

The major conclusion that can be drawn from these free radical addition reactions is that here the 9,10-double bond is not affected by the terminal group but is similar in reactivity to the double bond in 9-octadecene. The mechanism of these free radical reactions can therefore be represented in accordance with the concepts of Kharasch (7), Mayo (23), and Waters (26).

Summary

Oleic acid and a series of its esters were reacted with hydrogen bromide under conditions favoring addition by a free radical mechanism. Variations of the solvents, catalysts, temperatures, dilution, and the ester groups were investigated. Under all conditions a statistical 50:50 distribution of position isomers was found.

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Positional Isomers Formed During the Hydrogenation of Cottonseed Oil¹

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EARLY in the history of the hydrogenation of glycerides and their derivatives one finds reports of the migration of double bonds during hydrogenation. Lewkowitsch (14) claimed that iso-oleic acid was formed by the catalytic reduction of oleic acid under certain conditions and suggested that iso-oleic acid was a positional isomer of oleic acid. Later Moore (15), Hilditch and Vidyarthi (13), and others demonstrated the presence of positional isomers in partially hydrogenated methyl oleate or oleic acid as well as in hydrogenated linoleates. Compounds having double bonds in the 8-, 10-, and 11-position were reported. Invariably it was shown that one or more positional isomers of the oleoyl group were present in the reaction products. Because of serious limitations in the methods of analysis, no attempt was made to identify all of the isomers present.

Recently with the aid of improved methods of analysis, the migration of double bonds in several unsaturated fatty acids or their methyl esters has been followed quantitatively. Boelhouwer and co-workers (8) determined the positions of the double bonds in progressively hydrogenated samples of methyl oleate, elaidinate, petroselinic, and linoleate. Allen and Kiess investigated the migration of double bonds and the formation of *trans* isomers during the hydrogenation of oleic acid and methyl oleate (3) and linoleic acid and methyl linoleate (2). In another investigation Allen (1) established the manner in which

methyl *cis*-10, *cis*-12-octadecadienoate undergoes hydrogenation to form *cis*-10-, *trans*-11-, and *cis*-12-octadecenoates.

Heretofore the improved methods of analysis for establishing the position of double bonds have not been utilized to follow the hydrogenation of a natural oil or any triglyceride. While triglycerides would be expected to behave on hydrogenation like methyl esters, we have found differences (10, 11). Under comparable operating conditions triglycerides apparently hydrogenate much less rapidly than do methyl esters, and the amount of *trans* isomers formed is considerably less. In the current investigation the extent of the migration of double bonds during the hydrogenation of cottonseed oil is established. In addition, it is shown what influence the conditions of hydrogenation have on the extent of migration and the amount of *trans* isomers formed.

Experimental

Materials. The cottonseed oil used in the experiments was a commercially refined, bleached, and deodorized product. It was fresh oil, being obtained from the processor shortly after its production from seed grown in the current crop year. In all respects the oil was a normal product, having the following characteristics:

Iodine value.....	109.7
Free fatty acids, as oleic, %.....	0.04
Saponification value.....	196.4
Unsaponifiables, %.....	0.48
Content of linoleins, as trilinolein, %.....	49.6
Content of oleins, as triolein, %.....	27.6
Content of saturates, as trisaturates, %.....	22.8

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The hydrogenations were carried out with a commercially available nickel catalyst, which had been prepared by electrolytic precipitation and dry reduction (6). The hardened coconut oil, in which the catalyst was originally suspended, was removed and replaced with cottonseed oil before the catalyst was used.

Hydrogenation Apparatus and Procedure. The hydrogenator was of the dead-end type and consisted essentially of a vertical, cylindrical vessel of the type 316 stainless steel. The inside diameter was 8 in. and the inside depth was approximately 15 in. The vessel was provided with a motor-driven agitator of the paddle type, which was inserted through a stuffing box on the top cover, and with baffles to prevent swirling of the charge and to ensure good mixing. Hydrogen entered through a perforated ring in the bottom of the vessel. Oil samples were withdrawn through a flush type of valve in the center of the dished bottom.

The charge of oil was brought to hydrogenation temperature by circulating hot, liquid Dowtherm through a jacket covering most of the outer surface of the vessel. After hydrogenation commenced, the temperature was prevented from rising by circulating air or water through a loop of $\frac{1}{8}$ -in. pipe inside the vessel. With this heating and cooling arrangement the temperature could easily be kept constant to within 1 or 2°C.

Commercial, electrolytic hydrogen was metered into the oil from a small storage tank; the pressure drop in the tank served as a measure of the amount of hydrogen consumed. An adjustable reducing valve between the storage tank and the hydrogenator served to keep the pressure in the latter at a constant value.

At the beginning of each hydrogenation run the oil and catalyst, at room temperature, were put in the vessel. Air was removed with the help of a mechanical vacuum pump. With the vacuum pump working, the motor-driven agitator on the hydrogenator was started, and the charge was heated to the operating temperature. Then the vacuum pump was disconnected, and the hydrogen was introduced. As the hydrogenation progressed, successive samples were withdrawn at iodine values of approximately 75, 62, and 48. The samples which were withdrawn were protected from oxidation by keeping them under a protective blanket of nitrogen until they had cooled to about 80°C., at which temperature they were filtered to remove the catalyst. In all, three hydrogenation runs were made.

Methods of Analysis. The iodine values and linolein contents, together with the analytical values reported for the original cottonseed oil, were determined essentially according to the methods of the American Oil Chemists' Society (4). In determining the contents of linoleins the spectrophotometric method, Cd 7-48, was used even though it is recognized that the method may not be strictly applicable. A given amount of iso-linoleic acids, in which the double bonds are separated by several methylene groups, may appear in the calculated composition as twice that amount of oleic acid. However, under the operating conditions employed, the production of such iso-linoleic acid groups was considered to be quite low. The composition of the samples of hydrogenated oil in terms of equivalent amounts of trilinolein, triolein, and trisaturated glycerides was

calculated from the iodine values and contents of the linoleoyl group.

Trans isomers were determined as described previously (10); the method used was a slight modification of the infrared spectrophotometric method of Swern *et al.* (16).

To determine the positions of the double bonds in the unsaturated fatty acid groups, the hydrogenated samples generally were treated to obtain the free fatty acids, and the latter were ozonized. The free fatty acids were prepared by the following procedure. Ten grams of the sample were saponified with 2.13 g. of potassium hydroxide (10% excess of 85% assay) in 12.5 ml. of water and 237.5 ml. of absolute ethanol. The mixture was refluxed for 1 hr., after which it was placed on a steam cone and the ethanol was evaporated off under nitrogen. During this removal of ethanol, which sometimes required a day or more, water was added to maintain a constant volume. The ethanol-free soap solution was acidulated with hydrochloric acid and extracted with peroxide-free diethyl ether. The ether extract was washed with water and dried, and the ether was removed to obtain the free fatty acids.

For the ozonization step 1 g. of the free fatty acids was dissolved in sufficient ethyl acetate (usually 75 ml.) to yield a clear solution when cooled to -5°C. and held at this temperature for 15 min. Ozone (6 to 7% in oxygen) was then passed through the sample at -5°C. until the ozonization was completed. The reaction was judged to be completed when the excess ozone leaving the system darkened, or turned to a brownish-red color, a solution of potassium iodide. Then 10 ml. of 30% hydrogen peroxide were added to the solution of ozonides, and the mixture was refluxed for 1 hr. After the refluxing and while the solution was being heated on a steam cone, most of the volatiles were removed by evaporation under nitrogen. When most of the volatiles had evaporated, 20 ml. of ethyl acetate were added to the residual solution and the evaporation was continued. When most of the volatiles had evaporated a second time, another 20-ml. portion of ethyl acetate was added; and the solution was evaporated to dryness. This procedure removed any short-chain monobasic acids which would have interfered with the subsequent chromatographic analysis of the dibasic acids. The residue, usually about 0.9 g., left after the evaporation to dryness was prepared for chromatographic analysis by dissolving it in 100 ml. of a 5% tert-amyl alcohol-in-chloroform solution.

For those samples of hydrogenated oil containing a sizable proportion of the linoleoyl group it was found necessary to carry out the ozonization step before the saponification step. When the procedures were reversed, they were basically the same though for some samples the proportions of solvents used had to be changed. For example, in the ozonization of one sample, 2-2, it was necessary to use 1.5 liters of ethyl acetate per gram of fat. Also it was found desirable to use ethyl acetate instead of diethyl ether to extract the fatty and dibasic acids from the water solution.

The chromatographic methods used for determining the content of dibasic acids (C_6 through C_{14}) in the ozonized and hydrogen peroxide treated mixtures were modifications of methods developed by Higuchi *et al.* (12) and Corcoran (9). The principal modifications were these. The columns were packed by

simply pouring the slurry of silicic acid into the column and allowing the slurry to settle under gravity. Air bubbles in the column were removed by tapping the sides during settling. After settling, the entire column was pressed down thoroughly by using a cap or plug of filter paper and a glass rod.

In the citrate column used to determine the proportions of C_6 - C_{10} dibasic acids, 18.75 ml. instead of 25 ml. of 1 *M* citrate buffer (pH, 5.40) per 25 g. of silicic acid were used. In eluting the organic acids 100 ml. of each of the following solutions were used: pure chloroform, 1.5, 3, 5, 10, and 20% of butanol-in-chloroform. Elution was completed with 200 ml. of 35% butanol-in-chloroform.

In the glycine column used to determine the proportions of C_{11} - C_{14} dibasic acids, 16.5 ml. of the 2 *M* glycine buffer (pH, 8.50) were used per 25 g. of the specially prepared silicic acid. The column was eluted with 100 ml. each of pure chloroform, 1.5, 5, and 10% of butanol-in-chloroform and completed with 200 ml. of 25% butanol-in-chloroform.

In making the analyses, the citrate and glycine columns were always charged with 5-ml. portions of the mixed organic acids in the 5% tert-amyl alcohol-in-chloroform solutions described above. In other words, a column was always charged with about 45 mg. of the whole mixture of dibasic acids and long-chain monobasic acids obtained by the saponification, acidulation, and oxidation procedures. No attempt was made to separate the monobasic and dibasic acids before analysis or to put a fraction obtained from one column through a second column. Typical titration curves obtained with the citrate and glycine columns are reproduced in Figures 1 and 2.

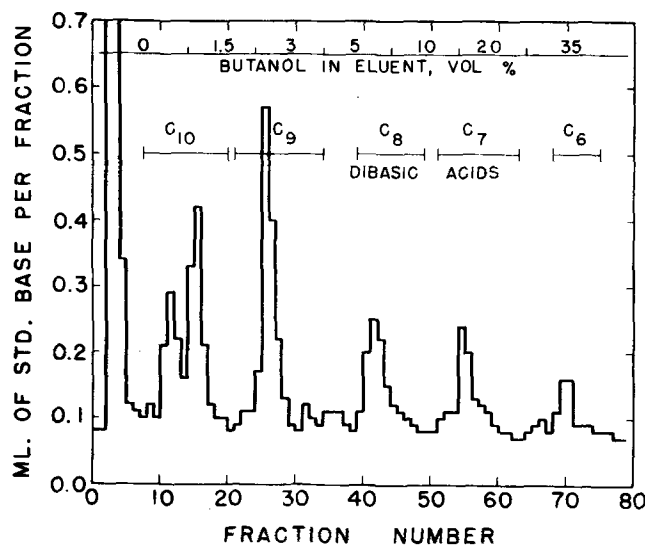


Fig. 1. Titration curve for mixed acids from sample 2-3 on citrate column.

Results and Discussion

Conditions of Hydrogenation. The operating conditions—temperature, hydrogen pressure, type of catalyst, catalyst concentration, and degree of agitation—employed in the hydrogenations were similar to the conditions frequently employed in commercial practice. In the present investigation the degree of agitation was varied by varying the amount of oil

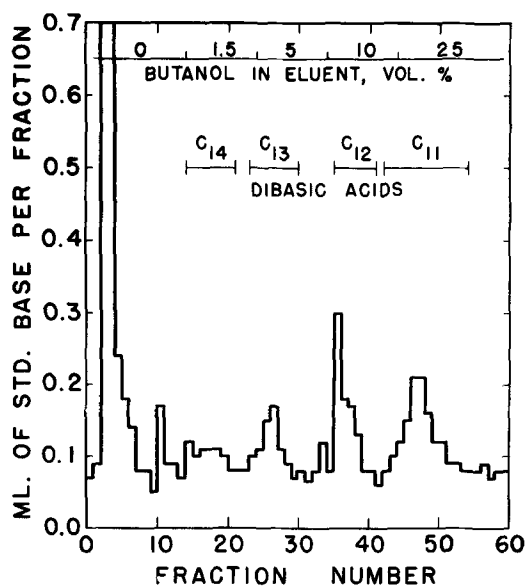


Fig. 2. Titration curve for mixed acids from sample 2-3 on glycine column.

charged to the hydrogenator; the speed of the mechanical stirrer was kept constant at 440 r.p.m.

A plot on rectangular coordinates of time *vs.* iodine value of the oil being hydrogenated produced in each instance a gently curved line (Figure 3), indicating that the reactions were not of zero order. In Table I there is recorded for each sample the hydrogenation time to the indicated iodine value and the rate of hydrogenation at that iodine value.

In an earlier investigation of the hydrogenation of cottonseed oil under similar conditions (7) it was demonstrated that the selectivity of the hydrogenation reaction was increased by increasing the temperature and catalyst concentration and decreasing the pressure and agitation, also that increasing the selectivity simultaneously favored an increase in the formation of iso-oleic acid groups. Selectivity here refers, of course, to the preferential conversion of the linoleoyl group to a monounsaturated acid group over the conversion of the monounsaturated acid group to the stearoyl group.

In the present investigation samples of oil were withdrawn at three stages of each hydrogenation. The first sample was withdrawn when the reaction was concerned largely with the linoleoyl group; the second was withdrawn when the linoleoyl group had about disappeared, and the third was withdrawn when the hydrogenation was concerned entirely with monounsaturated groups.

Accuracy of Analyses. The method of analysis used for determining the position of the double bonds in the unsaturated fatty acids is such that for diunsaturated acids only the position of the bond nearest the carboxyl end of the chain is determined unless the two bonds are separated by six or more methylene groups. In all probability, compounds of the latter type occur only to a very minor extent or not at all in hydrogenated cottonseed oil. With this method of analysis cottonseed oil should and does show practically 100% of the double bonds to be in the 9-position.

As mentioned above, samples containing an appreciable proportion of linoleins had to be ozonized prior to saponification. Failure to follow this order appar-

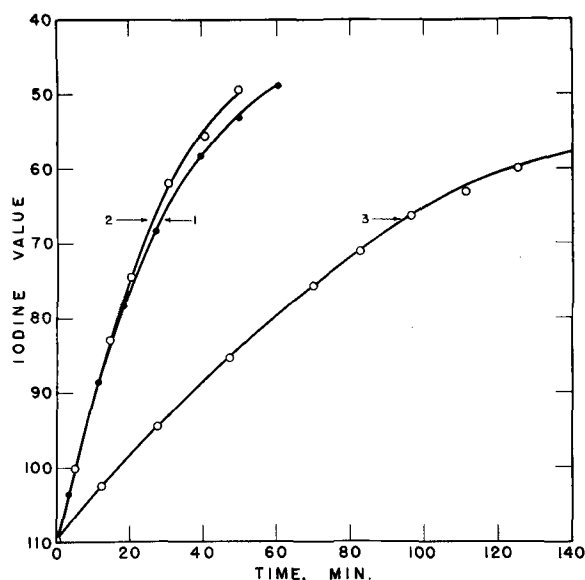


FIG. 3. Curves of time *vs.* iodine value for the three hydrogenation runs: (1) nonselective, (2) normal, and (3) selective.

ently resulted in a significant shift of double bonds during the analysis. For example, when the unhydrogenated cottonseed oil was saponified first and then ozonized, the following percentages of dibasic acids were found: C₆, 6.2; C₇, 4.0; C₈, 7.5; C₉, 64.0; C₁₀, 4.0; C₁₁, 6.6; C₁₂, 4.2; C₁₃, 1.8; and C₁₄, 1.7. Surprisingly, the fatty acids which were obtained by saponification and which yielded this mixture were quite low in contents of conjugated diene and *trans* isomers, contents of these components being equivalent to 1.7% conjugated linoleic acid and 0.72% elaidic acid, respectively.

When samples were analyzed in which the unsaturates consisted entirely of oleins, including glycerides of iso-oleic acids, the order of saponification and ozonization was unimportant. Also oleic acid in the absence of iso-oleic acids yielded only pelargonic and azelaic acids.

The recovery of mixed acids from the chromatographic columns was always very nearly equal to the total fatty acids placed on the columns. For these and other calculations the individual titrations were corrected by subtracting the number of milliliters of standard base required for blank titrations, usually 0.06 to 0.08 ml. For calculating the relative proportions of dibasic acids only those areas under the several peaks of the titration curves were employed. Using these procedures and taking into consideration certain other factors, it is estimated that the percentages of dibasic acids found are accurate to within about three units.

Migration of Double Bonds. In the samples of hydrogenated cottonseed oil, double bonds were found in the 6 through 14 positions (Table I). However the percentages of double bonds in the 6 and 14 positions generally were low. This degree of scattering is in sharp contrast to the belief generally held several years ago that the linoleoyl group exhibited a strong tendency to hydrogenate first at the 12:13 double bond and leave the 9:10 double bond untouched to yield the oleoyl group. The degree of scattering is even more pronounced than would be anticipated on the basis of data recently published by Allen and

Kiess (2, 3). They found only 9, 10, 11, and 12 monoenes in partially hydrogenated linoleic acid and methyl linoleate. In partially hydrogenated oleic acid and methyl oleate only 7, 8, 9, 10, and 11 monoenes were found in most samples though considerable amounts of 6 and 12 monoenes were found in one sample hydrogenated to a point where it contained about 70% of saturated components.

Boelhouwer *et al.* (8), on hydrogenating methyl oleate and linoleate, found in each case double bonds in the 8, 9, 10, and 11 positions as well as some bonds in positions higher than 11. The latter were not further identified. However, since the publication of their data, Boelhouwer *et al.* have prepared another article claiming that the method of analysis used in their original work was faulty. Their original data must now be accepted with some reservations.

Our data indicate a degree of scattering of double bonds which is greater than that found by other investigators. One factor in this difference may be that we hydrogenated triglycerides while the other investigators hydrogenated free fatty acids or methyl esters.

From the data in Table I it appears that the linoleoyl group in cottonseed oil did not hydrogenate to any large degree by simple saturation of either the 9:10 or 12:13 double bond. Consider sample 2-1. During the hydrogenation of the cottonseed oil up to the point where this sample was withdrawn, those linoleoyl groups actually undergoing hydrogenation should have been about the only ones involved in the reaction. This belief is based on the facts that the increase in saturated components was slight and that the linoleoyl group, which is from about 7 to 38 times more reactive than is the oleoyl group (5), was present at all times in appreciable amounts. If the hydrogenation up to the point at which sample 2-1 was withdrawn involved only linoleoyl groups actually undergoing hydrogenation and if the partially hydrogenated linoleoyl groups did not have the remaining double bond in the 9 position, then the proportion of C₉ dibasic acid appearing on analysis of sample 2-1 should have been about 54%, mole basis (about 35% from the oleoyl groups present in the original oil plus about 19% from the unhydrogenated linoleoyl groups). Actually 50.2% of C₉ dibasic acid was found. Hence the amount of 9 monoene produced by partial hydrogenation of the linoleoyl groups must have been relatively small. Simple reduction of the 9:10 bond of the linoleoyl group to yield a 12 monoene could not have been a main reaction because the proportion of 12 monoene produced was only 9.0% mole basis when linoleoyl groups equivalent to about 35%, by weight, of trilinolein were hydrogenated. It is concluded that the 9:10 and 12:13 double bonds of the linoleoyl group mostly were conjugated before one of them was hydrogenated. This conclusion seems reasonable on the basis of earlier work (10).

When the cottonseed oil was hydrogenated under selective conditions, another factor apparently became relatively important. The proportion of C₉ dibasic acid derived from hydrogenated oil sample 3-1 was found to be only 35.2%, based on the total number of moles of dibasic acid found. The original cottonseed oil contained oleoyl groups sufficient to yield about 35 mole % of C₉ dibasic acid, and very few of these had been reduced to the stearoyl group when oil sample 3-1 was taken. Sample 3-1 also contained the equivalent of 11.9% by weight of trilinolein, which if truly trilinolein, would be sufficient

TABLE I
Operational and Analytical Data on the Hydrogenated Cottonseed Oils

Type of hydrogenation	Operating conditions ^a	Sample No.	Hydrog. time, min.	Hydrog. rate, iodine value/min.	Iodine value	Trans isomers, %	Glyceride composition, wt. % ^c			Composition of dicarboxylic acids obtained on oxidation, mol. %									
							Tri-linolein	Tri-olein	Tri-saturates	C ₆	C ₇	C ₈	C ₉	C ₁₀	C ₁₁	C ₁₂	C ₁₃	C ₁₄	
Nonselective	0.05% nickel, 140°C., 40 p.s.i.g., 10 lb. oil	1-1	20.9	1.30	74.7	9.4	16.9	52.8	30.3	5.2	2.2	4.1	47.4	9.3	9.4	6.8	14.6	1.1	
		1-2	35.3	0.709	61.4	19.6	4.9	61.5	33.6	5.4	7.2	7.8	24.5	13.2	12.8	11.6	6.4	5.0	
		1-3	60.5	0.342	47.5	21.6	0.3	54.6	45.1	9.0	7.3	8.2	21.9	16.0	13.9	14.4	7.3	1.9	
Normal	0.10% nickel, 170°C., 20 p.s.i.g., 12 lb. oil	2-1	18.0	1.73	76.3	20.4	14.4	59.7	25.4	0.0	2.2	8.3	50.2	18.3	10.3	9.0	1.5	0.0	
		2-2	28.2	1.02	62.6	28.6	1.6	69.6	28.8	7.7	9.8	7.9	26.3	19.6	10.5	11.0	5.7	1.3	
Selective	0.20% nickel, 200°C., 5 p.s.i.g., 16 lb. oil	3-1	61.7	0.403	74.6	27.3	11.9	62.8	25.3	4.4	8.2	14.6	35.2	6.4	14.6	10.9	2.7	2.9	
		3-2	100.4	0.269	62.3	37.9	0.5	71.4	28.1	4.6	7.7	7.6	30.7	25.5	15.1	10.0	5.3	3.5	
		3-3	171.5	0.150	49.6	37.7	0.0	57.7	42.3	5.0	9.5	10.2	17.9	22.9	13.3	9.2	6.7	5.3	
Original cottonseed oil					109.7	0.0	49.6	27.6	22.8										

^a Increasing the amount of oil used decreased the rate of dispersion of the hydrogen in the oil.

^b Calculated in terms of the indicated triglycerides, using iodine values and experimentally determined contents of linoleic acid.

^c Calculated as trielaidin.

to yield about 16% of C₉ dibasic acid. Hence only about 2/3 of the C₉ dibasic acid which might be expected was found on analysis. Obviously a sizable proportion of the oleoyl groups originally in the cottonseed oil or the residual linoleoyl groups or both had isomerized when sample 3-1 was taken.

The pattern of double bond distribution was essentially similar for all three hydrogenation runs when the iodine value had been reduced to about 48. At this level of hydrogenation the positional isomers of the oleic acid group ranged from about 78 to 82% of the total content of the oleic acid group and its isomers.

Geometric Isomers. The selectivity of the hydrogenation reaction had more effect on the formation of *trans* isomers than it had on the formation of positional isomers. At an iodine value of about 75 the nonselective hydrogenation produced 9.4% of *trans* isomers, calculated as trielaidin, while the selective hydrogenation produced 27.3%.

It is apparent from the data in Table I that the 27.3% of *trans* isomers shown for sample 3-1 was produced while the content of linoleins, expressed as trilinolein, was reduced from 49.6 to 11.9% and the content of saturates was increased from 22.8 to 25.3%. If it is assumed that all of the *trans* isomers were formed on partial hydrogenation of the linoleoyl group, then one mole of this group produced about 0.72 mole of *trans* isomers of the oleoyl group. On the basis of information obtained in an earlier investigation (10), it is likely that the *trans* isomers were formed during the half-reduction of the linoleoyl group or by conjugation prior to the half-reduction. Even though large proportions of *trans* isomers are formed during hydrogenation of the oleoyl group (11), it does not seem logical that the oleoyl groups originally in the cottonseed oil were involved to any large extent in the isomerization at this stage of the hydrogenation.

Summary

A commercial cottonseed oil was hydrogenated under nonselective, normal, and selective conditions. The operating variables used were within the ranges of those ordinarily found in large-scale operations. For each run samples were withdrawn at iodine values of approximately 75, 62, and 48; and these samples were analyzed for the position of the double bonds, content of *trans* isomers, and content of linoleins.

Double bonds were found in the 6 through 14 positions of the monounsaturated fatty acid groups resulting from the hydrogenations. On the basis of the percentage distribution of the double bonds, there appeared to be no marked tendency for the linoleoyl group to form 9- or 12-isomers of the oleoyl group. In the early stages of the selective hydrogenation the rate at which double bonds shifted from the 9-position was greater than the rate at which double bonds were hydrogenated.

The conditions of hydrogenation did not have a marked effect on the distribution of the double bonds at iodine values of about 62 and 48.

The conditions of hydrogenation did have a marked effect on the percentage of *trans* bonds. At an iodine value of approximately 75 the content of *trans* bonds, expressed as weight percentage of trielaidin, was 9.4 for the nonselective hydrogenation and 27.3 for the

selective hydrogenation while at an iodine value of approximately 48 these values increased to 21.6 and 37.7, respectively.

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Antioxidative Activity of Derivatives of Vitamin B₆ and Structurally Related Compounds¹

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VITAMIN B₆ is believed to serve as a physiological antioxidant (1, 2, 3) and has also been reported to serve as an antioxidant for vitamin A *in vitro* (4).

Since the 3-hydroxyl group of the vitamin molecule may be responsible for the reported antioxygenic activity, an attempt was made to prepare pyridoxine 5-monopalmitate, which is completely soluble in fats, contains a free 3-hydroxyl group (5). Similarly ethyl *N*-pyridoxyl-*p*-aminobenzoate² (6) and pyridoxal isonicotinoylhydrazone (7) are of interest as possible oxidation inhibitors for fats. The latter compound is also known to form a chelate complex with various metallic ions, such as copper (8), and this property might serve to eliminate pro-oxidative metallic ions from fats.

In the present study various *N*-hydroxybenzyl and *N*-hydroxybenzylidene compounds were synthesized as preparations structurally related to vitamin B₆ derivatives, and they were tested for antioxidative activity in lard at 37°C. and at 80°C.

Experimental

Test Compounds. The synthesis of pyridoxine 5-monopalmitate as well as ethyl *N*-pyridoxyl-*p*-aminobenzoate² has been reported previously (5, 6). The derivatives of ethyl *N*-hydroxybenzyl-*p*-aminobenzoate were prepared according to the following procedures, using ethyl *p*-aminobenzoate and the respectively substituted benzaldehyde *via* reductive *N*-alkylation. Three and three-tenths grams of ethyl *p*-aminobenzoate and an equimolar amount of the properly substituted benzaldehyde were dissolved in 60 ml. of a mixture of methanol and dioxane (1:1, v/v). The solution was then hydrogenated under 20 lbs. of hydrogen pressure in the presence of 0.5 g. of platinum oxide catalyst at room temperature for 1 hr. After hydrogenation the catalyst was removed by filtra-

tion, and water was added to the filtrate until it showed slight turbidity. Upon standing, the product crystallized.

Ethyl *N*-anisyl-*p*-aminobenzoate (m.p., 128.5-130.0°C.) was recrystallized from isopropanol-methanol (9).

Ethyl *N*-(*p*-hydroxybenzyl)-*p*-aminobenzoate was recrystallized from methanol-water. M.p., 142.0-142.5°C. *Anal.* Calcd. for C₁₆H₁₇NO₃: C, 70.83; H, 6.32; N, 5.16. Found: C, 71.18; H, 6.41; N, 5.11.

Ethyl *N*-salicyl-*p*-aminobenzoate was recrystallized from methanol. M.p., 146.5-148.0°C. *Anal.* Calcd. for C₁₆H₁₇NO₃: C, 70.83; H, 6.32; N, 5.16. Found: C, 71.04; H, 6.50; N, 5.24.

Ethyl *N*-vanillyl-*p*-aminobenzoate was recrystallized from isopropanol-methanol. M.P., 149.0-150.0°C. *Anal.* Calcd. for C₁₇H₁₉NO₄: C, 67.75; H, 6.36; N, 4.65. Found: C, 67.85; H, 6.58; N, 4.85.

N-Vanillyl-β-phenylethylamine hydrochloride was also prepared in a similar manner from vanillin and 2-phenylethylamine. The hydrogenation product was treated with hydrogen chloride, and the hydrochloride was recrystallized from ethanol-ether. M.p., 185.5-186.5°C. *Anal.* Calcd. for C₁₆H₁₉NO₂·HCl: C, 65.41; H, 6.86; N, 4.77. Found: C, 65.07; H, 7.08; N, 4.55.

The isonicotinoylhydrazones and the nicotinoylhydrazones of pyridoxal, salicylaldehyde, and vanillin were prepared by treating the hydrazide with the proper aldehyde in ethanol-water in the presence of sodium acetate as a catalyst (7). The products were recrystallized from ethanol-water. Pyridoxal isonicotinoylhydrazone melted at 263.0°C. (decomposition) (7). Salicylaldehyde isonicotinoylhydrazone melted at 249.0-251.0°C. (10). The isonicotinoylhydrazone and the nicotinoylhydrazone of vanillin melted at 230.0°C. and 213.0-215.0°C., respectively (11, 12).

The synthesis of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde isonicotinoylhydrazone (BHB-INH) was carried out as follows. Twenty-five grams of 2,6-di-*tert*-butylphenol were treated with hexamethylene tetramine in glycerine containing boric acid, followed by diluted sulfuric acid as described by Duff (15). Five grams of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde were ob-

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² The term "pyridoxyl" denotes the 2-methyl-3-hydroxy-5-hydroxymethyl-4-pyridylmethyl radical. The term "pyridoxylidene" denotes the 2-methyl-3-hydroxy-5-hydroxymethyl-4-pyridylmethylene radical.