Solubilities of the Fatty Acids in Organic Solvents at Low Temperatures

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RECENT developments in the field of low temperature crystallization have proved very useful in the study of the fatty acids. Applications of this technic include methods for the following: the quantitative separation of the saturated and unsaturated acids (1), (2), (3); the preparation of very pure oleic (4), (5), (6), linoleic (7), (8), and ricinoleic acids (9); the partial purification of linolenic (10) and arachidonic acids (11), (12); and the separation of alpha-linoleic and alpha-linolenic acids into pure linoleic and linolenic acids and certain contaminating impurities (13). The technic has also been used by Cramer and Brown (14) in the resolution of the component methyl esters of the main fractions of human depot fat. Work previous to 1941 has been reviewed by Brown (15).

As useful as the crystallization procedure has been up to the present time, its success has depended in large measure upon trial and error for the choice of crystallization conditions. This state of affairs has been due in part to the fact that practically no authentic solubilities of the unsaturated acids have been reported in the literature, and in part to the fact that most investigators in this field have regarded the more common unsaturated acids as noncrystalline oils of practically infinite solubility in most organic solvents at temperatures above 0°. In the work previously mentioned, it has been shown repeatedly that exactly the opposite is the case with most of the naturally occurring monoethenoic acids, and with linoleic and linolenic acids, since they are in fact well defined crystalline solids of low solubility at sufficiently low temperatures. So far it has not been possible to crystallize acids, such as arachidonic acid, with four non-conjugated double bonds.

The review by Brown (15) includes previously reported solubilities, except for the work of Ralston and Hoerr (16), who have very recently described important additional data on the solubilities of several of the saturated acids and of some of their derivatives.

The present study was begun in 1939 with the objective of determining the solubilities of a number of the higher saturated and unsaturated acids in several organic solvents. It was hoped that the data thus obtained would be useful in discovering more favorable conditions for the separation of the components of fatty acid mixtures. A great deal of time and attention was paid to the preparation of acids of extremely high purity and to the development of apparatus for determining solubility at low temperature. As a matter of fact, precise data on solubility seem to be almost impossible of attainment, on account of the time required for solutions to come into equilibrium at very low temperatures, so that the values we have found are to some extent dependent on the technic. They do constitute, however, important information for workers in this field. Their usefulness, furthermore, will have to be qualified somewhat, because in employing these data for separations it is a recognized fact that solubilities in mixtures will depend to an important extent on the composition of the mixture, namely, on the presence of other acids in the solution.

EXPERIMENTAL

Materials and Apparatus

THE saturated and monoethenoic acids used in this study were specially prepared to attain products of very high purity. For the several methyl ester fractionations described below an extremely efficient column, packed with glass helices and electrically heated, was used.

In the preparation of lauric acid the C_{12} esters of cocoanut oil were isolated by distillation through this column. The resultant methyl laurate was saponified, the lauric acid set free and crystallized. Myristic and palmitic acids were similarly obtained from the corresponding fractions of bayberry wax and palm oil. These fractions gave practically zero iodine number showing absence of unsaturated esters. Stearic acid was obtained by repeated crystallization of a commercial stearic acid, subsequent preparation and distillation of the butyl esters and conversion of the butyl stearate to stearic acid.

Several pure acids were prepared from rapeseed oil. The C_{22} methyl ester fraction of this oil was recrystallized to give pure methyl erucate, from which erucic acid was made by saponification and recrystallization. Behenic acid was derived from erucic acid by hydrogenation. In the course of distillation of the esters of rapeseed oil, we were surprised to find a previously unreported fraction consisting mainly of C_{20} esters, which, in turn, were found to consist mainly of methyl eicosenoate. After purifying this ester by crystallization, eicosenoic acid was isolated and from it arachidic acid was prepared by hydrogenation.

An outline of our method for the isolation of very pure oleic acid has been reported previously (15). The C_{18} esters of olive oil were especially purified by two distillations through the column mentioned above. Methyl linoleate was then removed by five to six recrystallizations from methyl alcohol at —60°. The four per cent methyl stearate remaining in the oleate was then cautiously removed by cooling dilute solutions in methyl alcohol to about —30 to —34°. The constants of the final oleic acid indicated it to be about 99.8 per cent pure.

The linoleic and linolenic acids used in this work were the highly purified specimens described elsewhere by Matthews, Brode and Brown (13).

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TABLE I

Summary of Analytical Constants of the Fatty Acids

Acid	Molecular Weight		Iodine* Number	Melting Point °C.		Solidification Point °C.	
	Found	Theory		Observed	†	Observed	†
Lauric	200.8	200.3	0.0	43.6		43.5	
Myristic	228.7	228.4	0.0	54.3	54.4	54.0	53.9
Palmitic	256.3	256.4	0.0	62.75	62.9	62.45	62.4
Stearic	284.3	284.5	0.0	69.55	69.6	69.3	69.2
Arachidic	313.2	312.5	0.0	74.5	75.35	74.4	74.9
Behenic	340.3	340.6	0.0	79.9	79.95	79.85	79.6
Oleic	282.6	282.5	89.83	13.2	......	......	......
Linoleic	280.9	280.4	181.0	—5.2 to —5.0	......	......	......
Linolenic	278.1	278.4	274.8	—11.3 to —11.0	......	......	......
Eicosenoic	311.0	310.5	80.0	......	......	......	......
Erucic	338.7	338.6	74.1	33.5	......	......	......

* Wijs, one hour.
† Data from Francis and Piper (17).

A summary of the physical and chemical constants of these several acids is given in Table I.

THE purity of the saturated acids in Table I is evidenced by (1) the close agreement of the experimental and theoretical values of the analytical constants, (2) the correspondence of their characteristics with acids widely recognized to be of the highest order of purity, namely, the preparations of Francis and Piper (17), (3) the small differences between the solidification and melting points, and (4) no discoloration when heated in concentrated sulfuric acid to 70°.

The solvents used were C.P. grade methanol, which was carefully redistilled before use, Skellysolve B, a commercial petroleum fraction of boiling range 57-63°, and very pure acetone prepared by thermal decomposition of an acetone-sodium iodide complex after the method of Shipsey and Werner (18).

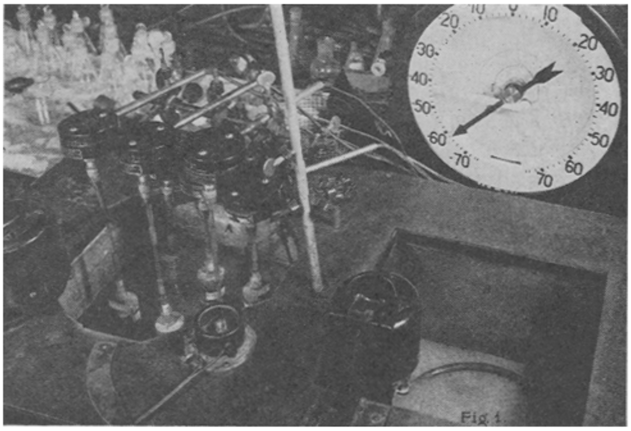

FIG. 1. Constant temperature apparatus.

A constant temperature bath was designed and built for use at low temperatures. Figure 1 gives a top view of the bath, showing the essential working parts and setup for a solubility determination. The overall dimensions of the bath are 95x40x55 cm. It is divided into two monel chambers 28x29x27 cm. each, insulated on all sides by 12.5 cm. of rock wool. Side A, as indicated in Figure 1 is the working unit; side B, the cooling unit. The heat transfer medium in both units is a mixture of ethanol and methanol, but, in order to avoid the inconvenience of excessive evolution of dissolved CO_2 when warm vessels are introduced into A, the liquid in A is kept separate from that in B. Dry ice is placed in the solvent mixture in B. The liquid from A is then forced by the circulating pump shown in B through the copper coil in B. Action of the pump is controlled by the bimetallic thermostat, shown on A, which starts and stops the pump through a time-delay relay. Temperature is measured and recorded with a Leeds and Northup Model R Micromax Recorder. It is possible by this apparatus to maintain a temperature to within ±0.5° over ten-hour periods at temperatures of —50° or lower. At somewhat higher temperatures the limits are within ±0.25°.

TABLE II

Solubilities of the Saturated Acids

Solvent	Acetone		Methyl Alcohol		Skellysolve B	
Temp. °C.	grams/ 1000 g. sol'n	grams/ liter sol'n	grams/ 1000 g. sol'n	grams/ liter sol'n	grams/ 1000 g. sol'n	grams/ liter sol'n
			Lauric Acid			
0°	....	....	....	....	15.1	10.2
—10°	....	....	28.3	21.9	10.7	7.20
—20°	17.4	13.6	17.0	13.3	3.79	2.56
—30°	12.3	9.7	8.23	6.47	....	....
—50°	0.25	0.19	....	....	....	....
			Myristic Acid			
0°	22.7	17.8	18.4	14.3	9.43	6.34
—10°	10.7	8.4	8.26	6.46	3.25	2.19
—20°	4.33	3.40	3.44	2.70	1.31	0.83
—30°	1.74	1.37	1.53	1.19	0.20	0.13
—50°	....	....	....	....	....	....
			Palmitic Acid			
10°	17.7	13.8	13.1	10.3	8.38	5.63
0°	7.15	5.59	3.96	3.35	1.25	0.84
—10°	2.80	2.18	1.46	1.13	0.26	0.17
—20°	1.34	1.05	0.63	0.48	0.12	0.08
—30°	0.48	0.38	0.20	0.16	0.09	0.06
			Stearic Acid			
10°	4.69	3.68	2.59	2.04	1.31	0.83
0°	2.19	1.71	0.92	0.72	0.24	0.16
—10°	0.38	0.29	0.32	0.25	0.10	0.07
—20°	....	....	0.10	0.08	....	....
			Arachidic Acid			
10°	1.83	1.43	0.96	0.75	0.56	0.38
0°	0.75	0.58	0.65	0.50	0.48	0.32
			Behenic Acid			
10°	0.51	0.39	0.42	0.32	0.29	0.22
0°	0.10	0.08	0.10	0.08	0.10	0.07

Procedure and Results of Solubility Determinations

IN the development of a suitable procedure, it was necessary to overcome several difficulties that arose because of the low temperatures at which the determinations were to be made. Among these were adequate temperature control, slowness in attaining equilibrium, removal of clear, saturated solution samples without disturbing the temperature, and condensation of moisture. In addition, it was necessary to adapt this procedure so that a large number of determinations could be run in a comparatively short time.

The determinations were carried out in 250 ml. wide-mouth Pyrex Erlenmeyer flasks. Solutions of the acids were made up so that the initial concentration was approximately twice the solubility at the highest temperature to be used in a given series of determinations. The same solution was usually employed for obtaining data at three different temperatures. The flasks containing the solution were clamped into the constant temperature bath, fitted with mercury seal stirrers to exclude moisture and the solutions stirred at the test temperature for 9½ hours. At this time the stirrers were removed and replaced with sintered glass filter disks (3.0 cm. in diameter) fitted into rubber stoppers. The filter disks were pressed down into the flasks until they rested barely above the surface of the solution. They were kept in this position for one-half hour, in order that they attain the same temperature as the test solution. The filters were then pushed down into the solution and samples forced out into 125 ml. tared glass-stoppered flasks by means of dry air cooled to the test temperature. Following this operation, the flasks were again fitted with their stirrers and stoppers and the temperature was lowered to the next level (usually 10° lower) at which the determination was to be made.

TABLE III

Solubilities of the Unsaturated Acids

Solvent	Acetone		Methyl Alcohol		Skellysolve B	
Temp. °C.	grams/ 1000 g. sol'n	grams/ liter sol'n	grams/ 1000 g. sol'n	grams/ liter sol'n	grams/ 1000 g. sol'n	grams/ liter sol'n
			(a) EICOSENOIC ACID			
—20°	16.1	12.6	10.6	8.30	....	....
—30°	4.58	3.60	3.48	2.73	2.95	1.99
—40°	1.47	1.15	1.29	1.01	1.07	0.71
			(b) ERUCIC ACID			
—20°	3.52	2.76	1.76	1.38	....	....
—30°	....	....	0.87	0.68	0.59	0.40
			(c) OLEIC ACID			
—30°	14.2	11.1	7.08	5.55	11.8	....
—40°	5.16	3.97	3.29	2.58	4.83	3.25
—50°	1.89	1.48	0.89	0.70	1.04	0.69
—60°	0.61	0.48	0.51	0.40	0.42	0.28
—70°	0.40	0.31	0.32	0.25	0.24	0.16
			(d) LINOLEIC ACID			
—50°	48.2	37.8	25.2	22.5	17.0	11.5
—60°	14.2	11.1	9.25	7.26	3.27	2.21
—70°	5.19	4.07	3.94	3.09	0.09	0.40
			(e) LINOLENIC ACID			
—62°	43.2	38.8	17.6	13.8	4.43	2.96

The flasks containing the samples of saturated test solution were stoppered carefully and kept at 16.5°. A 10 cc. sample, measured at this temperature, was pipetted off into another tared glass-stoppered Erlenmeyer flask, and weighed at room temperature. The solvent was evaporated, 25 cc. of neutral alcohol added and the solution titrated with 0.1 or 0.01 N sodium hydroxide, depending upon the amount of acid involved, with phenolphthalein as an indicator. Solubilities were calculated in grams per 1000 gms of solution and grams per liter, measured at 16.5°.

The solubilities of the acids in various solvents are presented in Tables II, III, and IV. The results are graphically presented in Figures 2, 3, and 4.

TABLE IV

Solubility Ratios of Fatty Acids Under Various Conditions*

Solvent	Temp.	Oleic	Palmitic	Ratio Oleic: Palmitic
Methyl acetate	—25°	10.0	0.74	14.7:1
Acetone	—30°	14.2	0.48	30.0:1
Methyl alcohol	—30°	7.08	0.20	35.4:1
Butyl alcohol	—25°	62.8	1.32	47.6:1
Ethylidene dichloride	—25°	26.8	3.24	82.7:1
Skellysolve B	—30°	11.8	0.09	130.0:1
Carbon disulfide	—30°	15.7	<0.1	>157.0:1
Toluene	—30°	50.2	<0.1	>500.0:1
Ethyl ether	—40°	43.7	<0.1	>450.0:1
Solvent	**Temp.**	**Linoleic**	**Oleic**	**Ratio Linoleic: Oleic**
Skellysolve B	—70°	0.60	0.24	2.5:1
Carbon disulfide	—62°	4.12	0.398	10.3:1
Methyl alcohol	—70°	3.94	0.32	12.3:1
Acetone	—70°	5.19	0.40	13.0:1

* Solubilities expressed g./1000 g. solution. Numerous other ratios can be derived from the preceding tables and curves.

Discussion of Results

IT was not practical in this investigation to attain complete equilibrium conditions with respect to saturation of the solution. Fatty acids are particularily prone to supersaturation and require long periods of time for equilibrium at a given temperature to be established between the acid in solution and the undissolved acid. It was the experience of Hehner and Mitchell (19) with palmitic acid in alcohol that the solubility was changing, even after 156 hours at 0° C. In our work it was found experimentally that after the standard ten-hour test period, equilibrium conditions were not attained, since solubility values, resulting when the test temperature was approached from the warm side, were a maximum of ten per cent higher than those resulting when the test temperature was approached from the cold side. Since these data are designed for use in crystallization experiments, namely, under non-equilibrium conditions, and because of our desire to make as large a number of determinations as possible, the ten-hour period was arbitrarily chosen as of the most practical value.

By careful control of conditions such as initial concentration of solute, time, temperature, purity of acids and solvents, moisture condensation, etc., it was

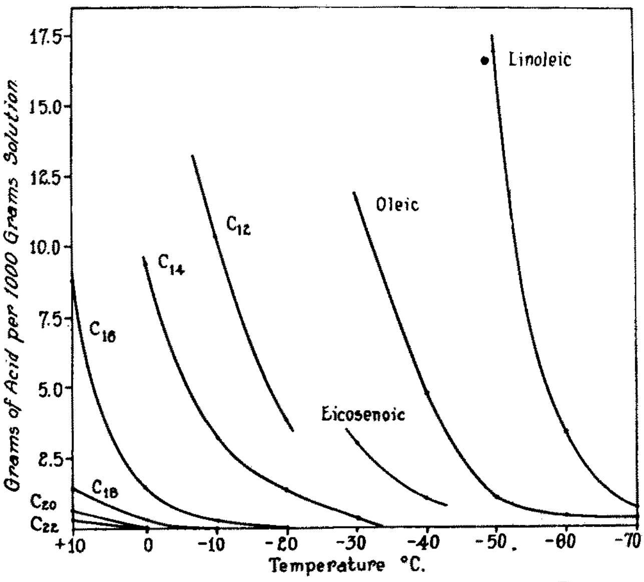

FIG. 2. Solubilities of fatty acids in Skellysolve B.

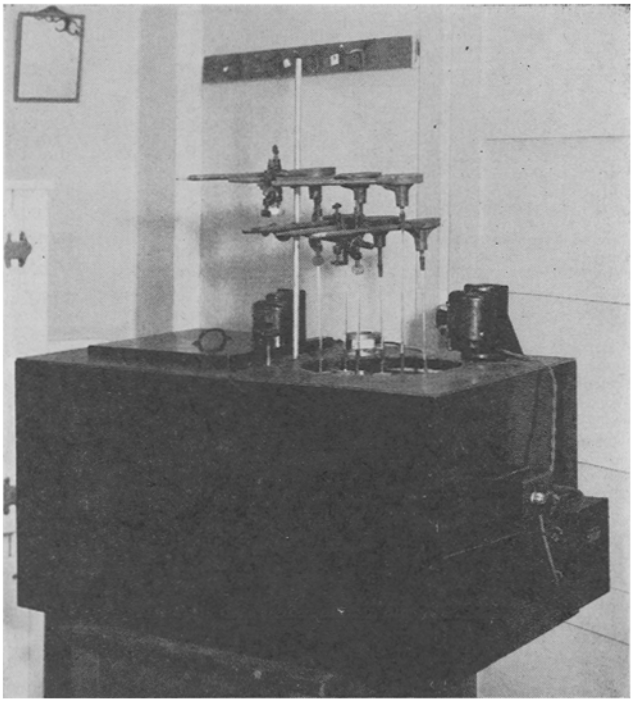

FIG. 1-a. Constant temperature apparatus.

possible to obtain consistent results by approaching the test temperature from the warm side, which were reproducible within three per cent.

In the application of solubility data for crystallization purposes, it is that portion of a curve where the solubility falls off rapidly to relatively low values that is the most important. The lower solubility limits are the most valuable for crystallization work. Thus, the curves show that at —32° practically all of the saturated acids above palmitic will be removed from solution in any of the three principal solvents. However, if the concentration of oleic acid present in an acetone solution is above 1.35 per cent, it, too, will be precipitated along with the saturated acids at this temperature. In this situation it is desirable to adjust the volume of the solution so that the concentration of the oleic acid is less than 1.35 per cent, provided, of course, that the amount of oleic acid is not so large that a tremendous volume of solution is necessary.* If this is the case, a smaller volume can be used and the resultant precipitated saturated acids can be recrystallized to remove oleic acid. The number of recrystallizations and the optimum concentrations can be calculated from the data.

An interesting illustration of the use of solubility data is the preparation of linoleic acid by Brown and Frankel (7). Although they used petroleum ether (30-60°) in their final purification, Skellysolve B is used in this illustration since data in this solvent are shown on the curves and the same effects apply as in petroleum ether. As the temperature of a solution of a mixture of oleic and linoleic acids in acetone is lowered, oleic acid is precipitated out faster than linoleic acid, with resulting concentration of the linoleic acid in the filtrate. However, at —70°, the purity of the linoleic acid, due to the solubility ratio of the acids of 13:1, is still only about 93 per cent. This is the maximum purity attainable at this temperature in this solvent. By changing to a solvent in which there is a different solubility ratio, it is possible to effect further purification. The solubility ratio of the two acids in Skellysolve B is only 2.5:1. It is possible by adjusting the concentration of the solution so that all the oleic remains in solution to precipitate out pure linoleic acid as the precipitate. This is done at the cost of some of the linoleic —namely, that which remains behind with the oleic—but considering the very poor yields and the possibility of isomerization on use of the alternate method of preparation, the bromination-debromination method, this procedure has decided advantages.

In order to extend the usefulness of our data, solubility ratios of oleic to palmitic and linoleic to oleic acids were determined in several additional solvents at given temperatures. These data, together with several ratios taken from the preceding curves, are shown in Table IV. Some of these values suggest certain solvents which should be very useful in separating saturated and unsaturated acids. The oleic to palmitic acid ratio in carbon disulfide at —30°, for example, is 157-1; in ether at —40°, the ratio is 450-1; in toluene at this temperature, it is 500-1. On the other hand, the most favorable ratio of linoleic to oleic acid is in acetone at—70°, the value being 13-1, which is about that found by Brown and **Frankel**. It may be pointed out further that the ratios of palmitic to oleic acids in Table IV, if they can be attained in practice, suggest separations of saturated and unsaturated acids which should be more efficient than the commonly employed lead soap

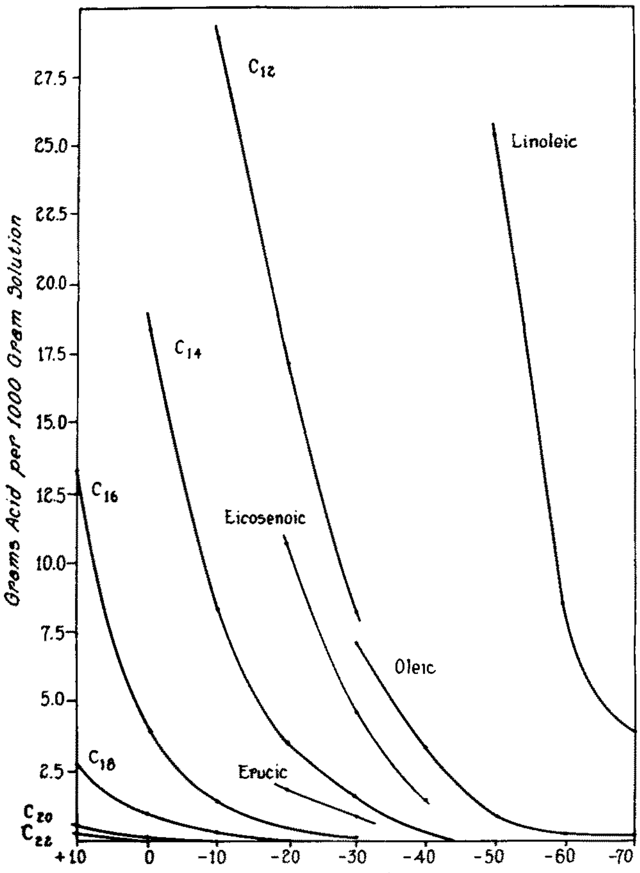

FIG. 3. Solubilities of fatty acids in methanol.

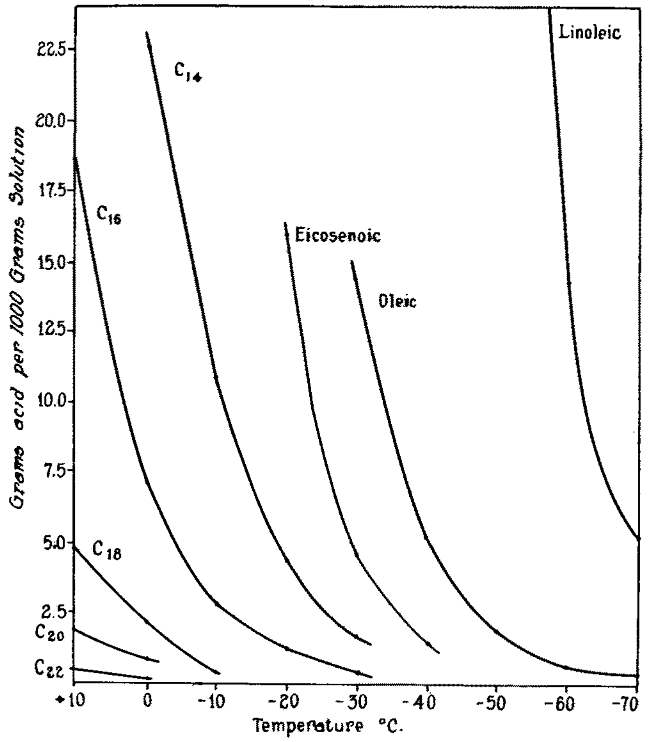

FIG. 4. Solubilities of fatty acids in acetone.

alcohol or ether methods. Again it should be emphasized, however, that in practice mutual solubilities may decrease the efficiency of the procedure.

A great deal of additional work should be done to extend the data presented above. The data, especially on lauric, erucic and linolenic acids, are needed in order that the curves of their solubilities may be extended. This is especially true of linolenic acid which has not yet been prepared pure by crystallization procedures. Maximum purity so far attained was 91 per cent by Guy in unpublished work from this laboratory (20). It would also be of especial interest to extend the series of monoethenoic acids to tetradecenoic and hexadecenoic acids and to study series of acids with one double bond to determine the effect of carbon series, double bond position and geometric isomerism on solubility. Furthermore, certain of the conjugated acids are high melting solids and their solubilities would be of great interest. Also, the data should be extended to include many more solvents. Unfortunately, in the current emergency it is not possible to continue these studies for the present.

The authors are greatly indebted to the Sherwin Williams Company, Cleveland, Ohio, for sponsoring this research through an open fellowship in the Ohio State Research Foundation. They also appreciate the interest and advice of Dr. Robert Ruthruff of that company during the entire course of this investigation.

Summary

A considerable series of pure fatty acids has been prepared and their solubilities have been determined at temperatures down to —70° in acetone, methanol and Skellysolve B. Solubility ratios have also been determined for oleic to palmitic acid and linoleic to oleic acid in a number of additional solvents at certain temperatures.

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Report of the Olive Oil Committee

ON account of the fact that the olive oil trade has called for no particular problem for our investigation, the chairman up to a short time ago could find nothing worthwhile for the committee to consider.

However there has appeared in the Journal of the Association of Official Agricultural Chemists a method by Dr. J. Fitelson, who is a member of our committee, on "The Detection of Olive Oil in Edible Oil Mixtures." This method determines the iodine value of that portion of the unsaponifiable matter which is not adsorbed by aluminum oxide. Since olive oil is notable in that it contains a greater iodine value from the higher squalene content than any of the common edible vegetable oils, the test shows promise of value in detecting small quantities of olive oil in the many types of oil blends appearing on the market, which have been the cause of considerable controversy regarding the true content of olive oil ingredient.

M. F. LAURO, *Chairman.*