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UREA COMPLEXES: PREPARATION AND PURIFICATION OF ELAIDIC ACID, AND ITS GLYCERIDE ESTERS

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The technique of urea complex formation was first employed by Schlenk and Holman[1] for the separation of saturated fatty acids from the unsaturated. They also demonstrated easy urea complex formation by elaidic acid in comparison to its corresponding *cis* isomer, oleic acid. Later, these procedures were used by other workers.[2-6] Khan[7-8] purified the unsaturated fatty acids with the ingenious application of multiple steps of fractionation through urea complexes. In a current investigation,[9] he has shown the reasons for preferential precipitation of elaidic acid over *cis*-oleic, as urea complex crystals.

In the present investigation, the conditions for elaidinization have been described for larger yields of elaidic acid. A new method has been developed that involves generation of nitrogen dioxide gas right inside the larger quantities of fatty acids by addition of potassium nitrite and nitric acid together. Such procedures have been found advantageous in many respects: (a) elimination of separate arrangements for generating nitrogen dioxide, (b) shorter duration of isomerization and (c) preparation of larger amounts of elaidic acid. The instrumental set-up is quite simple and the starting materials need not be very pure in such procedures.

The prospects of separating elaidic acid from the standard mixtures have now been established through urea complex formation. Such data indicated the trends of fractionation through urea complexes and subsequent isolation of particular fatty acid. The experiments showed that elaidic acid cannot be well separated from the acids, but when oleic acid has been added to such mixture, elaidic acid could be isolated together with oleic acid. The oleic acid with more affinity for elaidic helps urea complex formation away from the saturated acids. Such processes have been termed "amplified urea complex segregation", because a third agent accentuates the urea complex formation. The knowledge of amplified distillation[10] will clear the picture.[11] It is now obvious that in the presence of oleic acid, elaidic acid can be separated from mixtures containing the saturates through urea complex formation. Elaidic acid was thus prepared in a pure state from the isomerized reaction mixtures of original sesame oil acids. Elaidinization converts linoleic and linolenic acids into polymers.[12] Consequently, their presence in the starting materials within limits, does not hamper purification of elaidic acid. These findings allow preparation of elaidic acid in larger quantities from three grades of starting materials: (a) saturate-free sesame oil acids, (b) saturate-free sesame oil acids containing 6-14% linoleic acid and (c) sesame oil acids containing 3-9% linoleic acid. The saturate-free sample does not contain any detectable amounts of the saturated acids, while the (c) sample contains the saturates with the unsaturates cut down, as noted.

The recovery of elaidic acid from (a) and(b) products was 15.0% and 22.0%, respectively, in the carefully conducted experiments. The isomerized (c) acids, through amplified urea complex segregation, yielded 11.0% pure elaidic acid. One advantage with (c) is that it involved one clear-cut series of procedures at one stretch. The urea complex formation (Tables 1-3) has been found in all these experiments to remove elaidic acid more effectively than simple crystallization methods. The data in Table 1 relate to the points that elaidic acid can be concentrated by urea complexes and then isolated in pure form

TABLE 1.—STANDARD MIXTURES AND SEPARATION. ELAIDIC: OLEIC ACIDS=1:1.

Fractions	% of original mixture	Iodine number	*Trans* contents (I.R.) % in fractions
1	18.0	88.9	81.0
2	24.7	89.4	72.5
3	22.0	89.6	44.1
4	20.0	89.4	36.3
5	15.3	89.8	12.9

TABLE 2.—STANDARD MIXTURES AND SEPARATION. STEARIC : ELAIDIC ACIDS=1:2.

Fractions	% of original mixture	Iodine number	*Trans* contents (I.R.) % in fractions
1	15.0	33.5	29.5
2	37.0	58.0	61.3
3	32.0	78.2	80.4
4	16.0	77.5	76.5

Table 3.—Standard Mixtures and Separation. Stearic: Elaidic: Oleic Acids=1:1:1.

Fractions	% of original mixture	Iodine number	*Trans* contents (I.R.) % in fractions	% pure elaidic acid, recovered
I	18.0	31.2	12.0	—
II	24.0	40.7	18.0	—
III	11.4	58.9	34.0	—
IV	10.7	78.6	45.0	—
V	25.6	86.2	51.0	98.5%
VI	10.3	88.5	42.0	100.0%

through crystallization in acetone (25% solution) due to differences in solubility. Table 2 indicates that elaidic acid cannot be separated from stearic, because of their similar properties and solubilities in acetone. Table 3 shows that elaidic and oleic acids precipitate out together in the last fractions as urea complexes, free of stearic acid as the unsaturation(I.N.) provides the clues. The fifth and sixth fractions (Table 3) yielded on crystallization 98.5 and 100.0% elaidic acid. These results on the fractionations of elaidic acid helped the development of the procedures, "amplified urea complex segregation" for facilitating isolation of elaidic acid from the saturates by addition of a known unsaturated acid.

The tri-elaidin from pure elaidic acid and other types of glycerides with different contents of elaidic acid were also obtained by interesterification with refined sesame oil.

Experimental

Preparation of Saturate-Free Sesame Oil Acids.—Sesame oil acids(500 g.) in the presence of 600 ml. methyl alcohol were freed of the saturates in three steps, using (a) 200 g., (b) 100 g. and (c) 100 g. of urea in succession. The filtrate from the last step was slowly cooled in a bottle-cooler, in order to obtain a crop of urea complex crystals (about 40-50 g.). After quick removal of crystals under cold conditions, the filtrate was freed of the solvent. The yields of residual saturate-free acids were in the range of 65-72% in several batches of preparation.

The acid samples were esterified to methyl esters and then subjected to acetone-permanganate oxidation.[13] The oxidized esters were taken up in petroleum ether, (b.p., 80-90°C., freed of the unsaturates by the methods of Cooper and Melville[14]) and passed through a column of alumina (2.0 cm. × 60.0 cm.). The alumina was prepared by treating with 2N hydrochloric acid, washing with water until neutral and finally with alcohol. The acitivation was done at 150°C. for 6 hrs. The column eluants, first with pretoleum ether and then with petroleum ether containing 1% ethyl ether, did not give any neutral saturated fatty acid esters, palmitic or stearic in origin. This means that the above samples contained only unsaturated acids that got cleaved by permanganate oxidation into lower monobasic and dibasic fragments absorbed strongly by the alumina column.

Preparation of Saturate-Free Sesame Oil Acids containing 6-14% Linoleic Acid.—The above saturate-free sesame-oil acids in alcohol were again subjected to urea complex formation with an additional amount of 300 ml. methyl alcohol in one step. About 750 g. of urea in this boiled mixture was thoroughly stirred with strong glass rod for half an hour. The urea complexes were freed of adhering liquids and washed thoroughly with mixed (ether+petroleum, 1:1) solvent. The complexes gave the required acids with yields of 42-54% of the original sesame oil acids. The saturate-contents were checked as in the foregoing section. The linoleic acid contents were roughly determined by the bromide number methods with the relevant correction.[15]

Preparation of Sesame Oil Acids containing 3-9% Linoleic Acid.—Five hundred grams of sesame oil acids and 1 litre of methyl alcohol were boiled together and thoroughly stirred with 1200 g. of urea for over 30 minutes. The rest of the procedures were same as in the last preparation. The yields were in the range, 45-57%. The linoleic acid contents were checked through the procedures[15] mentioned above.

Preparation of Elaidic Acid in Large Quantities.—Several types of elaidinization reactions were carried out. (i) Nitrogen oxide gases were passed through 500 g. of crude sesame oil acids contained in a cylinder and agitated by air without any success. (ii) In another attempt, the oxides of nitrogen were mixed with oxygen in a tower filled with glass wool and were then passed through fatty acids with slightly better isomerizaton. (iii) The solid surface was considered necessary for *cis-trans* interconversion. However, the added pumice stone and powder gave unexpectedly low isomerization. (iv) The last and successful attempt was to use potassium nitrite and other salts formed from it as solid surfaces. Hence, the successful process of preparation was accomplished after a series of reactions by generating nitrogen oxides in the presence of oxygen with all reagents manipulated properly in the medium of fatty acids, as noted below.

Five hundred grams of sesame oil acids of grades (a) to (c) were successively placed in a cylindrical tube (2½″×20″). Both potassium nitrite (140 g.) and concentrated nitric acid (60 ml.) were added in proportions to leave a slight excess of potassium nitrite in the mixture. The time of addition was between 10 to 15 min. Dried air was bubbled through the mixture during the addition and for 12 hours thereafter. In the last stages, precaution was taken not to stir the accumulated salts in the bottom of the tube too vigorously . The reaction mixture was left open to air in layers in glass trays for another 12 hours in a hood, and then washed into a 5-litre Florence flask with hot water. The wash liquid appearing as the bottom layer was siphoned off. After three washings, the residue was dissolved in petroleum ether (25-30% solution), left over anhydrous sodium sulphate for 24 hours and then filtered. One crystallization from the above solution, followed by several crystallizations (about 4 times) from acetone solution (30-40%) below 0°C., gave white crystalline products (yields in the range of 11-22%). Some solid acids were also recovered from the filtrates. The elaidic acid samples except from (c) batch were distilled under vacuum and subjected to physical and chemical analysis. Elaidic acid in one characteristic sample had the following properties: I.N. (iodine number), 89.4; n.e. (neutral equivalent), 282.4; nitrogen, 0.0%; C, 76.36; H, 11.82. Calculated for $C_{18}H_{34}O_2$: C, 76.59; H,12.05. Usually, elaidic acid samples had I.N. in the range of 88.9-89.6 and nitrogen 0.0%, indicating reasonable degrees of purity.

From the above crystallizations, the final filtrates were freed from the solvent. The unreacted oleic acid plus some elaidic acid were recovered from the filtrate residues of all batches through urea-inclusion compounds, since the polymers were unaffected by such treatment. The monomer portion of these residual acids was separated in seven fractions. One thousand grams of the acids (approx.) from different batches of experiments already described were mixed with 100 ml. of methyl alcohol and added to the boiling methyl alcohol (1 litre) containing 300 g. of urea. The mixture was well stirred during addition and overnight. The crystals were filtered, washed with ethyl ether and freed of the solvent. Six more fractions of crystals of urea-inclusion compounds were obtained by adding first II, 400 g. of urea to the above filtrate mildly boiling, and then III, 500; IV, 500; V, 400; VII, 200 g. of urea to the successive boiling filtrates from the similar crystallization processes in each fraction. The fractions of crystals were combined, freed from the urea by water, saponified and hydrolyzed in order to reconvert certain esterified portions to free acids. These acids were recycled for isomerization.

The (c) batches of sesame oil acids, containing saturated and 3-9% lionleic acids, gave fractions of elaidic acid contaminated by saturated acids through the above procedures of crystallization. A novel approach somewhat different from previous procedures,[1-5] has been made to isolate elaidic acid from the saturates by obtaining data on the degrees of separation in the standard mixtures during urea complex formation: elaidic acid (150 g.) and oleic acid (150 g.)-Table 1; elaidic acid (200 g.) and stearic acid (100 g.)-Table 2; elaidic acid (100 g.), stearic acid (100 g.) and oleic acid (100 g.)-Table 3.

The alcohol and urea used in each fractionation are: first mixtures, 600 ml. methyl alcohol; (1) 300, (2) 350, (3) 250, (4) 250 and (5) 200 g. of urea; second mixtures, 800 ml. methyl alcohol; (1) 250, (2) 450, (3) 350 and (4) 250 g. of urea; third mixtures, 500 ml. methyl alcohol; I, 200; II, 150; III, 200; IV, 200; V, 250; and VI, 200 g. of urea. Chemical and physical analyses were carried out for characterizing the fractions. The unsaturate-free stearic acid was obtained by only one course of hydrogenation, as described by the method of Khan.[16]

The valuable information available from the elaidic acid fractionations through urea complexes gave the clues to the new methods of elaidic acid separation. This has been accomplished by the addition of the unsaturated acids to the samples of elaidic acid contaminated with the saturates. Elaidic acid with saturates from (c) batch was mixed with oleic acid in the same amounts as those of elaidic acid in the sample and subjected to urea complex formation with successful results. The greater portion of elaidic acid came together with oleic acid fraction. Since 300 g. of the mixture was taken, the other reagents did not vary from the third standard mixture. The added substance, oleic acid, was removed in the filtrate by crystallization, while elaidic acid crystals easily separated out. This process may be given the name "amplified urea complex segregation" (AUS).

Preparation of Tri-elaidin and Mixed Trans-glycerides.—Elaidic acid (300 g.) was esterified with 95% glycerol (34 g.) in the presence of $SnCl_2,2H_2O$ (0.5 g.) at 200°C. under 20 mm. of mercury for 6 hours. Such reaction was carried out in 0.5-litre 3-necked flask with standard joints and the outlet for a long stem thermometer (standard joint) of 0°-250°C. The low pressure was obtained by means of a vacuum pump with two traps, one 1-litre filtering flask in chipped ice and the other in the freezing mixture. A glass stirrer with a ground glass vacuum seal and a 20/40 joint was used for agitation. The reaction mixtures were refined according to the A.O.C.S. official methods[17] and tri-elaidin was recovered (yield, approx. 95-97%).

The refined[17] sesame oil was mixed with tri-elaidin in the proportions 65:35, and heated with sodium ethoxide (0.05%) as catalyst for interesterification at 180-200°C. under the atmosphere of hydrogen for 4 hours. The mixtures were then well washed and dried as a 30% solution in petroleum ether over anhydrous sodium sulphate. The mixed *trans* glyceride with about 30-35% isolated *trans* double bonds (10.36 μ) was obtained. Elaidic acid of 70-78% purity was also used for the same preparation of mixed *trans* glycerides with due allowance for the other acids present. In the case of preparing the synthetic triglyceride with about 10% isolated *trans* double bonds, the proportions of refined sesame oil and elaidic acid were 90:10. There was no difference in rest of the procedures. Iodine number (I.N.) was determined by Hanus method.[18]

Spectrophotometric Analysis.—Infrared absorption analysis was performed with the Perkin Elmer instrument, Model 21. The base line technique in conjunction with the absorption of pure elaidic acid, and tri-elaidin was used to determine the percentage *trans* contents.[19] The test wave lengths were 9.0-12.0 (particularly, 10.36 μ) and the solvent used was carbon disulphide. The infrared analyses were carried out under the courtesy of the Department of Chemistry, University of Minnesota, Minneapolis, Minnesota, U.S.A.

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