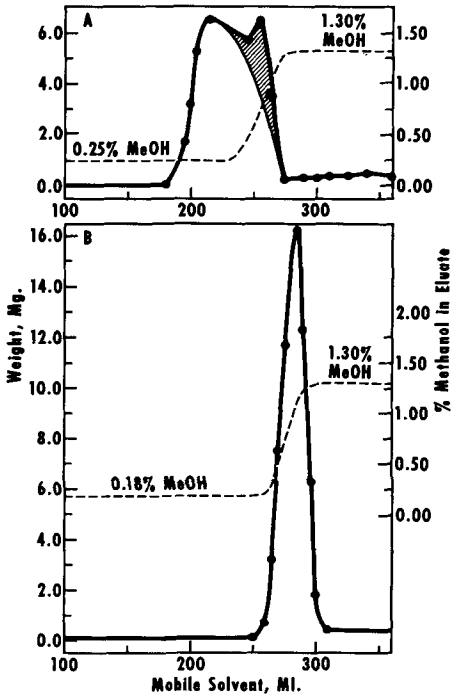

FIG. 5. Chromatography of reduced methyl linoleate hydroperoxide: A, 40 ml stationary phase and B, 34 ml stationary phase. (Dotted lines represent the methanol content of effluent solvent determined with control column without sample.)

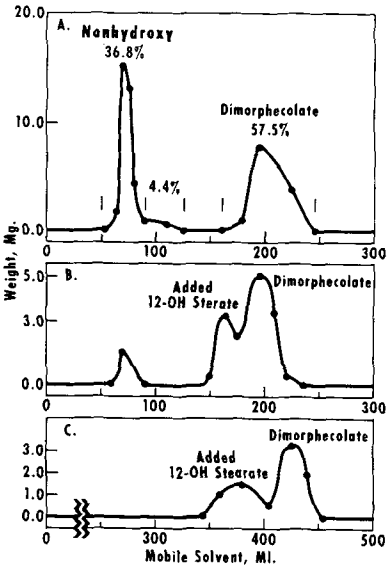

FIG. 6. Chromatographic fractionation of methyl esters of:
A. *Dimorphotheca* oil.
B. Same as A plus methyl 12-hydroxystearate.
C. Same as B using 1% methanol in benzene as mobile solvent.

as shown in Figure 5A, to the sharp rise in the methanol concentration of the eluate during elution of the material. The hydroxy fatty ester of *Strophanthus* oil was more polar than methyl ricinoleate and could be separated from it by using 1% methanol in benzene as mobile solvent (Fig. 7B).

Morris *et al.* (14) were also able to separate the hydroxy fatty ester of *Strophanthus* oil from methyl ricinoleate using thin-layer chromatography. The greater polarity of the hydroxy fatty esters of *Dimorphotheca* and *Strophanthus* oils was related by them to the position of the hydroxyl group and the double bond with respect to the ester group. They observed when the oxygen group is between the ester group and the double bond the fatty ester is more polar than when the double bond is between the oxygen and the ester groups. The conjugated dienoic system in methyl dimorphecolate would be expected to increase the polarity of this ester, irrespective of the position of the hydroxyl group. Any interaction

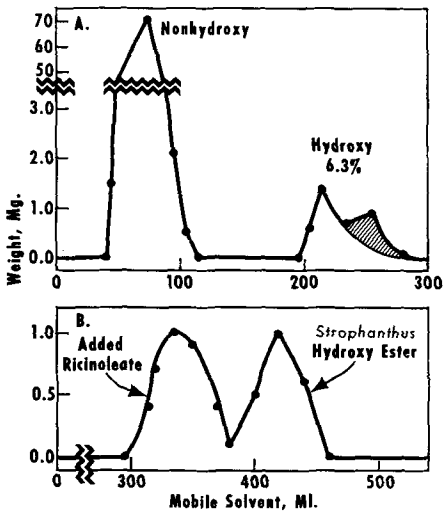

FIG. 7. Chromatographic fractionation of methyl esters of:
A. *Strophanthus* oil.
B. Hydroxy ester of *Strophanthus* oil plus methyl ricinoleate using 1% methanol in benzene as mobile solvent.

or hydrogen bonding of the hydroxyl group would further lower the polarity of this ester.

Two types of interactions are possible: either the hydroxyl group may hydrogen bond with the carbonyl oxygen of the ester, or it may associate with the π electrons of the double bond. Reduced polarity would be expected with ring formation and optimum spacing for the closing of a strainless ring would produce the maximum effect. Hydrogen bonding to an oxygen is a relatively strong bond (5 kcal) compared to the olefin associated bond. Both types give greatest effects with small rings. Schleyer *et al.* (17) showed that bonding occurs in unsaturated primary alcohols only when 1 or 2 methylene groups separate the hydroxyl from the unsaturated group and that no bonding occurs when 3 methylene groups are interposed. The same optimum ring size was shown for the association with an aromatic nuclei. Evidence for this type of association is also given by UV and NMR spectroscopy and has been used to elucidate the structure of steroids.

The highest polarity is expected in reduced linoleate hydroperoxide and dimorphecolic acid esters, since optimal ring closure is not possible because of the positional distance of the hydroxyl and a possible configurational effect of the *trans* bonds. The double bonds and hydroxyl group are therefore both available to increase the polarity of these esters. This effect is borne out from the similarity in retention volumes of methyl dimorphecolate and reduced linoleate hydroperoxide, which contain equal amounts of the 9-hydroxy-10,12-dienoic ester and 13-hydroxy-9,11-dienoic ester. This type of evidence indicates that the intermediate position of the hydroxyl, with respect to the ester and unsaturated group, is not important in exerting any polarity effect.

The greater polarity of the 9-hydroxy-12-enoic ester over that of the 12-hydroxy-9-enoic ester (ricinoleate) is explained on the basis of greater hydrogen bonding in ricinoleate where the spacing of the hydroxyl to the double bond is optimum for the formation of the most stable ring. Showell (19) obtained infrared evidence which indicates that an interaction occurs between the hydroxyl group and the double bond of methyl ricinoleate. This interaction would be expected to decrease polarity. The hydroxyl double bond interaction would be less favored in the 9-hydroxy-12-enoic ester of *Strophanthus* oil because these groups are separated by two methylene groups, whereas in methyl ricinoleate they are separated by only one methylene group. Sufficient model compounds are not available to us for the examination of hydrogen bonding with the carbonyl oxygen; however, it would be predicted that any hydroxy ester capable of this type of bonding would be the least polar. Studies on the positional effect of the hydroxyl group in the fatty acid chain and its relationship to the unsaturated groups on the polarity of the ester are needed.

Table II summarizes the spectral analyses of vari-

TABLE II

Spectral Characteristics of Hydroxy Fatty Esters Purified Chromatographically

Methyl esters	Diene $k_{232\,m\mu}$	Hydroxyl $k_{2.80\,\mu}$
	(1/g, cm)	
12-Hydroxystearate	.....	0.069
Ricinoleate	.....	0.059
Dimorphecolate	106.4	0.083
Reduced linoleate hydroperoxide	75.5	0.083
Reduced linolenate hydroperoxide	85.8	0.052
9,10-Dihydroxystearate	.....	0.122

ous hydroxy fatty esters isolated chromatographically. The diene content of the reduced hydroperoxides agrees well with values reported in the literature (1,5,16). The diene content of the chromatographed methyl dimorphecolate is higher than reported by Smith *et al.* (20) for their preparations but agrees well with the value reported by Chipault and Hawkins (6) for pure *trans-trans* conjugated methyl linoleate. The extinction coefficient of methyl 12-hydroxystearate at 2.8 μ is higher than that reported for ricinoleate and the absorption band is much sharper. Because of these two conditions no association of the hydroxyl groups is indicated. These results also confirm the purity of the hydroxy fatty esters obtained by LPC. This method has been a valuable adjunct to the study of various oxygen-containing fatty acid and esters and was used to characterize the hydroxy esters obtained from the hydrogenation of methyl linolenate hydroperoxides (9). This work offers a basis for the development of analytical methods to determine the hydroxy and other polar acid content of fatty glycerides and their derivatives.

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The Hydrogenation of Fatty Oils with Palladium Catalysts. VI. Hydrogenation for Margarine

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Abstract

Satisfactory margarine stocks have been made with a palladium on carbon catalyst in laboratory, pilot plant, and plant processing. The catalyst was shown to make a satisfactory product even when, on continued re-use, the ratio of oil to metal used reached 400,000 to 1.

TWO PREVIOUS ARTICLES (1a,1b) of this series dealt with the palladium catalyzed hydrogenation of various fatty oils for shortening stocks. The processing factors controlling the composition of the products were elucidated, and it was shown that with proper processing satisfactory products could be obtained both in the laboratory and in the pilot plant.

This article is concerned with the use of palladium catalysts in hydrogenation of various oils for margarine in laboratory, pilot plant, and plant processing. By proper choice of conditions and of catalysts margarine stocks with satisfactory plastic properties, having widely different compositions, were made. Satisfactory margarine stocks having both a high and a low *trans* isomer content were made in the pilot plant. Margarine stocks with a high *trans* isomer content could be obtained with continued re-use of the catalyst even when the weight ratio of oil to metal reached 400,000 to 1 (Table II).

Experimental

The oils used in the work were soybean oil, I.N. 130; a mixture of 70% soybean, 30% cottonseed oil, I.N. 124; and peanut oil, I.N. 93. Laboratory and pilot plant experiments were done in the 1 gal (1c) and 50 gal (1b) stainless steel reactors described earlier. Processing was controlled in both cases by determination of refractive index at 45C. Analyses of the products were made according to A.O.C.S. official methods (2). Ubbelohde's drop point was determined by the official German method (3) except that the time on ice was shortened to 2 hr. The *trans* content of the hydrogenated fats was estimated from the infrared absorption of a carbon disulfide solution according to the procedure recommended by the A.O.C.S. Spectroscopy Committee (4). The spectrophotometer was a Perkin-Elmer Model 21.

Results and Discussion

The plastic properties of margarine are set by its uses to within rather narrow limits, which in turn determine the permissible range of *trans* isomers, solid acids, etc. Dilatometry has become, since its first use for fats in 1931 (5), the chief analytical method for the determination of plastic properties of fats, especially margarine and shortening. Various modifications in procedure and in desirable dilatometric numbers have been made (6–11) reflecting the widely different standards for margarine stocks in various countries. But in general, numbers, as measured by the American method, in excess of 2 at 36C give a waxy margarine. Taste and aroma are improved as this number is lowered. The dilatometric number at 42C must be zero. Dilatometric points at 21.1C and at 26.7C characterize the softness of the product at room temperature, ease of shaping, and ability to retain shape without refrigeration.