208. *Higher Aliphatic Compounds. Part VIII. Purification of Oleic and Elaidic Acids. Binary Systems from Oleic, Elaidic, Palmitic, and Stearic Acids. The Technique of Low-temperature Crystallisation.*

By J. C. SMITH.

Oleic and elaidic acids (*cis*- and *trans*-$CH_3\cdot[CH_2]_7\cdot CH{:}CH\cdot[CH_2]_7\cdot CO_2H$) have been carefully purified and tested; the difficulty of eliminating stearic acid is demonstrated. The technique of filtration at low temperatures is described, and the methods are applicable to the crystallisation of other low-melting substances.

From an examination of the binary system it is concluded that considerations of symmetry reduce the tendency to form mixed compounds of the type elaidic acid–palmitic acid.

DESPITE the importance of oleic acid and the large number of measurements which have been made on the acid, it is unlikely that any specimen of above 99% purity has been prepared. An extended study of methods of purification was made by Lapworth, Pearson, and Mottram (*Biochem. J.*, 1925, **19**, 7) and important contributions have been made by Robinson and Robinson (J., 1925, **127**, 175), Holde and Gorgas (*Z. angew. Chem.*, 1926, **39**, 1443), Bertram (*Rec. Trav. chim.*, 1927, **46**, 397), Raymond (*Chim. et Ind.*, 1929, Special Number, p. 523), Skellon (*J. Soc. Chem. Ind.*, 1931, **50**, 131T), and Keffler and McLean (*ibid.*, 1935, **54**, 178T, 362T). The procedure of Brown and Shinowara (*J. Amer. Chem. Soc.*, 1937, **59**, 6) for removing saturated acids is a great advance on the older methods; by fractional crystallisation from acetone of crude oleic acid they obtained specimens which, judged by the m. p.'s, reached 97—98% of oleic acid.

In the present investigation methyl oleate was fractionally distilled at low pressure through an efficient column. This removed most of the palmitate and some of the stearate (the linoleate and linolenate were not separable from the oleate by distillation) and the regenerated acid was crystallised by Brown and Shinowara's method to constant m. p. It is possible that mere crystallisation of crude oleic acid from acetone at various temperatures may yield pure oleic acid in some cases, but a preliminary esterification and fractional distillation is generally recommended. In view of the claims so frequently made for the purity of oleic acid specimens, it was thought desirable to apply stringent tests. The iodine value when determined with suitable precautions was easily reproducible and indicated less than 0·5% of saturated acids. (The ease with which the polyethenoid acids were removed in the crystallisation from acetone left no doubt of their absence from the final product.) That the oleic acid contained less than 0·2% of palmitic acid was clearly shown by catalytic reduction to stearic acid of high m. p.

There is yet no really satisfactory way of detecting stearic acid when only 1% is present; oxidation of the oleic acid to dihydroxystearic acid while the stearic acid remains unchanged (Robinson and Robinson, *loc. cit.*; Lapworth and Mottram, J., 1925, **127**, 1628) is the best available method. In this case oxidation failed to show definitely the presence of stearic acid and indicated that not more than 1% could have been present.

From the data available for comparison, this oleic acid of (α) f. p. 13·34°, m. p. 13·36° ± 0·04°, (β) m. p. 16·25° appears to be the best so far obtained; Bertram's product had f. p. 13·2°, and Brown and Shinowara's f. p. 13·0°. Unfortunately, many authors have recorded m. p.'s correct only to ± 1°; when it is realised that 1% of stearic acid lowers the m. p. of oleic acid by only 0·13°, the necessity for taking the f. p. or m. p. with the thermometer *in the liquid* and correct to ± 0·05° is obvious.

Elaidic Acid.—Isomerisation of oleic acid with nitrous acid gave a mixture containing approximately 65% of elaidic acid (the *trans*-isomeride), in agreement with Griffiths and Hilditch (J., 1932, 2315). Elaidic acid, because of its high m. p., is easily separated from linoleic and linolenic acids, but stearic acid is a very persistent impurity; 1% of stearic acid, moreover, lowers the m. p. by only 0·1°.

Dr. S. H. Piper, who kindly examined the purest specimen of elaidic acid, reported that it had an *X*-ray spacing at room temperature of 48·95 A., which corresponds to a double molecule with a " vertical chain." The longest spacing observed for stearic acid is 46·2 A. (Piper, private communication), and is that of a metastable form.

Binary Systems.—The binary systems of mixtures of saturated and unsaturated acids have been examined because of their usefulness for analysis and in the hope of obtaining information on compound formation.

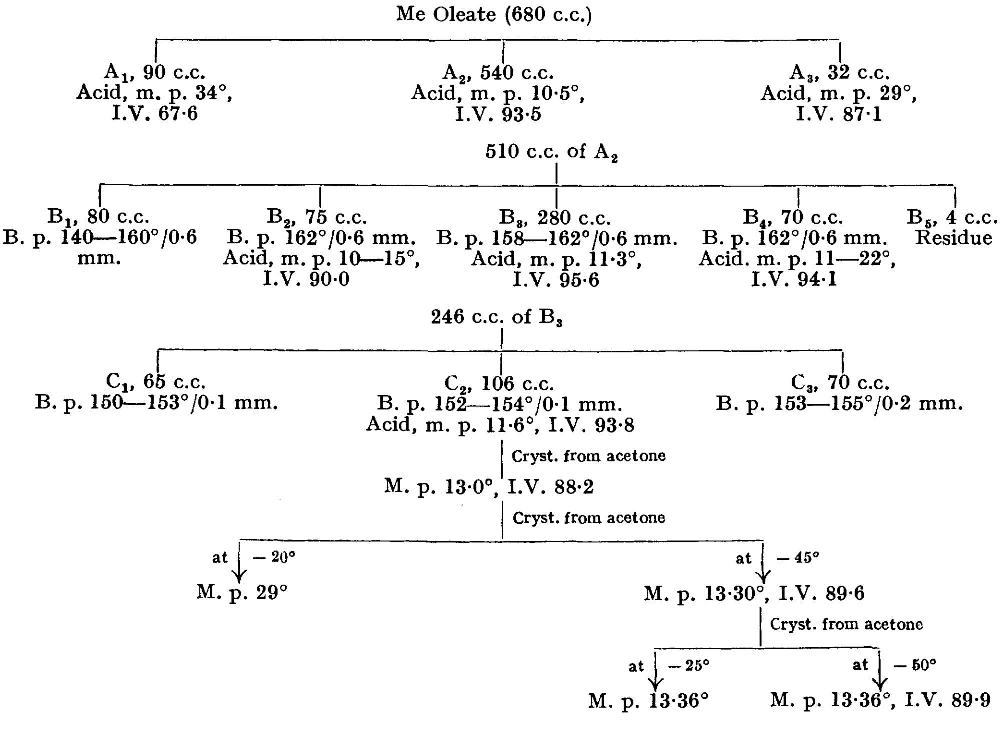
The systems are all of the eutectic type, and in the oleic–stearic system the eutectic is reached at 2—3% of stearic acid. None of the systems (nor the oleic–elaidic system, Griffiths and Hilditch, *loc. cit.*) gives in the liquidus curve any indication of compound formation such as is almost always found in mixtures of long-chain saturated acids (see Part VI, J., 1936, 625). It was not expected that oleic acid would readily yield compounds, but elaidic acid because of its higher m. p. and because of the shape of the *trans*-chain (see Marsden and Rideal, J., 1938, 1165) might more easily combine with palmitic or stearic acid. For this reason the thaw point method of Rheinboldt and Kirscheisen (*J. pr. Chem.*, 1926, **113**, 348), which is useful in detecting compound formation in aromatic systems, was applied to various mixtures of elaidic and palmitic acids; the results indicated a very small proportion of an equimolecular compound.

The behaviour of oleic acid in solution shows that it is less completely associated than stearic acid (Robertson, J., 1903, **83**, 1425; Brocklesby, *Canadian J. Res.*, 1936, **14**, *B*, 222); on the other hand, the *X*-ray measurements of elaidic acid crystals certainly show that the units are double molecules, as are those of palmitic and stearic acids. The reason why very few mixed double molecules crystallise as a compound is probably to be found in the lack of symmetry of a mixed molecule containing one " bent " methylene chain.

EXPERIMENTAL.

Distillation of Oleic Acid.—The oleic acid (" reinst ") of Messrs. Fraenkel and Landau had iodine value (I.V.) 90·4 (Hanuš; theory, 90·0) and m. p. 9·2° (thermometer in the liquid). The methyl ester from this acid was distilled from a Claisen flask at 0·6 mm., and an almost colourless distillate obtained. This methyl ester (680 c.c.) was then distilled through a 60-cm. column of Raschig rings (Part I, J., 1931, 804): the fractionation is summarised in Table I.

TABLE I.



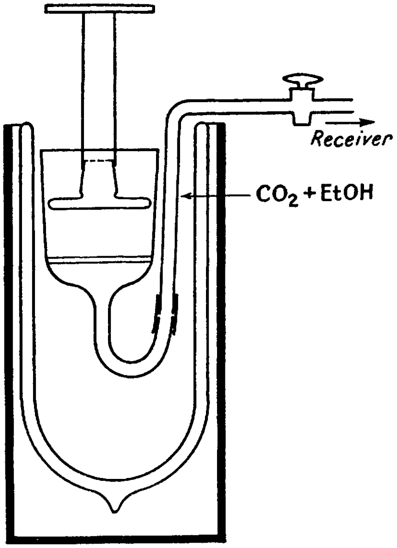
Portions of the fractions were hydrolysed, and the resulting specimens of oleic acid were tested (m. p. and iodine value).

Fraction A$_1$ obviously contained much palmitic acid, and A$_3$ much stearic acid. Most of the main fraction (A$_2$) was redistilled in the same apparatus, and fractions B$_1$ to B$_5$ were collected. B$_2$ yielded an acid melting indefinitely from 10° to 15° and appeared to contain some palmitic acid; similarly B$_4$ contained stearic acid. The presence of saturated acids is not indicated by the m. p. in the acid obtained from B$_3$ or A$_2$ or in the original oleic acid because a *mixture* of saturated acids has much less effect in raising the m. p. of oleic acid than has either of the pure saturated acids.

B_3 appeared to be a mixture of methyl oleate with more highly unsaturated esters and with small amounts of palmitic and stearic acids. (The presence of the saturated acid was shown by acetone crystallisation of a portion of hydrolysed B_3.) When B_3 was redistilled with rejection of large head and tail fractions, the main fraction (C_2) yielded an acid still of low m. p. and high I.V. (93·8). It was obvious that even with an efficient column the separation of oleates from linoleates and linolenates was almost impossible. Subsequent crystallisation from acetone showed also the presence of saturated acid in C_2, so distillation alone is not a satisfactory method of purification of oleic acid.

Low-temperature Crystallisation.—Bertram (*loc. cit.*) crystallised partly purified oleic acid from acetone at − 15°, and Raymond (*loc. cit.*) similarly used alcohol and described a piston-filter. Brown and Shinowara (*loc. cit.*) made a study of the crystallisation of crude oleic acid from acetone at various temperatures. The technique of low-temperature crystallisation developed by the author during the last 15 years seems worth describing, especially as methods of filtration seem to have given trouble in some laboratories.

FIG. 1.



(i) For very small quantities (up to 50 c.c. of solution and 2 g. of precipitate), a conical filter with a sintered-glass plate may be used. A cold jacket is made by cutting the whole stem off a plain glass funnel which is then fixed with a short rubber stopper or ring to the stem of the filter. The jacket is partly filled with the liquid used for cooling the solution, and the filter (covered with a watch glass to exclude moisture) is connected to a filter flask.

(ii) For filtration at − 75° of quantities up to 8 g. of precipitate and 100 c.c. of solution, a Jena-glass filter (Büchner type, 40 mm. plate, 60 c.c. capacity, No. 11 G. 1) is kept in a wide-top Dewar vessel (Thermos 1-pint fruit jar) with carbon dioxide–alcohol (see Fig. 1). The filter has the stem bent through 180°, and a piece of Jena glass tube bent at right angles is sealed on (or attached with rubber) in order to lead horizontally to the receiver and the pump. A glass stopper fitting the funnel closely is useful for pressing out the solvent and for keeping moist air away from the filter plate. This filter and stopper are left for at least 15 mins. in the cooling mixture while the solution in a 100-c.c. conical flask is being cooled to − 75° in a second Dewar vessel (15—30 mins. with occasional shaking). Gloves and cloths must of course be used in handling the cold vessels. When the crystals cease forming in the solution and the temperature is steady at − 75° (toluene thermometer), the receiver is connected to the cold filter, the pump is turned on, and the well-shaken mixture poured into the filter, the crystals being pressed with the cold stopper. The solid is then transferred with the aid of a cold spatula to a thin-walled glass funnel and allowed to melt into a 100-c.c. conical flask; warm solvent is poured into the Jena filter to remove any solid and then allowed to run over the melting crystals so that a solution is made up for a second crystallisation. Finally, the residual solvent is removed from the crystals by melting and heating in a vacuum, or by distillation.

(iii) For larger quantities than 10 g. of solid, and when temperatures not so low as − 60° to − 80° are desired (impurities are often too sparingly soluble at − 80°), a low-temperature bath is convenient; it has another advantage in that the temperature can be lowered slowly when large crystals are desired. A 5-litre jar containing about 1 litre of methylated spirit is placed on cardboard resting on a block of solid carbon dioxide, the whole being surrounded by an insulated container. By adjusting the thickness of the materials under and around the jar, temperatures from − 20° to − 60° can readily be maintained to within 5—10°. The solution and the sintered-glass Büchner funnel can be simultaneously cooled in this alcohol-bath; if necessary, the alcohol is washed off the filter with some of the same solvent (cold) as is used for the crystallisation. The filter is then attached to the filter flask (which need not be cooled) and the filtration carried out quickly, the crystals being pressed with a cold stopper. Acetone has proved the best solvent in nearly every case.

Crystallisation of Oleic Acid.—A solution of the acid (30 g., m. p. 11·6°, I.V. 93·8) in acetone (360 c.c.) was left for 12 hours at − 30° in the cooling bath. The copious precipitate was readily separated from the liquid and had m. p. 13·0°, I.V. 88·2 (theory 90·0); the whole precipitate was then dissolved in acetone (200 c.c.), and the solution cooled to − 20° with frequent stirring. After having stood for 20 mins., the fine precipitate (4 g., m. p. 29°) was removed. The filtrate on cooling to − 45° for 8 hours deposited oleic acid of m. p. 13·30°, changing to β-form, m. p. 16·18°, I.V. 89·6. A solution of this product in acetone (200 c.c.), cooled to − 25° with stirring, deposited 6 g. of crystals, m. p. 13·36°, and the filtrate after 3 hours at − 40° to − 50° gave 9 g., m. p. 13·36°, I.V. 89·9, 89·8; *M*, 282. Further crystallisation from ether, ethyl acetate, or hexane failed to change the m. p. of this oleic acid, the final values being f. p. 13·34°, m. p. 13·36° (α) and m. p. 16·25° (β); I.V. 89·8 and 90·0 ± 0·4; $n_D^{20°}$ 1·4597, $n_D^{15°}$ 1·4616.

Other experiments showed that oleic acid, m. p. 11·0—11·6° after 3 crystallisations from acetone and 1 elimination of saturated acids, gave 50—60% yields of pure oleic acid; thus an acid corresponding to that from Fraction A_2 (Table I) readily gave oleic acid of f. p. 13·34°. But the crude oleic acid, m. p. 9·2°, if not esterified and distilled, gave only a 40% yield of acid, m. p. 13·0°. Brown and Shinowara's specimens also melted at 13·0°.

Even the purest oleic acid oxidises on standing in corked vessels, and the m. p. falls about 0·1° per month. Also, water is absorbed, and on distillation of old specimens there is a partial recovery in m. p. If the acid is kept solid in a refrigerator there is little change of m. p., but it is preferable to seal it in evacuated tubes.

Estimation of Saturated Acids.—Iodine numbers. Estimation of the residual fatty acids in oleic acid is difficult when these amount to less than 1%. In determinations of the iodine value there is usually an error of ± 0·5%, partly due to the physical properties of iodine chloride or bromide solutions (compare Keffler and Maiden, *J. Soc. Chem. Ind.*, 1933, **52**, 242T). By working at 16° and checking the volume of the added iodine bromide solution (Hanuš) by weighing, values consistent to ± 0·2% were obtained.

Catalytic reduction. An experiment with hydrogen at 1 atm. in presence of palladium–barium sulphate showed that approximately 97% of the calculated quantity of hydrogen was absorbed by oleic acid in hexane solution in 4 hours, and 99% in 8 hours, but that the volume of hydrogen could not be accurately measured. Reduction in hexane solution was slow, but the product was more likely to be free from by-products than if other solvents were used.

Oleic acid (3·0 g., m. p. 13·36°) in hexane (60 c.c.) was shaken for 30 hours with hydrogen (4 atm.) in presence of the catalyst (from 0·25 g. of palladium chloride and 3 g. of barium sulphate). The main product had m. p. 69·58° : pure stearic acid has m. p. 69·62° ±0·05° (Part VII, this vol., p. 615) and the m. p. is lowered by 0·24° by 1% of palmitic acid. On complete evaporation of the filtrate, a slightly yellow residue (0·11 g.) was obtained. This melted at 67·2—67·6° and was unsaturated to warm permanganate solution; on further reduction this gave 0·08 g., m. p. 68·3°. Even if the impurity causing the depression of 1·3° in the m. p. was all palmitic acid, only 6% could have been present, corresponding to 0·2% in the 3 g. of oleic acid reduced.

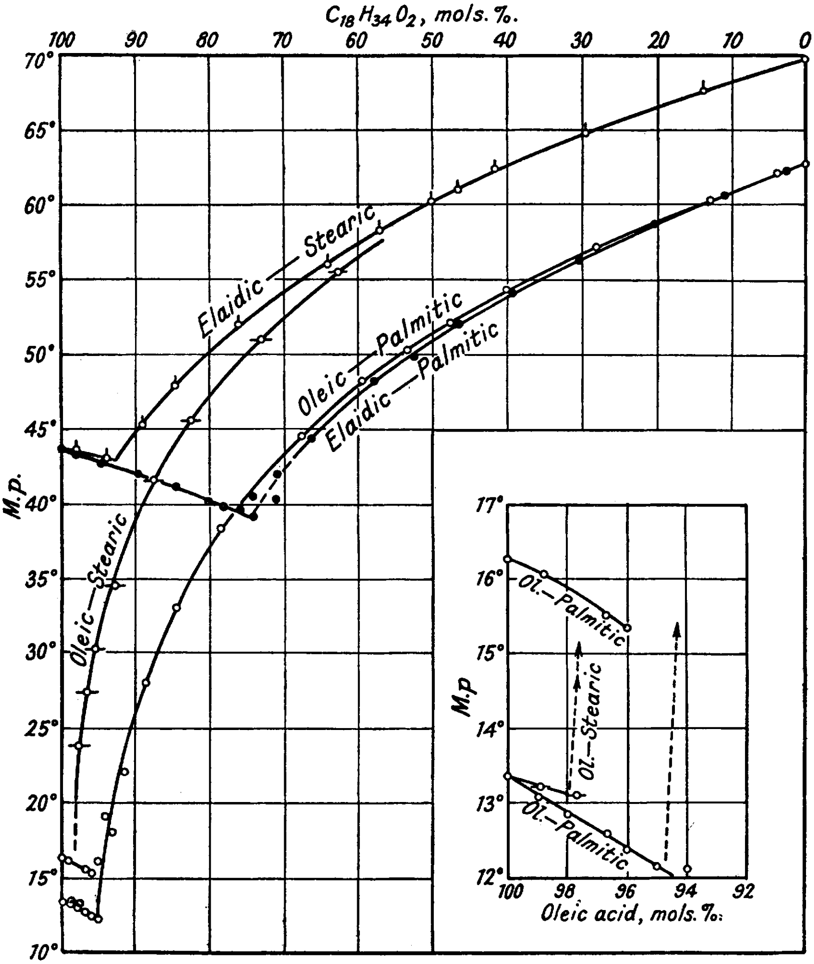
Oxidation of Oleic Acid.—The conditions used by Lapworth and Mottram (*loc. cit.*; compare Robinson and Robinson, *loc. cit.*) for conversion of oleic into dihydroxystearic acid were slightly modified. Oleic acid (4·00 g., m. p. 13·36°), dissolved in a solution of sodium hydroxide (4·0 g.) in water (350 c.c.), gave a clear solution on dilution with water (2 l.). Potassium permanganate solution (400 c.c. of 1%) was stirred in, and oxidation proceeded at 9° for 16 minutes. After sulphur dioxide had been passed in and concentrated hydrochloric acid (30 c.c.) added, the precipitate floated on a clear liquid which was siphoned away. The precipitate, collected on a tared filter, washed well with water, and dried in high vacuum over calcium chloride for 5 days (weight constant after 2 days), weighed 4·48 g., m. p. 126—128° (theory for dihydroxystearic acid, 4·48 g., m. p. 132°), but was not homogeneous.

In order to remove the impurity, the solid was boiled for ½ hour with two lots of light petroleum (50 c.c., b. p. 100—120°) and then melted at 130—131·5°. The extracts were cooled to 30°, filtered, and the filtrate worked up as described by Lapworth and Mottram. The residue (0·202 g., m. p. 30—46°, unsaturated to permanganate) was dissolved in sodium hydroxide solution (30 c.c.). This solution was diluted to 200 c.c. and oxidised at 10° with permanganate (25 c.c. of 1% solution). The product (m. p. 110—123° after drying in a vacuum) on extraction with hot light petroleum yielded 0·05 g. of an intractable mixture, m. p. 40—75°. Some keto-hydroxy-acid may have been present (compare King, J., 1936, 1788). Even if the mixture contained 50% of stearic acid, only 0·6% of stearic acid would have been present in the oleic acid.

Polymorphism.—Oleic acid crystallises first in the form with f. p. 13·36°, and this on keeping may change into the slowly crystallising, stable form, m. p. 16·25° (17° in a capillary tube).

Kirschner (*Z. physikal. Chem.*, 1912, **70**, 759) gives m. p. 16°. These forms are quite definite and have been recorded by most observers. Lapworth, Pearson, and Mottram (*loc. cit.*) state that a specimen of original m. p. 12·04°, after some months in winter, had m. p. 17·45°, and that one of f. p. 12·8° changed to m. p. 17° after some days. An acid of original m. p. 12·04° is near the eutectic of the palmitic–oleic system, and the 17·45° observed may represent the m. p. (on the palmitic branch of the system) of a mixture containing 4—6% of saturated acid. The other specimen of f. p. 12·8° probably changed into the stable form, m. p. 17° (in a capillary tube). In the present research some inconclusive evidence has been obtained of the existence of a form of m. p. 18—19°.

FIG. 2.



Elaidic Acid.—Isomerisation of oleic acid by nitric acid and sodium nitrite (Rankoff, *Ber.*, 1931, **64**, 619) gave a solid product which on purification, finally by crystallisation from alcohol at − 5°, had m. p. 43·68° ± 0·05° (in a capillary tube, 43·5—44·5°), unchanged by crystallisation from hexane. Marsden and Rideal (*loc. cit.*) give m. p. 43·6° ± 0·2°. If impure oleic acid is used it is difficult, especially in small-scale preparations, to free the product from stearic acid. The best product obtainable from oleic acid, m. p. 13·17°, melted at 43·56°.

Elaidic acid changes very little on standing : a specimen after 6 months in a loosely-corked tube showed a fall in m. p. of only 0·05°.

Binary Systems (see Fig. 2).—Although pure oleic and pure palmitic acids crystallise readily, and the f. p.'s are within 0·03° of the m. p.'s, mixtures of the two acids crystallise slowly and the f. p. varies with the conditions. Satisfactory m. p.'s (clearing points) can usually be obtained in the Beckmann apparatus with the bath 1—2° above the temperature of the melt. On the oleic side of the system, the higher-melting form can be obtained either by seeding or by keeping the mixture just above the first m. p. for some time. Between 96% and 90% of oleic acid even the clearing points are indefinite; from 50% up to 100% of palmitic acid the crystals change gradually from a fine powder to large plates.

In the oleic–stearic system there is an indefinite portion between 98% and 93% of oleic acid, and in the elaidic–palmitic system between 74% and 66% of elaidic acid; mixtures of elaidic and stearic acid give the sharpest m. p.'s.

For the thaw-point determinations on mixtures of elaidic and palmitic acids, 0·1—0·2 g. of mixture was made for each composition. The acids were carefully melted together, the liquid stirred while cooling, and the resultant solid powdered. This powder was immediately introduced into a 1·5-mm. tube, tapped down with a rod, and the thaw-point taken as described by Rheinboldt and Kirscheisen (*loc. cit.*).

Oleic Acid–Palmitic Acid.

Oleic acid, mols. %.	M. p.		Oleic acid, mols. %.	M. p.	Oleic acid, mols. %.	M. p.
	α.	β.				
100	13·36°	16·25°	91·4°	22°	47·3	52·1°
99·0	13·08	16·05	88·3	28	40·0	54·3
98·0	12·83	—	84·3	33	28·0	57·15
96·7	12·60	15·50	78·3	38·3	12·55	60·4
96·0	12·37	15·35	67·4	44·5	3·7	62·15
95·0	12·15—12·3—16*	—	59·3	48·2	0·0	62·75
94·0	12·1, 16—19*	—	53·1	50·25		
93·2	18*	—				

Eutectic, 94—95% oleic acid, 12·1°.

* Indefinite.

Oleic Acid–Stearic Acid.

Oleic acid, mols. %.	M. p.	Oleic acid, mols. %.	M. p.	Oleic acid, mols. %.	M. p.
100	13·36°	95·2	30·2°	82·3	45·6°
98·9	13·20	92·8	34·5	72·9	51·0
97·7	13·10 and 23·8	87·1	41·6	62·5	55·5
96·45	13·10 and 27·4				

Elaidic Acid–Palmitic Acid.

Elaidic acid, mols. %.	M. p.	Elaidic acid, mols. %.	M. p.	Elaidic acid, mols. %.	M. p.	Elaidic acid, mols. %.	M. p.	Elaidic acid, mols. %.	M. p.
100	43·68°	80·0	40·15°	70·8	40·2—42·0° *	49·6	51·0°	20·15	58·75°
98·0	43·37	78·0	39·85	66·2	44·4*	46·35	52·1	10·95	60·6
94·6	42·7	75·85	39·55	57·7	48·2	39·4	54·05	2·6	62·2
89·3	42·0	74·0	39·0—40·7*	52·5	49·8	30·05	56·4	0·0	62·76
84·3	41·15								

* Indefinite.

Elaidic Acid–Palmitic Acid.

Elaidic acid, mols. %.	Thaw point.	Elaidic acid, mols. %.	Thaw point.	Elaidic acid, mols. %.	Thaw point.	Elaidic acid, mols. %.	Thaw point.	Elaidic acid, mols. %.	Thaw point.
14·3	48·0°	45·0	34·2°	50·5	35·1°	54·2	34·5°	84	33·8°
25·0	35·4	48·0	34·6	51·0	34·9	57·7	34·0	88	36·2
29·8	34·2	49·5	35·3	52·5	34·4	73	33·5	92	39·5
37·7	34·4	49·6	35·1						

Elaidic Acid–Stearic Acid.

Elaidic acid, mols. %.	M. p.	Elaidic acid, mols. %.	M. p.	Elaidic acid, mols. %.	M. p.	Elaidic acid, mols. %.	M. p.	Elaidic acid, mols. %.	M. p.
100	43·68°	88·8	45·35°	63·8	56·0°	46·4	61·0°	13·7	67·6°
98·0	43·45	84·5	47·95	56·9	58·3	41·4	62·4	0·0	69·6
93·75	43·05	76·0	52·0	50·1	60·2	29·3	64·8		

THE DYSON PERRINS LABORATORY, OXFORD UNIVERSITY.

[*Received, April 27th,* 1939.]