hot ethyl acetate, this product was thought to be either a leucoanthocyanin or the colorless anthocyanin pseudobase.

No red pigment was obtained when the cold-pressed oil employed in these studies was acid-treated.

It was considered important to check whether the presence of an anthocyanin pigment which would separate out on acid treatment was peculiar to the particular screw-pressed oil under investigation or whether other dark oils from different seed or processed under different conditions would contain similar pigmentation. Therefore two more darkly-colored screw-pressed oils, from another mill in West Texas and from a South Texas mill, and three darkly-colored commercially hydraulic-pressed oils from different mills were treated with acid in the same manner. The same red precipitate was obtained in the case of the other two screw-pressed oils but in smaller amount than the original West Texas oil. No red precipitate was obtained from any of the hydraulic-pressed oils tested. The indication implied by the results of these trials of acid-treating highly-colored oils of different histories is that the conditions of the screw-pressing operation alone are favorable to the formation of anthocyanins in the oil or to the extrusion of such pigments from the flakes during pressing. Therefore it seems highly possible that anthocyanins, their precursors, or their decomposition products contribute greatly to the dark color of "red" screw-pressed cottonseed oils which are difficult to refine and bleach.

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Binary Freezing-Point Diagrams for Alpha- and Beta-Eleostearic Acids with Each Other and with Acetamide

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BINARY freezing-point diagrams of adjacent pairs of even-numbered members of a homologous series of long-chain saturated fatty acids indicated that 1:1 molecular compounds are formed (7). However binary systems of fatty acids with one or both components unsaturated seem always to be of the simple eutectic type (7). It has previously been demonstrated by freezing-point data that acetamide forms such equimolecular compounds not only with long-chain saturated fatty acids (6) but also with monounsaturated fatty acids (8). The present report deals with the freezing-point diagrams for the binary systems between two conjugated triply-unsaturated fatty acids, alpha- and beta-eleostearic acids, and between each of these acids and acetamide.

Preparation of Materials

Alpha-eleostearic acid. About 200 g. of fresh tung oil was saponified in a 4-l. flask containing 1,200 ml. of 8% KOH in ethanol by heating at 70-80°C. until 10 minutes after the solution became clear. The solution was cooled to 50°C., diluted with 2,000 ml. of distilled water, and acidified with conc. HCl. The aqueous layer was drawn off with vacuum, leaving the crude acids, which were washed three times with 500-ml. portions of distilled water. The crude acids were then dissolved in 2,000 ml. of Skellysolve B,[2] transferred to a separatory funnel for the removal of the water layer, and the solution filtered through absorbent cotton. After cooling the solution to —30°C. the precipitated acids were filtered off and redissolved in 400-500 ml. of acetone. The acetone solution was slowly cooled, and the first 10-15 g. of acids which precipitated were filtered off and discarded. The solution was cooled to 0°, held there for 1-2 hrs. and the precipitate filtered off. This second precipitate was recrystallized twice more from acetone, using just enough acetone to dissolve the acids at 25°C. and then removing the precipitate formed on cooling the solution to 0°C. The precipitate obtained from the third recrystallization was dried for 20 minutes under vacuum and then dissolved in enough Skellysolve F to give a 10% solution, which was filtered at room temperature with vacuum through a Buchner funnel. This filtrate was cooled to —30°C., and the crystals were filtered off and dried under vacuum at room temperature. Throughout all of the above operations solutions were kept blanketed with nitrogen, and filtrations were carried out rapidly to minimize exposure to air. The product, about 50 g., was stored in

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[2] Mention of trade products does not imply that they are endorsed or recommended by the Department of Agriculture over similar products not mentioned.

TABLE I

Binary Freezing-Point Data

Mole % acetamide	Freezing point, °C. Stable	Freezing point, °C. Metastable
Acetamide—alpha-eleostearic acid system		
0.00	48.4	
12.72	45.9	
22.47	44.4	
30.83	42.6	
39.79	40.0	
(40.4)[a]	(40.0)[a]	
45.19	48.5	38.5
(47.4)[a]		(37.7)[a]
50.65	56.6	43.7
54.95	61.6	49.7
57.88	64.9	53.5
70.21	74.1	63.5
81.15	77.6	67.3
90.00	79.0	68.5
100.00	79.7	69.5
Acetamide—beta-eleostearic acid system		
0.00	70.5	
8.04	69.2	
13.08	68.5	
22.33	66.9	
26.39	66.3	
30.56	65.5	
37.78	63.8	
41.03	63.0	
49.50	60.2	
(52.5)[a]	(59.1)[a]	
58.11	65.3	56.9
(59.7)[a]		(56.2)[a]
70.14	74.8	64.1
82.91	78.3	68.1
90.18	79.2	68.7
100.00	79.7	69.5

Alpha-eleostearic acid—beta-eleostearic acid system

Mole % beta acid	Freezing point, °C.
0.00	48.4
10.11	46.3
14.65	45.3
18.58	44.6
(20.0)[a]	(43.9)[a]
23.04	46.1
29.55	50.2
35.33	52.8
37.26	53.9
43.41	56.2
49.93	58.6
60.39	61.8
66.16	63.2
72.51	64.9
80.17	66.6
89.46	68.4
100.00	70.5

[a] Values in parentheses are eutectic temperatures and compositions obtained by graphical extrapolation.

evacuated ampules at —50° to —70°C. and, when used, had an estimated purity of 100% by the spectrophotometric method.

Beta-eleostearic acid. Three 200-g. batches of tung oil that had been isomerized to the beta form with iodine were saponified, and the crude acids were recovered and crystallized from Skellysolve B as described for alpha-eleostearic acid. The acids removed from the Skellysolve B at —30°C. were dissolved in the minimum amount of acetone at 25°C. Three approximately equal crops of crystals were obtained by chilling this solution down to —40°C. These were subjected to "double withdrawal" fractional recrystallization (16) from acetone at 0°C. The series of fractions obtained were analyzed spectrophotometrically for beta-eleostearic acid, and those of 95-97% purity were combined and recrystallized once more from acetone, rejecting the first crop of crystals. The product so obtained was dried under vacuum and dissolved to give a 10% solution in Skellysolve F, which was clarified by filtration at room temperature, and cooled to —40°C. yielding about 30 g. of crystals. The same precautions against exposure to air were taken as for the alpha acid, and whenever coarse crystals were formed, decantation was used in preference to filtration. The vacuum-dried product was stored under vacuum at —50°C. and, when used, had an estimated purity of about 99%.

Acetamide. The acetamide was the purest Eastman Kodak Company product dried in vacuum over P_2O_5.

Freezing-Point Determinations

The freezing points were determined by the static method. For each composition, weighed amounts of the components were sealed in vacuum in a glass tube after flushing twice with nitrogen. A glass bead was included to ensure efficient stirring as the sample tubes were turned end-over-end in a constant temperature bath. Two temperatures a few tenths of a degree apart were found, one at which the last crystals just disappeared and the other at which a few crystals remained undissolved after prolonged agitation. The freezing point was taken as the mean of these two temperatures, corrected for both thermometer calibration and emergent stem, and had a probable accuracy of at least ± 0.2°C. The freezing points were redetermined on a number of the same tubes after standing at room temperature for two weeks and were found to be unchanged.

Results and Discussion

The freezing-point data are given in Table I and are represented graphically in Figures 1 and 2. All three systems were of the simple eutectic type. For the acetamide side of the diagrams two freezing points were obtainable. The solid lines represent the temperatures at which the various liquid compositions are in equilibrium with the stable polymorphic form of acetamide, and the dotted lines represent the corresponding metastable equilibria between the liquid and either the respective acids or the low-melting form of acetamide, as the case may be.

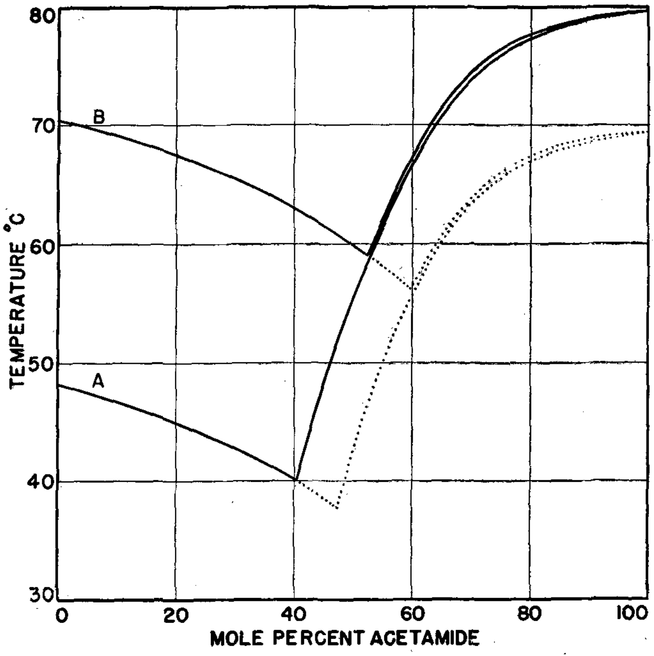

Fig. 1. Binary freezing-point diagram for acetamide with: A, alpha-eleostearic acid; B, beta-eleostearic acid. Dotted curves represent metastable equilibria.

The freezing points could be made to fall on the solid or the dotted lines at will by proper manipulation of the temperature. Since the stable form of acetamide was used in making up the mixtures, the higher freezing point was always obtained on the initial melting of the samples, but after heating 10 to 15 degrees above this temperature the freezing points invariably fell on the lower (dotted) curves. In order to obtain the higher freezing point again it was then found necessary to shock-chill the molten sample in an alcohol-dry ice mixture and heat slowly to the melting point.

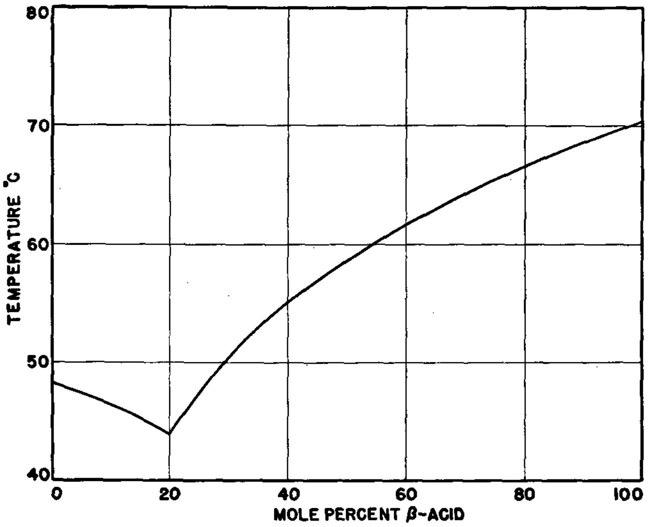

FIG. 2. Binary freezing-point diagram for alpha-eleostearic acid with beta-eleostearic acid.

Each acetamide system thus exhibits two eutectic temperatures and compositions, one stable and the other metastable. For mixtures between these two compositions the acid can be caused to crystallize from the liquid which is supersaturated with respect to the stable form of acetamide.

The fact that alpha- and beta-eleostearic acids form simple binary eutectic systems with each other and with acetamide suggests that the tendency to form crystalline molecular compounds decreases with the degree of unsaturation of the fatty acid. For example, stearic acid forms molecular compounds with other saturated fatty acids; *e.g.*, palmitic (2, 3) or arachidic (11), but not with mono-unsaturated acids; *e.g.*, oleic or elaidic acids (14). Similarly binary systems of unsaturated acids, such as the oleic-elaidic (4) and the oleic-linoleic acid (15) systems, fail to show compound formation. Acetamide seems to have a stronger tendency to form crystalline molecular compounds with the acids for such compounds are formed not only with saturated fatty acids (6) but also with mono-unsaturated fatty acids (8). On the basis of the present data it would be concluded that this tendency is not great enough to cause compound formation with a triply-unsaturated acid.

For the binary system between alpha- and beta-eleostearic acids straight lines are obtained when the logarithm of the mole fraction of either acid is plotted against the reciprocal of the absolute freezing temperature in the usual way. The heats of fusion, ΔH_f, calculated from the slopes of these lines are 10.5 kcal. per mole for alpha-eleostearic acid and 13.2 for beta-eleostearic acid. Similar calculations were made based on all of the available freezing-point data in the literature for binary systems between pairs of C_{18} fatty acids of different degrees of unsaturation (4, 14, 15). In cases where straight lines were not obtained, the slope of the curve near the 100% mole fraction was used. The calculated values of ΔH_f so obtained, excluding those for which the data were erratic or otherwise inadequate, are listed below with the second component of the binary system in question being represented by the following symbols: *(a)* stearic acid, *(b)* oleic acid, *(c)* elaidic acid, *(d)* linoleic acid, *(e)* linolenic acid, *(f)* alpha-eleostearic acid, and *(g)* beta-eleostearic acid.

Stearic acid	16.8 kcal./mole *(c)*
Oleic acid (unstable) m.p. 13.5°C.	11.0 *(a)*, 10.1 *(d)*, 9.8 *(e)*
Oleic acid (stable) m.p. 16.3°C.	8.3 *(d)*, 8.8 *(e)*
Elaidic acid	17.5 *(a)*, 16.0 *(b)*
Linoleic acid	12.3 *(b)*
Linolenic acid	9.4 *(b)*
Alpha-eleostearic acid	10.5 *(g)*
Beta-eleostearic acid	13.2 *(f)*

Although the data are scant and the calculation of ΔH_f involves a number of simplifying assumptions, it is interesting to note a few consistent trends. In the few cases in the literature where ΔH_f values have been reported for both the cis and trans modifications of mono-unsaturated compounds, the value for the trans form is higher than that for the cis form. This is true, for example, for betachlorocrotonic acid (13), 2-butene (5, 12, 18), and 2-pentene (17). It is therefore not surprising that ΔH_f for elaidic acid is higher than for its cis modification, oleic acid. The fact that ΔH_f for beta-eleostearic acid is larger than for alpha-eleostearic acid is therefore in harmony with the fact (1, 9) that the beta acid is trans-trans-trans and the alpha acid is cis-trans-trans. It can be seen from molecular models that of the two, the trans-trans-trans molecule would more nearly have the elongated shape of the saturated acid and would therefore be comparable to elaidic as related to oleic acid. If the length of the molecule were to be considered one of the pertinent factors involved, it might be expected on the basis of molecular models that ΔH_f for the completely trans modification of di- and tri-unsaturated fatty acids would be considerably greater than for any of the various modifications having one or more cis linkages. The structure of the linoleic and linolenic acids used in the determination of the above heat of fusion data is unfortunately not known. Considering their method of preparation however, they are in all probability not of the completely trans type (10) and they would therefore be comparable to oleic rather than elaidic acid. Comparison of the heats of fusion to stearic, oleic, linoleic, and linolenic acids shows that the values alternate as the number of double bonds increases from zero to three. Additional data will be necessary to establish the significance of this trend. The same is true of the observation that both modifications of the fully conjugated, triply-unsaturated eleostearic acid studied have higher heats of fusion than the nonconjugated, triply-unsaturated linolenic acid.

Summary

Complete binary freezing-point data have been obtained for each of the polymorphic modifications of acetamide with alpha-eleostearic acid and beta-eleostearic acid and for alpha-eleostearic acid with beta-eleostearic acid. In the light of previously published data the fact that all of these systems are of the simple eutectic type suggests that the tendency of the long-chain fatty acids to form 1:1 molecular compounds with acetamide and with each other decreases as the degree of unsaturation increases.

The fact that the heat of fusion of beta-eleostearic acid, 13.2 kcal. per mole, calculated from the freezing-point data is greater than that for the alpha acid, 10.5 kcal. per mole, corroborates the conclusion that the beta acid is the trans-trans-trans modification.

Calculations of heats of fusion have been made, based upon all the available freezing-point data in the literature for binary systems between pairs of C_{18} fatty acids of different degrees of unsaturation, and certain trends have been dsicussed.

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Flavor Reversion in Soybean Oil. VII. The Autoxidation of Isolinoleic Acid[1,2]

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THE problem of flavor reversion in fats and oils has been investigated over a period of years by many workers (1, 2), and a number of theories have been proposed to account for the development of these objectionable flavors and odors. These are generally based upon either the linolenic and polyunsaturated acid content, the non-glyceride fraction, or various nitrogenous constituents.

Previous investigators (3-7) have isolated from the deodorization condensate of reverted oils a number of saturated and unsaturated carbonyl compounds which contribute to the reversion flavors in hydrogenated and unhydrogenated materials. Recent organoleptic studies (8) have supported polyunsaturated acids as precursors of reversion products and, as suggested by Lemon (9-12) in an investigation of the hydrogenation of both linseed and perilla oils, isolinoleic acid, formed during the course of the hydrogenation, may be responsible for the presence of reversion flavors. Isolinoleic acid, by definition (13), may be a mixture of isomeric octadecadienoic acids which does not give a conjugated double bond system on isomerization with alkaline ethylene glycol as determined by the spectrophotometric procedure (14). The purpose of this work was to prepare isolinoleic acid by the partial catalytic hydrogenation of methyl linolenate and to investigate the products from the autoxidation of this compound in order to gain some insight into the reversion process and to determine, if possible, the validity of the proposal that isolinoleic acid may be a precursor of reversion products.

Experimental

Preparation of Methyl Isolinoleate. Methyl isolinoleate was prepared by the partial catalytic hydrogenation of a highly purified methyl linolenate (I.V. 260.0 ± 0.5) obtained from linseed oil linolenic acid which had been purified by bromination-debromination procedures according to the method of Frankel and Brown (15). The hydrogenation was conducted, using a Parr Medium Pressure Series 4,500 Hydrogenation Unit with limited stirring, 2.0% Harshaw Nickel Chip Catalyst Ni-0501f (0.5% nickel), a temperature of 175° ± 5°C. and a pressure of 45 ± 5 pounds. The hydrogenation was discontinued when spectroscopic examination of the reaction mixture showed negligible methyl linolenate present.

The crude methyl isolinoleate was concentrated by low temperature fractional crystallization from methanol according to the method of Rebello and Daubert (13). After removal of the solvent from the —75°C. filtrate, the residual ester was molecularly distilled to give a fraction (2 g., I.V. 170.4 ± 1.0, b.p. 55° — 7°C./3 — 4 microns pressure) which was designated as S.N. 1-2H-99. The composition of this material (theoretical I.V. 172.4), as summarized in Table I, was obtained through determination of the Iodine Value for total unsaturation, linoleic and linolenic acids by spectrophotometric procedures (14) and using methods of calculation (13) for the isolinoleic acid and combined oleic and isooleic acids.

TABLE I

Analysis of Methyl Isolinoleate (S.N. 1-2H-99)

Constituent	Percentage Composition
Methyl isolinoleate	94.8 ± 1.5
Methyl linolenate	0.5 ± 0.1
Methyl linoleate	1.5 ± 0.1
Methyl oleate and isoleate	3.2 ± 1.7

Reversion and Deodorization. The reversion and deodorization of the methyl isolinoleate was carried out, using the small scale, all-glass laboratory deodorizer previously described (4), by first heating over a 30-minute period to 200°C. under normal atmospheric conditions and then removing the reversion

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