Phase Behavior in the Solvent Winterization of Crude Cottonseed Oil in 85-15 Acetone-Hexane Mixture as Related to Reduction in Refining Loss and Color

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PRELIMINARY experiments in connection with previous investigations (10, 11) revealed that the solvent winterization of crude cottonseed oil in a solvent consisting of 85 parts by weight of acetone and 15 parts of hexane resulted in a considerable reduction in its refining loss and color. For hexane-extracted oils the direct winterization of the crude oil in 85-15 acetone-hexane mixture would offer an added advantage if solvent winterization is to be used. The appropriate amount of acetone could be added to the partially concentrated crude hexane miscella and the mixture winterized at this stage, thus eliminating one stripping step in the over-all process.

As was found for refined cottonseed and peanut oils (2, 3, 4, 5, 9) and for crude peanut oil (6), this mixture of acetone and hexane has distinct advantages over either solvent alone as a winterization solvent. Voogt and Seeles (14) used a mixture of acetone and benzene in the winterization of refined neat's foot oil. The present report deals with the pertinent phase behavior of a crude cottonseed oil in 85-15 acetone-hexane mixture and with the effect of oil-solvent ratio, chilling temperature, duration of chilling, and the presence of adsorbents on the degree of winterization, the refining loss, and the color of the winterized oil obtained.

Materials. The commercial crude cottonseed oil used in this investigation had an iodine value close to the average iodine value for cottonseed oils (12). It gave the following analysis: iodine value (Wijs) 105.1; free fatty acids as oleic, 1.98%; peroxide value, 0.01 millimoles per kg.; unsaponifiable matter, 0.83%; phosphorus, 0.066%; and the moisture and volatiles, 0.13%. A commercial C.P. acetone was used, containing 0.5% of moisture as determined by a special Karl Fischer reagent for ketones (8). The commercial hexane was Skellysolve B.[3] The adsorbents used were Celite [3] analytical grade filter aid, Norite [3] decolorizing carbon, fuller's earth powder, ignited aluminum oxide, and 70-100 mesh activated silica gel.

Procedures. Laboratory bench-scale winterization tests were carried out by essentially the same procedure as previously described (2, 9). In brief, duplicate weighed samples of oil in definite oil-solvent ratios were chilled in a bath at the desired temperature for a definite holding-time. They were then centrifuged at that temperature for 10 min. The solid-free supernatant liquid was quickly decanted into a tared flask, and the residual solid fraction was weighed. From the weights and oil contents of the supernatant and solid fractions the total amount of solid which had separated during the chilling was calculated. When adsorbents were added before or during the chilling period, the calculation of the amount of solid was based on the difference between the concentration of the supernatant liquid and the original solution, a method of calculation which had been found to be equally reliable. The "percentage of solid removed" is reported as the percentage of the total oil in the original solution. The term "solid" refers to all the material separating during the chilling period. All concentrations are in weight percentage.

It was necessary to refine the winterized crude oils before submitting them to the cold test. Since most of the samples were too small to be given a standard refining treatment, they were refined, unless otherwise mentioned, by a modification (6) of the chromatographic method (7), which was found to result in the same cold test. A modification (9) of the A.O.C.S. Official Method Cc 11-42 Cold Test was used. For the crude cottonseed oil used a cold test of 24 hrs. or better was obtained when 9% of solid was removed.

Effect of Holding-Time, Oil-Solvent Ratio, and Chilling Temperature

Experiments were performed to determine the percentage of solid separating at various holding-times, ranging from 1 to 16 hrs., at oil concentrations of 25, 35, and 50% by weight in 85-15 acetone-hexane, and at chilling temperatures of —12° and —16°C. The data obtained are represented by the solid-line curves in Figure 1. Only two 16-hr. values were determined, and these are not shown in the figure. For a 35% concentration at —12°C. with a 16-hr. holding-time

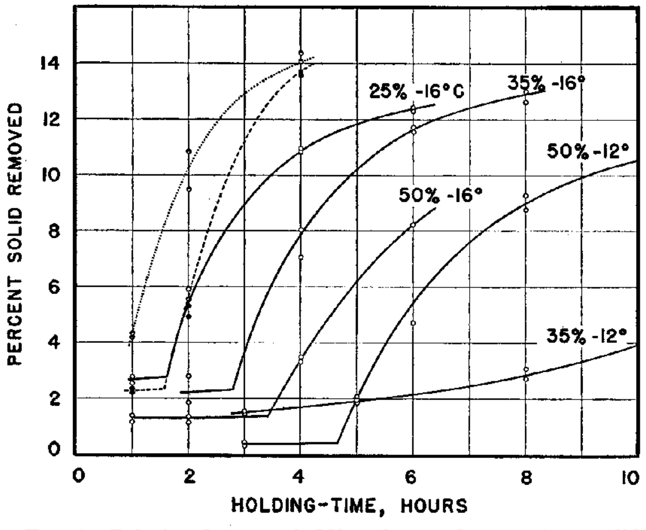

FIG. 1. Relation between holding-time and percentage solid removed from solutions of crude cottonseed oil in 85-15 acetone-hexane as affected by oil concentration, chilling temperature, intermittent agitation, and adsorbent carbon:
———— without agitation or carbon;
– – – – – – with agitation and no carbon;
.... with agitation and carbon.

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[3] The mention of names of firms or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

the duplicate values for the solid removed were 8.0 and 7.5%, and for a 50% concentration under the same conditions they were 12.8 and 13.1%.

There is an induction period of from 2 to 4 hrs. or more—represented by the flat portion of the curves—during which there is very little increase in the amount of material separating and after which there is a rapid increase, the rate of increase being more marked for higher degrees of supersaturation. The "solid" separating during this induction period is a dark brown, viscous, gummy material rich in phosphatides, and that separating later, indicated by the rise in the curve, consists of glycerides, which are readily distinguishable as white crystals. The percentage of material separating during the induction period is greater at the lower chilling temperature and at the lower oil concentrations. An increased separation of this material favors a shorter induction period (see Figure 1). It is apparent that the presence of the gummy material in the crude oil causes delayed formation of the first few crystals of the stearines, after which crystallization proceeds at a more normal rate, depending upon the degree of supersaturation.

This effect explains the marked difference in the winterization behavior of the crude cottonseed oil as compared with that previously found for the refined oil in the same solvent. For the refined oil at comparable temperatures (2, 5) relatively complete separation was attained with holding-times of one to three hours or even less, and at a given temperature a larger percentage of solid separated from the more concentrated solutions, as would normally be expected. The experiments with the crude oil, on the other hand, showed that crystallization was still far from complete after even a 4-hr. holding-time. Thus, because of the difference in induction periods, a larger percentage of solid separated from the more dilute than from the concentrated solutions (see Figure 1). With sufficiently long holding-times however the larger percentage of solid would probably separate from the more concentrated solutions. This is indicated, for example, by the fact that the curves for 25% and 35% at —16°C. would probably cross at a holding-time of about 8 hrs.

Nature of the Gummy Material

The gummy material is completely soluble in hexane but not in acetone. When a sample of the original crude oil was progressively diluted at room temperature, incipient precipitation—as shown by a turbidity—was reached when the crude oil concentration was about 45% with acetone as the diluent and about 30% with 85-15 acetone-hexane mixture. The precipitation was progressively more pronounced at higher dilutions or lower temperatures.

A sufficient quantity was collected by centrifugation and decantation in a series of replicate experiments, using a 25% mixture of the crude cottonseed oil in 85-15 acetone-hexane mixture with a 1-hr. holding-time at —16°C. As predicted from the corresponding curve in Figure 1, there was no evidence of separation of white stearine crystals. After decantation of the clear supernatant layer the gummy residues were combined; no effort was made to separate residual or dissolved oil. The residue, after removal of solvent in the usual way, gave the following analysis: iodine value (Wijs), 69.9; unsaponifiable matter, 2.8%; sterols as sitosterol, 0.15%; nitrogen, 0.58%; and phosphorus, 1.62%, which corresponds to 40.5% of phosphatide, using 25 as the conversion factor (1b). The original oil contained 0.33% of sterols and only 1.65% of phosphatides.

Effect of Adsorbents and Agitation

To aid in the removal of the gummy material a weight of decolorizing carbon approximately equal to 5% of the weight of the oil present was added to 25% oil solutions in the mixed solvent. These samples were chilled at —16°C. for 1-, 2-, and 4-hr. holding-times, during which they were agitated by swirling gently every 10 min. to redistribute the carbon throughout the solution. The results are represented by the dotted curve in Figure 1. The data obtained for parallel experiments under identical conditions with agitation but without the added carbon are shown by the broken curve.

Comparison of these two curves with that for the 25% sample chilled at —16°C. with neither carbon nor agitation shows that the presence of the carbon reduced the induction period from about 1½ hrs. to about one hour. Agitation alone did not affect the length of the induction period appreciably but did increase the rate of crystallization of the stearines after they had once started to crystallize. Thus, for the 25% mixture at —16°C., the holding-time required to remove 9% of solids—*i.e.*, enough to obtain a well-winterized oil—was less than 2 hrs. with carbon and agitation, about 2½ hrs. with agitation alone, and 3 hrs. with neither carbon nor agitation.

The effect of the carbon is enhanced by preliminary addition of an amount of filter aid equivalent to 5% of the weight of the oil. The addition of the carbon is delayed until the mixture has been chilled for 15 min. Using this modification of the procedure, with agitation at the 10th and 15th minute and at 10-min. intervals thereafter, data were obtained for 25, 35, and 50% oil-solvent mixtures with holding-times of 1, 2, 3, and 4 hrs. at —10°, —12°, and —14°C.

The results are shown in Figures 2, 3, and 4. Considerably more data would be necessary to establish the exact length of the induction period under the various conditions. In all instances however it was relatively short and with the exception of the 25%

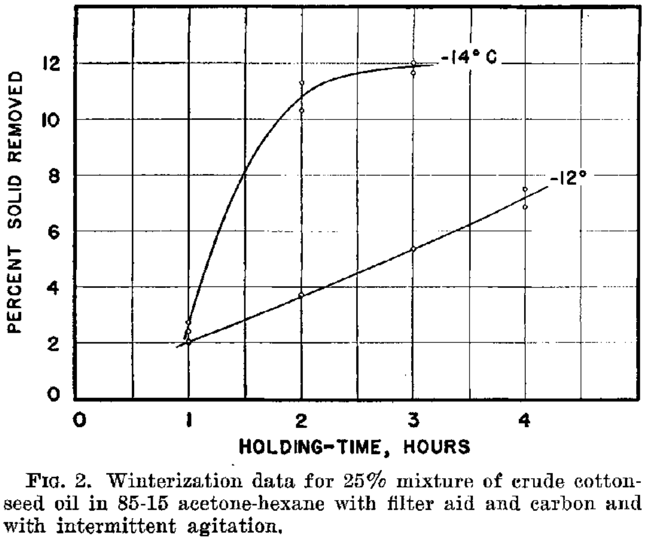

FIG. 2. Winterization data for 25% mixture of crude cottonseed oil in 85-15 acetone-hexane with filter aid and carbon and with intermittent agitation.

TABLE I

Refining Loss and Spectrophotometric Data for Crude Cottonseed Oil After Various Treatments of 35% Solutions in 85-15 Acetone Hexane

Sample no.	Treatment				Properties of recovered oil				
	Chilling temp.	Holding-time[a]	Adsorbents[b]	Per cent solid removed	Refining loss	Extinction coefficients in isooctane			
						360 mμ	450 mμ	560 mμ	670 mμ
	°C.	hours		%	%	$E^{g./l.}_{1 cm.}$	$E^{g./l.}_{1 cm.}$	$E^{g./l.}_{1 cm.}$	$E^{g./l.}_{1 cm.}$
1	+25	3	None	None	9.6	0.28	0.091	0.013	0.0037
2	+25	3	C + FA	None	8.5	0.21	0.074	0.012	0.0039
3	−12	3	FA	1.0	8.0	0.18	0.069	0.0096	0.0031
4	−12	5	None	5.2[c]	6.4	0.16	0.060	0.0088	0.0026
5	−12	2	C + FA	5.1[c]	6.2	0.15	0.058	0.0079	0.0023
6	−12	3	C + FA	10.8[d]	6.0	0.13	0.052	0.0079	0.0022

[a] With intermittent agitation.
[b] C = decolorizing carbon; FA = filter aid.
[c] Contained considerable stearines.
[d] Sufficient to produce well-winterized oil.

mixture at −12°C. and the 35% mixture at −10°C. the separation of the 9% of solids required to winterize this oil was reached in less than 3 hrs. As in Figure 1, it is apparent that the amount of material separating during the induction period tends to increase as the dilution is increased and as the temperature is lowered.

The results for a 35% mixture with a 3-hr. holding-time at −14°C., using carbon without filter aid, as shown by the crosses in Figure 3, indicate that the addition of the filter aid is advantageous. That is, more solid separated in 2 hrs. by the filter aid-carbon procedure than in 3 hrs. with carbon alone. Similarly, comparison of Figures 1 and 2 shows that with a 2-hr. holding-time about the same percentage of solid is removed from a 25% solution at −14°C. with filter aid and carbon as at −16°C. with carbon alone.

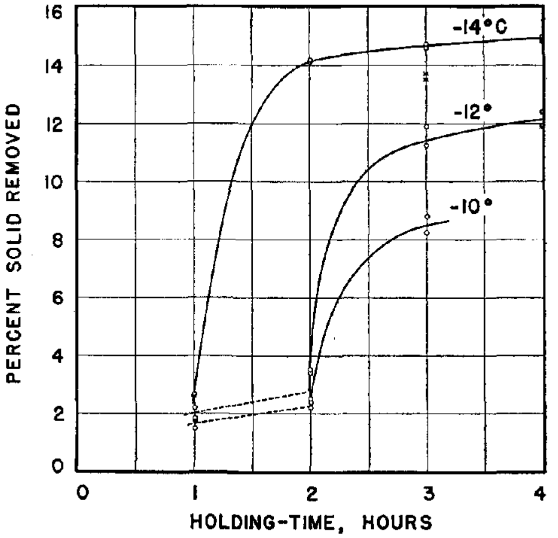

FIG. 3. Winterization data for 35% mixture of crude cottonseed oil in 85-15 acetone-hexane with intermittent agitation: ○, with filter aid and carbon; ×, with carbon at −14°C.

A number of factors must be considered in choosing the best conditions for a practical continuous solvent-winterization process. The holding-time should be short. For the particular oil here used 10 or 11% of solids would be removed, allowing for a small safety factor over the 9% required. It is also important that the percentage of solid removed be only slightly affected by small variations in holding-time, temperature, and concentration in order that the yield and degree of winterization can be properly controlled. For example, though the required 10 to 11% of solid would be removed with a holding-time of less than 1½ hrs. from a 35% solution at −14°C., the rate of crystallization at this time is still very rapid (see Figure 3), and a small difference in holding-time or temperature would cause considerable variation in the yield. For the concentrations and temperatures investigated, and reported in Figures 2, 3, and 4, the most suitable is the 25% mixture at −14°C., which would require a holding-time of about 2 hrs. The use of a slightly lower temperature and concentration would be expected to shorten further the induction period and the required holding-time.

Similar parallel experiments were performed on 35% oil-solvent mixtures at −14°C. with a 2-hr. holding-time, substituting fuller's earth, aluminum oxide, silica gel, and filter aid for the carbon in the filter aid-carbon procedure. While none was as effective as carbon, all except filter aid seemed to shorten the induction period. The results showed 12.4% solid removed for the fuller's earth sample, 11.4% for aluminum oxide, 8.5% for silica gel, and 2.6% for filter aid, as compared to 14.1% for the corresponding carbon experiment.

Reduction in Refining Loss and Color

It was apparent from visual observation that considerable color was removed by winterizing the crude

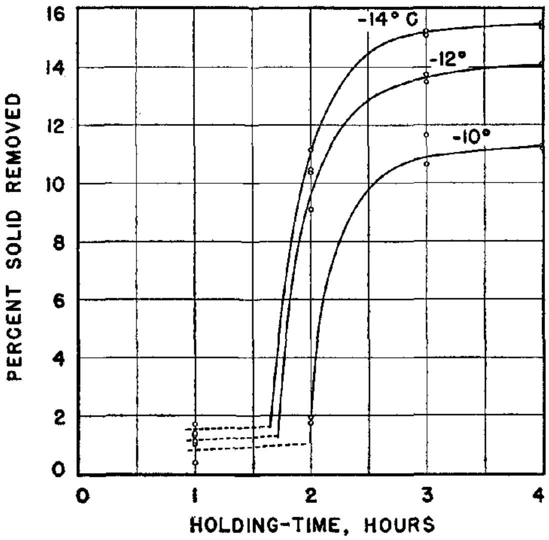

FIG. 4. Winterization data for 50% mixture of crude cottonseed oil in 85-15 acetone-hexane with filter aid and carbon and with intermittent agitation.

cottonseed oil. Preliminary tests also showed that the winterized crude oil had a lower refining loss than the original oil. A systematic series of parallel experiments was therefore performed to determine more or less quantitatively the relative effect of the filter aid, the carbon, the precentage of solid removed, and the chilling temperature on this reduction in refining loss and color.

In each instance, including the control (Sample 1), a 35% solution of the crude cottonseed oil in 85–15 acetone-hexane mixture was subjected to the conditions indicated in Table I.

The general procedure was the same as that used in the winterization experiments, including agitation at 10-min. intervals. The final steps involved centrifuging at the temperature in question, decanting the clear supernatant, and freeing it from solvent, first by distillation on a steam bath and finally under a partial vacuum with a stream of nitrogen. The oils so obtained were then refined by a standard cup refining method after first removing a small sample for spectrophotometric analysis. In order to obtain sufficient oil for the standard refining treatment it was necessary to combine the products of a number of replicate experiments. The resulting winterization data and the refining loss and spectrophotometric data for the recovered oil are given in Table I.

The refining loss of the original crude oil, 9.6%, was reduced about 11% by treatment of the oil-solvent mixture for 3 hrs. with filter aid and carbon at room temperature (Sample 2). Filter aid alone with a holding-time of 3 hrs. at —12°C. (Sample 3) resulted in the removal of 1.0% of solid (gummy material) and a total reduction of 17% in the refining loss. The results for Samples 4 and 5 show conclusively that the presence of adsorbents does not contribute appreciably to the reduction in refining loss when the conditions are such that even a small amount of stearines has crystallized. With no carbon or filter aid present (Sample 4) essentially the same reduction in refining loss was attained as with both adsorbents present (Sample 5) though the percentage of solid removed was substantially the same in both cases. This is confirmed by Sample 6 (with carbon and filter aid), in which enough solids were removed to give a well-winterized oil passing a cold test of more than 24 hrs. when refined. The reduction in refining loss was 33% for Sample 4, 35% for Sample 5, and 37% for Sample 6. Thus the reduction in refining loss attained by winterization was almost the same whether adsorbents were used or not.

The removal of color under the various conditions more or less paralleled the reduction in refining loss. Table I shows the extinction coefficients in isooctane, $E^{g./l.}_{1\,cm.}$, at 360, 450, 560, and 670 millimicrons, the positions of maximum absorption for the principal pigment types in crude cottonseed oil, *viz.*, gossypols, carotenes, gossypurpurin, and pheophytins, respectively. Partial winterization of the oil with no adsorbents (Sample 4) removed more color than treatment with filter aid and carbon at room temperature (Sample 2) or with filter aid alone at —12°C. (Sample 3). The presence of filter aid and carbon in the partial (Sample 5) and complete (Sample 6) winterization of the oil resulted in relatively small additional increases in color removal.

Discussion

It can be concluded that the phosphatide-rich gummy material separating during the induction period contributes very considerably to the refining loss and color of the original oil, that its presence in the oil inhibits crystallization of the stearines, and that it is removed along with the first portion of stearines crystallized whether adsorbents are present or not. Thus the major function of the adsorbents is to shorten the induction period.

Judging from Figure 1, the amount of gummy material separating could be increased and the induction period reduced without addition of adsorbents, *e.g.*, by the use of lower chilling temperatures. At these temperatures crystallization of the stearines would start sooner and proceed more rapidly. Considerably more stearines would eventually separate than required for adequate winterization, but this could be prevented by raising the temperature sufficiently during the early part of the crystallization so that only the desired amount of stearines would crystallize. Such a procedure would accomplish the reduction in holding-time and in refining loss and color without adsorbents. By centrifuging the chilled mixture before the stearine crystals start to form, the phosphatide-rich fraction could be isolated and thus the refining loss of the stearine fraction and therefore the over-all refining loss for the crude oil would also be markedly reduced.

The results show that it is possible to winterize crude cottonseed oil in 85-15 acetone-hexane mixture, using a chilling period of 2 hrs. and that the color and refining loss are at the same time markedly reduced. This has some important implications, assuming the process could be translated to an industrial scale, in the light of the present trend toward the use of hexane extraction in the recovery of oil from cottonseed. It is apparent from the extinction coefficients in Table I that the intensity of the absorption in the gossypol region was reduced by about 50% during winterization (Sample 6). These gossypol-like pigments are responsible for the color fixation caused by heating the miscellas to the temperatures usually employed in removing the last portion of solvent (1a, 13).

On the basis of the above results a process for winterizing crude cottonseed oil could be visualized which would involve mixing the proper proportions of the concentrated crude hexane miscella and acetone to make up a winterizable mixture 25% by weight of oil in 85-15 acetone-hexane mixture. Such a composition could be arrived at, for example, by adding approximately 64 parts by weight of acetone to 36 parts of a 69% hexane miscella or, if the prepress process is used, to a mixture of 16.5 parts of prepressed crude cottonseed oil and 19.4 parts of 42.8% hexane miscella. Performing the winterization step at this stage, before stripping, refining, bleaching, and deodorizing, would present a number of advantages. It would remove a large proportion of the color which becomes "fixed" during the stripping operation; it would reduce the refining loss markedly —by about 35% in the present case; it would make possible the isolation of a phosphatide-rich by-product; and it would eliminate the subsequent addition of solvent and restripping otherwise necessary if solvent winterization is used.

Summary

Fundamental physical chemical data have been obtained which indicate on a laboratory scale the feasibility and advantages of solvent winterization of crude cottonseed oil in 85-15 acetone-hexane mixture. The results show the effect of oil-solvent ratio, chilling temperature, duration of chilling, and the addition of adsorbents on the degree of winterization, the refining loss, and the color of the winterized oil. Crystallization is markedly inhibited by the presence of a phosphatide-rich material in the crude oil, but this can be overcome by the proper control of the oil-solvent ratio and temperature and by the addition of adsorbents. Winterization in this solvent with or without adsorbents results in the separation of a large proportion of the phosphatides, and a marked reduction in refining loss and color. The advantages of winterizing hexane-extracted cottonseed oils before refining are discussed.

Acknowledgments

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Infrared Studies on the Isomers of Kamlolenic Acid

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KAMALA OIL, obtained from the seeds of *Mallotus philippinensis* Muell. Arg., has recently been found to be a rich source of α-kamlonenic acid, to which the constitution 18-hydroxy, 9,11,13-octadecatrienoic acid has been assigned (5). The acid is transformed to its β-isomer (6) on irradiation with ultraviolet light. Kamlolenic acid has attracted a great deal of attention during recent years because the hydroxyl group is present at the terminal carbon atom.

It is the purpose of the present paper to establish the *cis-trans* configuration of α- and β-kamlolenic acids by studying their infrared spectra, their acetyl derivatives and maleic anhydride adducts, and the chemistry of the addition compounds. Quantitative absorption data in the 10 μ-11 μ region are also presented. A preliminary note in this connection has already been published (4). Infrared studies on the *cis-trans* isomerism of α- and β-elaeostearic acids, which are very similar to α- and β-kamlolenic acids with three double bonds in the 9,11,13-positions, have already been carried out by Ahlers, Brett, and McTaggart (1), Bickford, DuPré, Mack, and O'Connor (3), and Paschke, Tolberg, and Wheeler (8).

Experimental

PREPARATION OF THE MATERIALS

α-Kamlolenic Acid (6). Kamala oil fatty acids (15 g.), prepared by the saponification of the oil with alcoholic potash in the usual manner, were shaken with 150 ml. of petroleum ether (40-60°C.) and occasionally warmed. A pale white solid (4.9 g.), which separated on cooling, was filtered and crystallized three times from ethyl acetate as white needles (m.p. 78-79°C.).

β-Kamlolenic Acid (6). A suspension of α-kamlolenic acid in petroleum ether (40-60°C.), containing traces of iodine, was irradiated by ultraviolet light in a quartz flask for 3 hrs. and then cooled in ice water. When the separated acid was crystallized from ethyl acetate, white needles melting at 90-91°C. were obtained.

Acetyl Derivatives of α- and β-Kamlolenic Acids (5). α-kamolenic acid (10 g.) was placed in a flask in dry ethyl ether (100 ml.) and cooled to 0°C. Acetyl chloride (20 ml.) was slowly added with constant shaking and cooling. The mixture was left overnight at 0°C. and then at room temperature for 2 hrs. By the end of this time all suspended unreacted material was in solution. Crushed ice was added, and the ether solution was washed with cold water several times. The solvent was distilled off, and the last traces were removed under vacuum. The residue on crystallization from ethyl acetate yielded a white crystalline solid m.p. 43-44°C. The similar acetylated product obtained from the β-acid was found to melt at 58-59°C.

Maleic Anhydride Adducts of α- and β-Kamlolenic Acids (5). To 3 g. of α-kamlolenic acid, 25 ml. of 6% maleic anhydride solution in toluene were added, and the mixture was refluxed for 3 hrs. with occasional shaking. The flask containing the above material was cooled for a few minutes, and 10 ml. of distilled water were added and the boiling continued for 10 min. The contents of the flask were then cooled to about 25°C. and transferred to a separatory funnel with ethyl ether. The solution was thoroughly shaken a number of times with cold water and dried with anhydrous sodium sulphate, the solvents were distilled off, and the last traces were removed under high