TABLE II

Relative Sensitivity Factors for Fatty Amines

Amine	Response per gram	
	Apiezon L	Silicone Grease
Octylamine	......	1.126
Decylamine	......	1.093
Dodecylamine	1.068	1.063
Tetradecylamine	1.033	1.036
Hexadecylamine	1.000	1.000
Octadecylamine	0.946	0.946

approximates a linear function of molecular weight. This is in agreement with other available data on homologous series of fatty components (3,8).

Results. The results of the analyses of three known mixtures are shown in Table III. Two of the samples were run by employing the Apiezon L column, and one was run by using the Silicone Grease column. In all three mixtures the calculated area percentages are in good agreement with known sample composition.

TABLE III

Analysis of Known Mixtures of Amines

Amine	Mixture 1 [a]		Mixture 2 [a]		Mixture 3 [b]	
	Known wt., %	Determined wt., %	Known wt., %	Determined wt., %	Known wt., %	Determined wt., %
C_{12}	29.3	29.5	21.9	21.8	30.0	30.3
C_{14}	27.2	27.0	28.8	28.8	30.0	29.6
C_{16}	22.7	23.0	24.5	24.8	40.0	40.1
C_{18}	20.8	20.5	24.8	24.6	......	......

[a] Apiezon L column.
[b] Silicone Grease column.

Saturated fatty alcohols may also be run on the Apiezon L column under the same conditions as the corresponding fatty amines, and the symmetry of the peaks is comparable. No quantitative data is yet available concerning fatty alcohol mixtures.

The limiting temperatures of the Apiezon L column when used for the separation of both amines and alcohols appears to be 230°C. Above this temperature the liquid substrate deteriorates, but at 225°C. the column is stable for relatively long periods of time.

No deterioration has been observed with the Silicone Grease column when operating at temperatures in the range of 175–180°C. Preliminary data indicate that the life expectancy of a column prepared with silicone grease on treated Chromosorb is shorter than one prepared by using a support phase which is not pretreated to reduce its adsorptivity.

Summary

The separation of fatty amines has been carried out with nonpolar substrates on solid supports of Chromosorb and Chromosorb W, which were previously treated with potassium hydroxide to overcome adsorptivity. In this manner well-resolved symmetrical peaks are obtained. Untreated supports give peaks which trail and prohibit precise quantitative measurements. Both Apiezon L on Chromosorb W and Silicone Grease on Chromosorb have proved effective, the latter at 176°C. and the former at 225°C. Relative detector sensitivity factors have been determined for the amines C_8 to C_{18}, thus permitting accurate analyses. The relative response for primary alkyl fatty amines is a linear function of molecular weight.

REFERENCES

1. Cropper, F. R., and Heywood, A., in "Vapour Phase Chromatography," London, 1956, edited by Desty, D. H., Academic Press, New York, 1957.
2. Decora, A. W., and Dineen, G. U., presented at ISA's 2nd International Symposium on Gas Chromatography, Michigan State University, June, 1959.
3. Dimick, K. P., *et al.*, data presented at the American Oil Chemists' Society meeting, Los Angeles, Calif., September 28–30, 1959.
4. James, A. T., Biochem. J., *52*, 242 (1952).
5. James, A. T., Martin, A. J. P., and Smith, G. H., Biochem. J., *52*, 238 (1952).
6. Johns, T., Beckman Gas Chromatography Manual, Bulletin 756, *Beckman Instruments Inc.*, 1959.
7. Johnns T., in "Gas Chromatography," edited by Coates, V. J., Noebels, H. J., and Fogerson, I. S., Academic Press, New York, 1958.
8. Killheffer, J. V., *et al.*, data presented at the American Chemical Society meeting, Atlantic City, N. Y., September 13–18, 1959.
9. Knight, H. S., Anal. Chem., *30*, 2030 (1958).
10. Link, W. E., Hickman, H. M., and Morrissette, R. A., J. Am. Oil Chemists' Soc., *36*, 20 (1959).
11. Link, W. E., Hickman, H. M., and Morrissette, R. A., J. Am. Oil Chemists' Soc., *36*, 300 (1959).

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Acidolysis of Vegetable and Marine Oils with Phthalic Acids

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THE CONVERSION of vegetable and marine oils to partial esters by alcoholysis with polyhydric alcohols is a process that is used extensively in the manufacture of oil-modified alkyd resins. Acidolysis is a term that describes a similar alkyd manufacturing process in which oil is heated with a dibasic acid at high temperature to obtain an exchange reaction and liberation of monobasic acids from the oil. Both processes have the same purpose, that is, to change the neutral triglyceride oils into compounds that can be esterified with the other alkyd components to form an homogeneous oil-modified alkyd resin. It is the purpose of this paper to present some data on the reaction of triglyceride oils with the isomeric phthalic acids and the conversion of acidolysis products to alkyd resins. Orthophthalic acid dehydrates to the anhydride when heated, but the other two isomers, isophthalic and terephthalic acids, are heat-stable and readily react with oils at elevated temperatures.

Acidolysis Reaction

Procedures. All experiments were conducted in a 5-liter, four-neck, round-bottom flask equipped with stirrer, thermocouple, nitrogen gas inlet, and reflux condenser. The condenser contained a special baffle-type of packing and was heated with boiling water in the jacket; this arrangement permitted rapid heating without loss of glycerol during esterification. The flask was heated with an electric mantle. Alkyd-grade nonbreak and alkali-refined oils, and commercial grades of phthalic anhydride, isophthalic acid, and terephthalic acid were used.

Rates of acidolysis of triglyceride oil with the three phthalic acids were measured by heating soybean, linseed, or safflower oil with orthophthalic, isophthalic, or terephthalic acid at various temperatures while samples were withdrawn periodically. A ratio of about 3.5 moles of phthalic acid to 1 mole of oil was used; this produced an alkyd resin of about 55% oil content after the necessary amount of glycerol was added and esterified. The samples that were obtained at intervals during the acidolysis reaction were cooled and filtered to remove the insoluble, unreacted acid or anhydride; and the clear oils were titrated with 0.1 N KOH in ethanol to determine their acid numbers. Filtration to remove unreacted dibasic acid was facilitated by dilution of the samples with toluene.

Since the acidolysis reaction produces a mixture of monobasic acids, experiments were conducted to determine the effect of additional fatty acid on the rate of acidolysis of triglyceride oil. A 1:1 mole mixture of oil and fractionated tall oil fatty acids (low rosin content) was used. Acid numbers of the filtered samples were corrected by subtracting the acid number of the initial oil-fatty acid mixture.

Application of the acidolysis reaction to alkyd resin preparation was studied first by determining the minimum amount of conversion of oil that is necessary to obtain an homogenous alkyd resin free from haze cloud, or other signs of gel structure. The same 55% oil-alkyd formulas used in the rate studies were used in these experiments, as follows:

	Oil formula A, grams	Oil-fatty acid formula B, grams
Oil	1,375	1,043
Fatty-acid	......	338
Phthalic acid isomer	915	915*
Glycerol, anhydrous	414	451

* *830 g. of phthalic anhydride were substituted for orthophthalic acid in most runs.*

Acidolysis of the oil was conducted by heating all the ingredients, except the glycerol, for different times at different temperatures. After the acidolysis mixture was cooled to about 200°C., a sample was taken and analyzed for acid number of the oil, after which the glycerol was added; heating was resumed to complete the alkyd by esterification. A sufficient amount of oil conversion by acidolysis was obtained if the completed resin was bright and clear without any sign of haze, cloud, or gel structure. An insufficient amount of acidolysis resulted in alkyd resins that contained various forms of gel particles. Rapid esterification was accomplished in these alkyd resin preparations by using a 600-watt heat input to the electric heating mantle and reflux condenser to separate the water of esterification from entrained glycerol. Foam caused by this rapid esterification procedure was effectively controlled by slowly adding a small amount of mineral spirits to the kettle during the period of rapid water evolution. After the esterification slowed down, additional mineral spirts were added, the alkyd resin was completed by a solvent reflux procedure in which the vigorously refluxing solvent was condensed in the reflux condenser, and a water-solvent azeotrope was passed overhead.

A novel method of applying the acidolysis reaction to oil-modified alkyd preparation was developed, in which the oil and isophthalic or terephthalic acid mixture was heated to 260°C. or higher and the glycerol was added slowly while continuing to heat, adjusting the rate of glycerol addition to prevent cooling the kettle contents below the selected temperature. Esterification occurred simultaneously with acidolysis. Foam was controlled, as before, by adding mineral spirits at a slow rate during the period of rapid water evolution, and the alkyd resin was completed by the same solvent reflux procedure.

Results

Rates of acidolysis at 300°C. and 280°C. are shown in Figure 1 for the phthalic acid isomers and soybean oil. Isophthalic acid reacts rapidly with oil at these temperatures while terephthalic acid reacts more slowly, as expected, because of lower solubility. Orthophthalic acid dehydrates to the anhydride before any appreciable amount of acidolysis takes place.

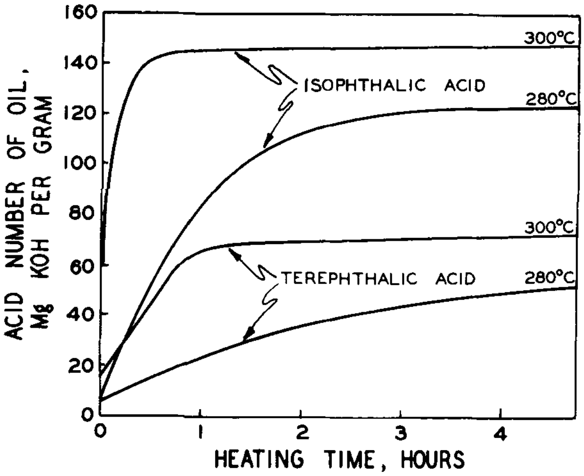

Fig. 1. Acidolysis of triglyceride oil with isophthalic and terephthalic acids.

Figure 2 shows rates of acidolysis with isophthalic acid at temperatures of 280°C. and lower, and a comparison between acidolysis of oil alone and in the presence of fatty acid. The data show that the presence of fatty acid does not significantly change the initial rate of acidolysis; but, as expected, equilibrium occurs at a lower conversion of oil.

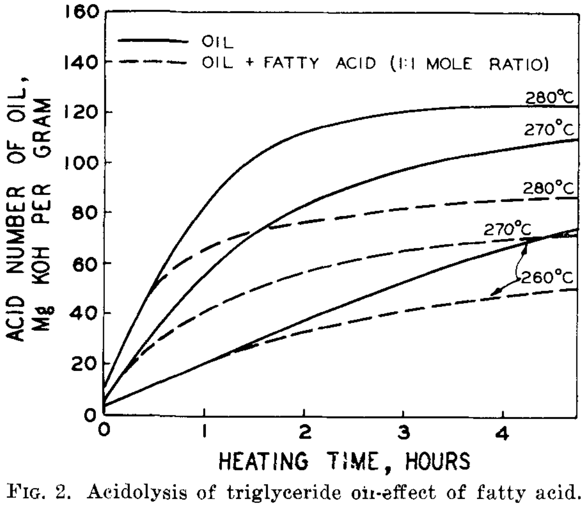

Fig. 2. Acidolysis of triglyceride oil-effect of fatty acid.

TABLE I

Isophthalic Alkyd Resins Produced by Acidolysis. Glycerol Added aftr Cooling Acidolysis Product

Formula	Temperature of acidolysis, °C.	Time for acidolysis, minutes	Acid number of oil	Resin appearance
A	270	60	58	Cloudy
A	270–280	120	92	Cloudy
A	270	180	108	Bright
A	280	60	101	Bright
A	300	2	122	Bright
B	270	2	......	Cloudy
B	270	15	27	Bright

All resins were esterified to an acid number below 10.

Table I contains data on isophthalic alkyd resin preparations which show the effect of different amounts of oil conversion on the formation of incompatible glyceryl isophthalate gel particles. The conditions for these experiments were selected so that the acidolysis and esterification reactions were separate, that is, the acidolysis products were cooled to below *esterification temperature* before the addition of glycerol. The data show that an oil with an acid number of about 100 must be obtained before glycerol is added in order to produce an alkyd resin which is bright and free from any sign of gel structure. When a mixture of oil and fatty acid was used, considerably less conversion of oil was required. Phthalic anhydride did not react with oil, and terephthalic acid reacted too slowly to be useful.

Table II shows results of the simultaneous acidolysis-esterification method of alkyd preparation. Bright alkyd resins, free from any sign of gel particles, were readily produced when glycerol was added slowly and continuously to a mixture of oil and isophthalic acid while maintaining the temperature at 260°C. A typical cooking log is shown in Figure 3. Terephthalic acid also reacted well by this procedure although a slightly slower addition of glycerol was required in order to avoid a cloudy resin. Phthalic anhydride formed glyceryl phthalate gel particles, and satisfactory alkyd resins were not obtained. However all three phthalic isomers reacted well, and bright resins free from any sign of gel particles were obtained when a mixture of fatty acid and oil (1:1 mole ratio) was used instead of oil alone. This also permitted lowering the temperature of the acidolysis-esterification reaction to 240°C., compared to a minimum of 260°C. when oil alone was used, except in the case of terephthalic acid, which still required the higher temperature.

TABLE II

Alkyd Resins Produced by Adding Glycerol Slowly During Acidolysis

Formula	Temperature of acidolysis, °C.	Time for glycerol addition, min.	Resin appearance	Phthalic isomer
A	260	65	Cloudy	IP
A	260	79	Bright	IP
A	260	82	Cloudy	TP
A	260	102	Bright	TP
B	250	73	Bright	IP
B	240	86	Bright	IP
B	250	70	Cloudy	TP
B	260	72	Bright	TP
B	240	89	Bright	PA
B	250	39	Bright	PA

All resins were esterified to an acid number below 10.

Following the experiment with the 1:1 oil-fatty acid mixture in which a bright alkyd resin was obtained from phthalic anhydride by the simultaneous acidolysis-esterification procedure, another run was made with reduced fatty acid content (1:0.75 mole mixture of oil and fatty acid); and the resulting resin was cloudy.

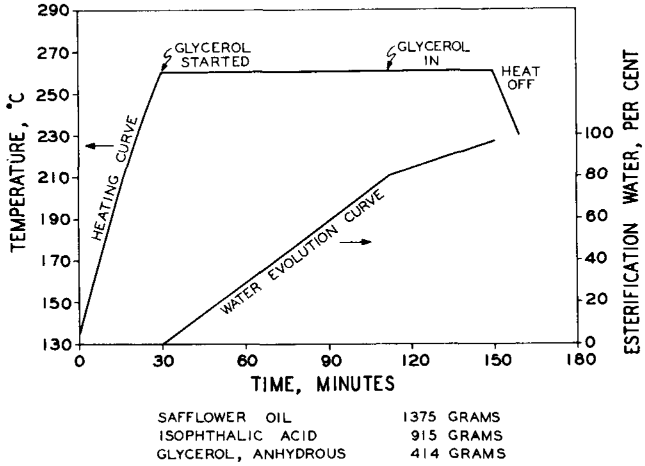

FIG. 3. Acidolysis method of alkyd-resin preparation, simultaneous esterification-acidolysis.

The procedure of alkyd preparation shown in Figure 4 was next developed. Oil and isophthalic acid were heated to 275°C. and held at this temperature for 30 min. to produce an oil with an acid number of 51; then the heat was turned off, and glycerol was added slowly during a period of 10 min. while the kettle cooled. Reheating and completing the resin preparation at 260°C. produced a bright resin, free from any sign of gel particles. A similar experiment, in which the initial heating was 30 min. at 270°C. and the acid number of the oil was 36, produced an alkyd resin with considerable gel particles. Raising the temperature to 280°C., holding only 15 min., and adding the glycerol slowly again produced a bright resin. These experiments showed that a considerable part of the acidolysis reaction occurred during the slow addition of glycerol at high temperature and that the time or temperature for the reaction of oil and isophthalic acid shown in Table I can be reduced if this procedure of adding glycerol is followed.

This work was all done with alkyd resins of 55% oil content. Resins of greater or less oil content were

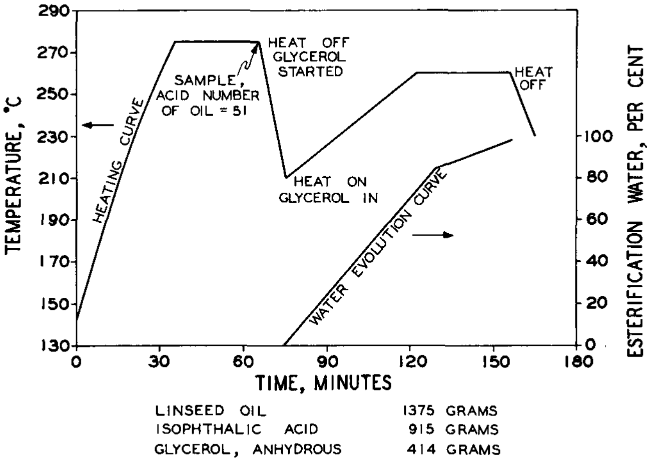

FIG. 4. Acidolysis method of alkyd-resin preparation, acidolysis followed by esterification.

not studied in detail, but enough was done to conclude that conditions for obtaining bright resins at all oil contents do not differ greatly from the conditions found for the 55% oil-content resin.

Acidolysis of oil in the presence of rosin instead of fatty acid was tried, according to the following formula: soybean oil, 1,043 g.; gum rosin, 385 g; isophthalic, 915 g.; and anhydrous glycerol, 451 g. All but the glycerol was heated 30 min. at 270°C., then the glycerol was added slowly over a period of 20 min. during cooling. The finished resin was bright and free from any sign of gel particles.

Acidolysis Combined with Other Operations

Linseed Oil Polymerization. The work described so far relates to the application of the acidolysis reaction to oil-modified alkyd resins, in which the unsaturated oils are relatively unbodied. An experiment was also conducted to determine the effect of acidolysis on oil polymerization. Previous work on the heat-bodying of alkyd oils made by alcholysis is published (1) so an experiment was designed to compare an alkyd oil made by alcoholysis with an alkyd oil identical in all respects except that it was made by acidolysis. The following formula was used (90% oil content): linseed oil, 2,640 g.; isophthalic acid, 249 g.; and anhydrous glycerol, 161 g. In one experiment the linseed oil and glycerol were heated with 0.05% litharge as alcoholysis catalyst for 30 min. at 230°C.; then the isophthalic acid was added and the temperature was increased to 295°C. and held at that temperature 4 hrs. for oil-bodying. In another experiment the linseed oil and isophthalic acid were heated to 295°C. and held at that temperature 4 hrs. for oil-bodying, followed by addition of glycerol and esterification at 230°C. Properties of the two alkyd oils are shown below:

	Alcoholysis alkyd oil	Acidolysis alkyd oil
Viscosity, poises	40	100
Acid number	6	8
Appearance	Cloudy	Bright

The much greater viscosity of the alkyd oil made by acidolysis is evident; furthermore this alkyd oil is bright, and filtration is not required.

Castor Oil Esterification and Dehydration. A comparison of isophthalic and terephthalic acids with phthalic anhydride in the esterification and dehydration of raw castor oil is shown in Figure 5. One mole of castor oil, 925 g., and one mole of phthalic anhydride, 148 g., were heated at 280°C., under xylene reflux to remove the water of reaction. Samples were taken periodically and titrated for total acidity, using t-butanol as a solvent and 0.1 N aqueous sodium hydroxide with phenolphthalein as an indicator. The samples were not filtered before titration so that total acidity could be measured. The experiment was repeated with 166 g. of isophthalic and terephthalic acids. Total acidity of the isophthalic samples was measured with 0.1 N potassium hydroxide in ethanol, using a toluene-isopropanol mixture as a solvent. The terephthalic acid run presented a special problem because the unreacted terephthalic acid was difficult to titrate because of poor solubility in the solvent used. Therefore unreacted terephthalic acid in the samples was measured by filtering and weighing; the filtrate was titrated with 0.1 N potassium hydroxide in ethanol. Total acidity of the samples was then calculated.

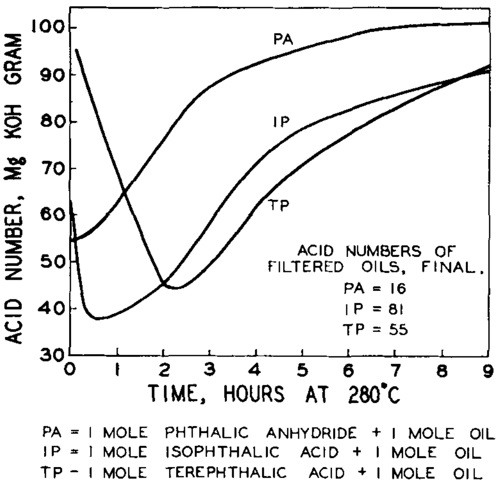

FIG. 5. Dehydration and acidolysis of castor oil at 280°C.

The data show that the orthophthalic ester of castor oil was least stable at 280°C. and that the isophthalic and terephthalic esters were slower to decompose to form the dehydrated castor oil. Acidolysis of the isophthalic- and terephthalic-treated castor oils also occurred, as shown by the high acid numbers of the filtered oils. With the phthalic anhydride oil however the acidity of the filtered oil is equal to the solubility of phthalic anhydride in the oil, so no acidolysis occurred.

The isophthalic dehydrated oil was esterified with glycerol, and tests showed good drying properties. A product of good drying properties was also obtained when the time and temperature of esterification and dehydration were changed to 1 hr. at 295°C.

Fish Oil Polymerization and Fatty Acid Separation. Since one of the products of interchange of triglyceride oil and isophthalic or terephthalic acid is fatty acid, an experiment was set up to remove some of the saturated fatty acids from fish oil during acidolysis and oil-bodying; 1,760 g. of alkali-refined menhaden oil were heated with 166 g. of isophthalic acid at 300°C., and the lower boiling fatty acids were removed by distillation. A high rate of nitrogen sparge was used to assist in the removal of fatty acid. After 4 hrs. of heating, samples of fatty acid totalling 235 g. were collected, which had acid numbers in the range of 217–207. After another 3 hrs. of heating, 160 g. more of fatty acid were collected; the oil in the kettle had an acid number 20 and a viscosity of 3 poises and a Gardner color of 12 when diluted with 50% by weight of mineral spirits; and the product had good drying properties. This process should be generally useful for the upgrading of semidrying oils by removing saturated and less unsaturated fatty acids. Lanson has published information of a similar nature on the selective acidolysis of oils (5).

Another experiment with coconut oil and the same proportion of isophthalic acid gave a larger yield of fatty acids at 300°C. Fatty acids from linseed oil were more slowly removed under similar conditions. In all of these experiments a small amount of isophthalic acid was sublimed and condensed with the fatty acids.

Tung Oil Polymerization. Tung oil gels in about 10 min. when heated at 280°C.; however a mixture of one mole of isophthalic acid and one mole of tung oil was heated at 280°C. for 1 hr. without gelation. This experiment shows that even highly reactive tung oil can be processed under acidolysis conditions.

Corrosion of Stainless Steel. The corrosion rate of stainless steel was measured under various acidolysis-reaction conditions by replacing the glass stirrer blade in the laboratory equipment with a stainless steel stirrer blade weighing approximately 58 g. The stainless steel was cleaned before and after each experiment by mild rubbing with a household type of abrasive cleanser, and weighings were made on an analytical balance. Weight losses were calculated as corrosion penetration per year, in thousandths of an inch, assuming two batches per day. Two types of stainless steel, 304 and 316, were compared and found to be similar. The reaction mixture contained 1,375 g. of oil and 915 g. of isophthalic acid. Results are shown in Table III.

The data show, and experience has proved, that considerable corrosion can occur at 300°C. if heating and cooling rates are slow. The procedures given in Figures 3 and 4 appear to be noncorrosive.

Terephthalic acid was substituted for isophthalic in another experiment, and even after 7 hrs. of heating at 300°C. there was no significant corrosion of stainless steel. This difference between isophthalic and terephthalic acid is believed to be related to the differences in their solubilities.

TABLE III

Corrosion Rates of Stainless Steel in Acidolysis Experiments

Cooking procedure	Temperature of acidolysis, °C.	Acidolysis time, min.	Penetration mils per year
Note 1	300	Note 1	10.1
Note 2	300	Note 2	0.8
	280	60	0.8
Figure 4	275	30	0.1
Figure 3	260	....	0.1

1. Total time in the range of 280–300°C. = 38 min.
2. Faster heating and cooling rates. Time at 280–300°C. = 22 min.

Discussion

Reference to old patents on various methods of making phthalic anhydride oil-modified alkyd resins without first preparing a monoglyceride may be found in Ellis (4). With the recent appearance on the market of the other two phthalic isomers, isophthalic and terephthalic acids, investigations of methods of preparing oil-modified alkyd resins from these materials were made. Data on the alcoholysis or monoglyceride method were published by Lum and Carlston (6). More recently the acidolysis method of preparing oil-modified alkyd resins from isophthalic acid was the subject of a paper and a patent by Carmody (2,3).

The data presented in the present paper show that the *meta* and *para* isomers of phthalic acid are much more adaptable to acidolysis methods of alkyd resin manufacture than is the *ortho* isomer. The acidolysis of triglyceride oils by isophthalic and terephthalic acids is time- and temperature-dependent, and no catalyst is required. This is an advantage since uncertainties of catalyst behavior that sometimes occur in alcoholysis in commercial practice are eliminated. Also, because of the absence of catalyst, resins made by acidolysis are bright and do not need the careful filter-pressing that is required to remove the cloud caused by catalyst residues. These characteristics of noncatalytic acidolysis procedures result in a desirable simplification of alkyd-resin manufacture. Another improvement occurs because glycerol can be pumped into hot acidolysis-reaction mixtures without opening the kettle. This eliminates the disagreeable and toxic fumes that escape from hot kettles when they are opened after alcoholysis for the addition of solid phthalic anhydride or isophthalic acid.

The interchange reaction of a triglyceride oil and isophthalic acid is very rapid at 300°C., reaching an equilibrium state in about 1 hr. However it is not necessary to carry the reaction to equilibrium for practical alkyd manufacture; sufficiently rapid rates of oil conversion are obtained at lower temperatures of 275–280°C. so that bright alkyd resins, free from haze, cloud, or gel particles, can be produced in a reasonable time. Acidolysis of oil also proceeds at a satisfactory rate in the presence of fatty acid or rosin.

The acidolysis method of alkyd manufacture is a simple heating of oil and isophthalic acid for a sufficient time at high temperature to obtain the required oil-acid interchange. A test that can be used to determine the amount of oil conversion is to dilute a sample with an aromatic solvent and filter at room temperature to remove unreacted isophthalic acid. The clear oil solution is titrated for acid number. When the acid number of the oil in the filtrate is about 100 or higher, a sufficient amount of acidolysis has occurred to permit cooling and addition of glycerol; if the glycerol is added slowly at 270–280°C., while cooling, the time or temperature for acidolysis can be significantly reduced. With mixtures of oil and fatty acid or rosin, even less conversion of the oil is required.

The cooling and reheating cycle can be eliminated, when glycerol is the polyol, by conducting the esterification and acidolysis reactions simultaneously. In this method of operation, oil and isophthalic acid are heated to a temperature in the range of 260–270°C.; glycerol is dropped into the kettle at a slow and steady rate while a constant temperature is maintained. Acidolysis of the oil occurs simultaneously with esterification; after the glycerol addition is completed, the esterification is continued in the normal manner. Terephthalic acid is also esterified rapidly by this procedure, and the resultant alkyds are bright. This is noteworthy, in view of the slow esterification and cloudy resins obtained by alcoholysis (6). Phthalic anhydride does not react with oil; however, when phthalic anhydride was heated at 240°C. with a 1:1 mole mixture of oil and fatty acid, glycerol was slowly added to form a bright, homogeneous alkyd resin.

Summary

The interchange reaction of the three phthalic acid isomers with triglyceride oils at high temperatures was studied. Orthophthalic acid dehydrates to the anhydride before any interchange occurs, but isophthalic and terephthalic acids are heat-stable and react easily with oils in the temperature range of 260–300°C. Isophthalic acid reacts more rapidly than terephthalic acid and is ideal for use in acidolysis processes of alkyd-resin manufacture. Various methods of application of the acidolysis reaction to isophthalic alkyd resin preparation were studied, and the minimum of oil conversion to monobasic acids that is required for successful alkyd preparation was found. Under certain conditions the acidolysis reaction occurs simultaneously with esterification; use is made of this fact to improve the process. Commercial use of the acidolysis reaction in alkyd-resin manufacture is economically attractive because it is noncatalytic; therefore no catalyst residues need to be removed by pressure filtration. Another advantage is that glycerol is readily pumped into hot acidolysis-reaction mixtures without opening the kettle, and this eliminates the disagreeable and toxic fumes that escape from hot kettles when they are opened after alcoholysis for addition of solid phthalic anhydride or isophthalic acid.

Applications of acidolysis in conjunction with oil polymerization, fish oil upgrading, and castor oil dehydration were studied briefly, and the results suggest that further work in these fields is desirable.

Acknowledgment

Commercial grades of phthalic anhydride, isophthalic, and terephthalic acids used in this work were supplied by the Oronite Chemical Company.

REFERENCES

1. Carlston, E. F., and Lum, F. G., Ind. Eng. Chem., *49*, 1051 (1957).
2. Carmody, R. F., preprint booklet, A.C.S., Div. of Paint, Plastics, and Printing Ink Chemistry, p. 180, April 1958.
3. Carmody, R. F. (Socony Paint Co.), U. S. 2,884,390 (1959).
4. Ellis, C., "The Chemistry of Synthetic Resins," Vol. 1, pp. 925, 927, 928, Reinhold Publishing Corporation, New York, 1935.
5. Lanson, H. J., J. Am. Oil Chemists' Soc., *33*, 68 (1956).
6. Lum, F. G., and Carlston, E. F., Ind. Eng. Chem., *44*, 1595 (1952).

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Conversion of Some C^{14} -Labelled Compounds into the Neutral Lipid of *Neurospora Crassa*

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Although there have been many biochemical investigations of the red bread mold, *Neurospora crassa,* few have been concerned with lipid metabolism. Previous studies have shown that the coloring matter consists of a variety of carotenoids (2,11). Ottke reported the presence of ergosterol in the mycelium (7) and studied its synthesis from acetate (8). Some of the cephalins have been fractionated (1). Lein *et al.* (6) isolated various mutants which required certain 18-carbon unsaturated fatty acids in the medium for normal growth. Information about the fatty acid composition of the fungus lipids also has been published recently (10).

This paper deals with the neutral lipid from conidia-free mycelia (that fraction of the total lipid which is not extracted from hexane by cold aqueous KOH solution), its incorporation of some C^{14}-labelled metabolites, and the nature of its fatty acid components.

Experimental

Neurospora crassa (cross of wild types E 5297a and E 5256A) was grown by the submerged culture technique previously described for the study of carotene biosynthesis (4). Eight C^{14}-labelled compounds were compared as possible precursors of lipids in the 2-day-old fungus under two sets of conditions: a) the culture was given the radio-active substrate and harvested 3½ days later, or b) the mycelial pad was washed gently, transferred to a sucrose-deficient medium complete in all other respects, given the radioactive material, and collected as in the first procedure. Preliminary experiments had shown that about two-thirds of the maximum dry weight of the mat was attained prior to addition of the labelled substrate.

The hexane-soluble fraction prepared as described elsewhere (5) was freed of acidic substances by extraction with the base (methanol-water-potassium hydroxide 90:10:20 v/v/w). Fatty acids esterified to the neutral lipid were then liberated by hot saponification in ethanol and recovered after acidification. Samples of the neutral lipid and its fatty acids were counted at infinite thinness on aluminum planchets in a windowless gas flow counter. Respired carbon dioxide, which was trapped in 40% potassium hydroxide and precipitated as barium carbonate, was also counted.

The fatty acids were determined qualitatively by chromatography on silicone-impregnated paper (9) and qualitatively and quantitatively by gas chromatography. The paper chromatograms were developed with 85% acetic acid-water (v/v) at 25° for 24 hrs. Autoradiograms were prepared by placing the strips on x-ray film for 60 days. Gas chromatography of the methyl esters, prepared by refluxing the unlabelled fatty acids in methanol with sulfuric acid, was performed at 200° with a 5-ft. polyester column (½ in. in diam.) of ethylene glycol and succinic acid (LAC-446)[3] as stationary phase and a helium flow rate of 75 ml./min.

Results and Discussion

Total fat, as crude hexane extract, produced by the mold *Neurospora crassa* in these experiments constituted about 25% of the weight of the dry mycelium. This crude extract was mostly neutral lipid.

Substantial differences were observed in the effi-

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[1] Journal Paper No. 1557 of the Purdue Agricultural Experiment Station, Lafayette, Ind.

[2] Present address: American Meat Institute Foundation, Chicago, Ill.

[3] Wilkens Instrument and Research Inc.