dure to commercial samples, two retained samples which had been analyzed by a different procedure (17) were run again. The results obtained are presented in Table III. The data show that although

TABLE III

Effect of Aging on Commercial Triphosphate

Sample	Method	Date of Analysis	% Na_2HPO_4	% $Na_4P_2O_7$	% $Na_5P_3O_{10}$	% $Na_3P_3O_9$
I	(a)	1952	0.40	3.7	95.4	0.63
	(b)	1956	0.55	14.4	82.9	0.56
	(a)	1956	0.79	14.2	84.4	0.25
II	(a)	1952	0.26	4.5	90.0	3.50
	(b)	1956	0.73	15.0	80.4	1.22
	(a)	1956	0.95	17.2	80.8	0.75

(a) See reference (17).
(b) Present chromatographic method.

only minor changes took place in the ortho- and trimetaphosphate content, approximately 10% of the triphosphate was converted to pyrophosphate in three and one-half years of standing in closed containers. The change was confirmed by re-running the samples by the same method used in 1952 (17). The data clearly indicate that in commercial triphosphate a direct conversion of triphosphate to pyrophosphate takes place at room temperature.

Pyrophosphate alone can be determined in about four hours elapsed time and all four components in about eight hours.

Application to Detergents. This method can be applied to triphosphate built synthetic detergents if an alcohol separation is used to remove the alcohol-insoluble portion (17). If the original sample is chosen to contain 2.5 g. of phosphate, the ion exchange procedure can be used as described except for changing the "sample weight" in the calculations. If a large portion of the triphosphate has hydrolyzed to pyrophosphate, it may be necessary to change the dilution of these fractions to get maximum sensitivity.

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[Received September 25, 1956]

Purification of Long-Chain Saturated Fatty Acids by Recrystallization of Their Molecular Compounds with Acetamide

FRANK C. MAGNE, ROBERT R. MOD, and EVALD L. SKAU, Southern Regional Research Laboratory,[1] New Orleans, Louisiana

IT HAS RECENTLY been shown by means of binary freezing point diagrams that acetamide forms molecular compounds of the general formula $RCOOH \cdot H_2NCOCH_3$ with long-chain saturated and mono-unsaturated fatty acids (2, 3, 4, 5, 6). It has now been found that the molecular compounds of the saturated fatty acids can be purified by recrystallization from concentrated solutions in suitable organic solvents and that the acids, freed from their homologs, can be regenerated by extraction of the acetamide with water.

Materials

Stearic Acid I, f.p. 68.5°C.; Palmitic Acid I, f.p. 61.4°C.; Myristic Acid I, f.p. 53.3°C.; and Lauric Acid I, f.p. 43.2°C. were Eastman[2] products. Stearic Acid II and Myristic Acid II were commercial grades obtained from Armour and Company. The former was Neo-Fat 1-65 (f.p. 66.8°C., iodine value 2.9), having the approximate composition of 90% stearic acid, 6% palmitic acid, and 4% of oleic acid. The latter was Neo-Fat 13 (f.p. 49.9°C., iodine value 2.0), purported to consist approximately of 90% myristic acid, 4% lauric acid, 4% palmitic acid, and 2% unsaturated acids. Reagent grade acetamide was dried in a vacuum desiccator over phosphorus pentoxide. The acetone was purified by treating with potassium permanganate, drying over anhydrous potassium carbonate, and distilling. Freezing points were obtained by the thermostatic, sealed tube method (2).

Results and Discussion

Equimolecular proportions of acetamide and fatty acid, based on the neutralization equivalent of the original acid, were dissolved by warming with an organic solvent in a centrifugal filtration tube (9). The tube was then assembled immediately to prevent evaporation and allowed to cool slowly to about 25°C. or, if necessary to obtain a satisfactory yield of crystals, to 0°C. The mother liquor was then separated from the crystals by exhaustive centrifugation at about 225 times gravity. Fresh solvent was added to the crystals without removing them from the container, and the process was repeated for two more recrystallizations. The resulting crystals of the acid-acetamide molecular compound were stirred with successive portions of hot water to extract the major part of the acetamide. After cooling, the solidified

[1] One of the laboratories of the Southern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture.

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

TABLE I
Summary of Purification Experiments

Acid sample	Original f.p.	Solvent	Solute-solvent ratio [a]	Times recryst.	Yield [b]	F.p. regenerated acid		Setting pt. previously reported [c]
						Crude	Recryst.	
	°C.				%	°C.	°C.	°C.
Stearic I	68.5	Benzene	1/1	3	60	69.06	69.36	69.29
Stearic II	66.8	Benzene	3/2	1		68.8		
				2		69.0		
				3	64 [d]	68.7	69.31	69.29
Palmitic I	61.5	Benzene	3/2	3	41	62.1	62.54	62.45
Myristic I	53.3	Acetone	2/1	3	22	......	53.90	53.85
Myristic II	49.9	Acetone	5/2	3	35	53.78	53.90	53.85
Lauric I	43.2	Acetone	3/1	3	34	43.22	43.82	43.77

[a] By weight.
[b] Based on amount of desired acid in original sample.
[c] Previously reported setting point values (2) obtained on samples of fatty acids purified by methyl ester fractionation method.
[d] Over-all yield; the yields for the individual recrystallizations were 71, 95, and 95%, respectively.

mass was transferred to a continuous liquid-liquid extractor and extracted with hot water to remove the rest of the acetamide. The apparatus used consisted essentially of an arrangement for refluxing water so that the condensate dripped down through the molten acid layer in a tube hung in the hot water vapor and equipped with a constant level syphon to control the liquid levels. The solidified acid was ground up and dried *in vacuo* over phosphorus pentoxide before determining its freezing point. Any residual acetamide was then removed by recrystallization from acetone, again using a centrifugal filtration tube, and the freezing point of the fatty acid was again determined.

The choice of a crystallization solvent is rather critical since the acid-acetamide compounds dissociate extensively into acid and acetamide molecules when melted or in solution (2) and because the solubility characteristics of acetamide differ greatly from those of the fatty acids. Furthermore the binary freezing point diagrams of the acids with acetamide show that an equimolecular mixture of either myristic or lauric acid with acetamide when cooled from the molten state becomes supersaturated first with respect to the acetamide (2). If sufficiently supercooled however, crystals of the molecular compound can be caused to separate. Such supercooling is favored by heating the melt to a temperature 10 to 15°C. above the melting point before dissolving to destroy the crystal nuclei of the acetamide.

Since benzene shows a low solubility for acetamide, its use as a crystallization solvent favors the separation of acetamide so that in the lauric and myristic acid systems the acetamide crystallizes before the molecular compound starts to separate. Anhydrous acetone or a one-to-one benzene-acetone mixture by volume were found to be satisfactory solvents for the recrystallization of the molecular compounds of these two acids. The presence of an appreciable amount of water in the acetone must be avoided since it tends to cause the fatty acid to crystallize first.

When acetone is used as the crystallization solvent for the palmitic or stearic acid compounds, the acids tend to crystallize first because of the lower solubility ratio of these acids with respect to acetamide at the crystallization temperature. Benzene however proved to be a satisfactory solvent in these cases, and good results were also obtainable with a two-to-one mixture of benzene and acetone by volume.

Since for all the systems investigated crystallization must be carried out from relatively concentrated solutions in order to obtain the crystals of the molecular compound, centrifugation was used in the separation of the crystals from the mother liquor. No solvent or solvent mixture was found which would permit crystallization in good yield from dilute solution. Therefore the method does not lend itself to the use of filtration for the separation of the crystals.

Table I summarizes the results obtained in the purification of a number of reagent and commercial grades of acids, showing the original freezing point, the solvent used, the solute-solvent ratio, the yield, and the freezing point of the regenerated acid before and after a final recrystallization from acetone to remove any residual acetamide. The last column shows the setting points previously reported (2) obtained by the Francis and Collins method (1) on samples of the fatty acids purified by the fractional distillation of their methyl esters through a Podbielniak column. It was found that the thermostatic method used in the present investigation for determination of the freezing points gave values about 0.10°C. higher than those by the Francis and Collins method. Comparison of the freezing point data in the last two columns of Table I indicates that the final acids obtained after three recrystallizations of the molecular compounds were very pure. In the case of the commercial Stearic Acid II, aliquot portions of the crystals were removed after each crystallization step and the freezing point of the regenerated acid was determined so as to follow the course of the purification.

The recrystallized fatty acid-acetamide compounds should be particularly useful in the preparation of highly pure fatty acid amides, using the method of Roe *et al.* (7, 8), which involves heating the fatty acid with acetamide. In this procedure the product is usually contaminated with amides of the homologous acids as a result of impurities in the original fatty acid, and subsequent purification is very difficult. By heating the recrystallized fatty acid-acetamide compounds with an excess of acetamide, the fatty acid amides obtained will be in a pure form free from homologs.

Summary

A method is described for the purification of lauric, myristic, palmitic, and stearic acid, based upon the recrystallization of the fatty acid-acetamide molecular compounds and subsequent regeneration of the acid by extraction of the acetamide with water. The choice of the crystallization solvent is rather critical because of the marked difference in the solubility characteristics of the acetamide and the fatty acids. For the purification of lauric and myristic acids acetone or a one-to-one benzene-acetone mixture by vol-

ume proved to be the best solvents, and for palmitic and stearic acids the most satisfactory results were obtained with either benzene or a two-to-one mixture by volume of benzene and acetone. Since the molecular compounds must be crystallized from concentrated solutions, the separation of the crystals from the mother liquor must be made by centrifugation. The recrystallized fatty acid-acetamide compounds provide a convenient intermediate for the preparation of pure acid amides free from homologs.

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[Received October 29, 1956]

Some Recently Discovered Constituents of Animal Fats[1]

L. HARTMAN, Fats Research Laboratory, Department of Scientific and Industrial Research, Wellington, New Zealand

UNTIL RECENTLY it was believed that fatty acids in natural fats were straight-chain molecules with an even number of carbon atoms. The occurrence of *iso*-valeric acid in the depot fats of dolphins and porpoises was regarded as a solitary exception (1). Whereas branched-chain fatty acids have been found in the past in the lipids of certain bacteria by Anderson (2) and more recently in wool grease by Weitkamp (3), these lipids were esters of high molecular alcohols and not glycerides.

Branched-chain Fatty Acids in Animal Fats. Accordingly isolation of branched-chain fatty acids from butterfat by Hansen and Shorland, first reported in 1950 (4), created widespread interest. This discovery originated from an investigation of the efficiency of separating liquid and solid methyl esters of butterfat by crystallization from acetone and fractional distillation. When concentrates of C_{18} liquid esters were oxidized with potassium permanganate in acetone, there was a residual neutral product which was saturated and yet liquid at room temperature. The product was resolved into several fractions, one of which suggested the presence of branched-chain fatty acids. In the course of further investigations Hansen and Shorland obtained evidence of the occurrence of several branched-chain fatty acids in butterfat (5a,b, 6a,b, 7a,b) and this was followed by the isolation of a number of such acids from the depot fats of oxen and sheep (8a,b,c,d,e).

The procedure for the isolation of these acids consisted in earlier studies in combining fractional distillation with hydrogenation and low temperature crystallization from acetone, which separates branched-chain from straight-chain saturated acids. However, in view of Hofmann and Lucas' findings (9) that hydrogenation could lead to the formation of branched-chain acids if the original product contained a cyclopropane ring, careful fractional distillation has been employed rather than hydrogenation. With the use of highly efficient fractionating columns the small amounts of branched-chain fatty acids present in natural fats could be sufficiently resolved to permit their subsequent purification by low temperature crystallization alone. The types and approximate amounts of branched-chain acids found in butterfat and depot fats of oxen and sheep are shown in Table I(a).

The Occurrence of Straight-chain Odd-carbon Numbered Acids in Ox, Sheep, and Butterfat. An essentially similar technique led to the isolation of straight-chain odd carbon numbered fatty acids in the above mentioned fats by the workers of this laboratory (7a,b, 10a,b, 11). The existence of such acids, and especially of margaric acid, in natural fats had been repeatedly reported in the past but never substantiated, as might be seen from Ralston's (12) following statement:

> Heptadecanoic acid does not occur in the natural fats and oils, although its presence has often been reported, and it has been only within recent years that such names as margaric acid and daturic acid have ceased to be the subject of scientific controversies. All the naturally occurring heptadecanoic acids which have been described, and subsequently investigated, have been shown to consist of mixtures of palmitic and stearic acids. Because of the frequent mutual occurrence of palmitic and stearic acids in fats, it is not surprising that a mixture of them has frequently been identified as a pure compound.

The identification of the above-mentioned acids was based on greatly improved methods of fatty-acid analysis by fractional distillation and low temperature crystallization, on measurements of long crystal spacings by X-ray diffraction, determination of mixed melting points with pure synthetic acids, and the like. Incidentally a qualitative test for the presence of saturated *n*-odd numbered fatty acids might be based on their characteristic property of shrinking from glass on cooling, which is not shown to any comparable extent by even-numbered fatty acids and their mixtures. Table I(b) shows the various straight-chain odd-numbered acids isolated up till now. It might be noted that their occurrence is not restricted to the saturated acids only.

The vapor-liquid chromatographic method of James and Martin (13), based on the automatic titration of fatty acids, was applied to the detection and estimation of both branched-chain and odd-numbered straight-chain volatile acids in the ox fat on a micro scale, and a consecutive series of these acids from C_2–C_{10} has been reported by Hansen and McInnes (14). Furthermore results obtained by Shorland and co-workers have been confirmed by James and Martin (15) with the use of their new chromatographic apparatus based on vapor density measurements, which

[1] Presented at the Fall Meeting of the American Oil Chemists' Society, Philadelphia, Pa., Oct. 10–12, 1955.