TABLE V
Run VI—Mustard Oil with Glycerol (98%) Temperature 250°C.

Sample No.	Time of reaction (min.)	Hydroxyl value	% Combined-glycerol[a]		% Mono-glyceride (p)	% Diglyc-eride (q)	% Tri-glyc-eride [100-(p+q)]
			Original	Reacted			
1	0	10.73	9.436	0.9145	0.9208	16.65	82.43
2	15	26.02	9.436	1.422	3.321	19.41	77.27
3	30	32.85	9.436	1.796	7.499	13.11	79.39
4	45	36.63	9.436	2.003	8.943	12.69	78.37
5	60	46.28	9.436	2.530	9.788	21.14	69.07
6	90	50.47	9.436	2.758	12.280	18.72	69.00
7	120	67.01	9.436	3.390	16.860	15.43	67.71
8	150	124.80	9.436	6.822	21.680	43.74	24.58
9	180	155.50	9.436	8.500	36.170	59.74	4.09
10	210	169.30	9.436	9.145	33.440	82.80	−16.24
11	240	169.70	9.436	9.276	26.07	108.50	−34.57

[a] Reacted glycerol, H/1829 × 100.

in Tables I and V. This difference in the behavior of mustard oil may be due to the presence of isothiocyanates (13), or it may be an inherent property of erucic acid oils.

Equilibrium monoglyceride contents of peanut and mustard oils are lower than those of linseed oil (58–60%), as reported by Runk. This may be due to the better solubility characteristics of the unsaturated fatty acids of linseed oil in glycerol. Further lowering of equilibrium monoglyceride content of mustard oil may be due to the presence of high molecular weight acid (erucic acid). Kawai (8) has also found that glycerolysis is selective and that fatty acids of low molecular weight and higher unsaturation are primarily re-esterified while those of high molecular weight and lower unsaturation are mostly left unchanged. Thus the nature of component acids present in the oil influences the relative amounts of mono- and diglycerides formed.

Summary

The glycerolysis of peanut and mustard oils has been carried out in the presence of catalysts such as lime and litharge. The effect of time and temperature on the rate of glycerolysis has been studied. The monoglyceride contents and hydroxyl values of the washed samples have been determined. The diglyceride and triglyceride contents of the products have been computed from the monoglyceride percentage and the hydroxyl values.

REFERENCES

1. Markley, "Fatty Acids," Interscience Publishers Inc., New York, p. 303 (1947).
2. Tsuchya and Akiyama, J. Soc. Chem. Ind., Japan, *36*, suppl. binding, 233 (1933).
3. Hilditch, T. P., and Rigg, J. Chem. Soc., 1774 (1935).
4. Blaganravova, Antipova, Savvina, and Svetlichnaya, J. Applied Chem. (U.S.S.R.), *14*, 192 (1941); Chem. Abst. *36*, 1590 (1942).
5. Ross, Bell, Arrowsmith, and Gebhart, Oil and Soap, *23*, 257 (1946).
6. Savary, Compt. rend., *226*, 1284 (1948).
7. Feuge and Bailey, Oil and Soap, *23*, 259 (1946).
8. Kawai, J. Soc. Chem. Ind., Japan, *44*, 705 (1941); Chem. Abst. *42*, 2118 (1948).
9. Runk, Ind. Eng. Chem., *44*, 1124 (1952).
10. Chinoy and Kamath, Paintindia, *3*, No. 5, 27 (1953); *3*, No. 6, 27 (1953).
11. Pohle, W. D., and Mehlenbacher, V. C., J. Am. Oil Chemists' Soc., *27*, 54 (1950).
12. Pohle, W. D., and Mehlenbacher, V. C., Oil and Soap, *23*, 48 (1946).
13. Pal and Mukherji, Indian Soap Journal, *13*, 291 (1948).

[Received July 12, 1954]

The Fractionation of Marine-Oil Fatty Acids with Urea[1]

CLAUDE DOMART,[2] D. T. MIYAUCHI, and W. N. SUMERWELL, Fishery Technological Laboratory,[3] Seattle, Washington

OILS obtained from marine animals such as the menhaden, herring, and seal are characterized by their substantial content of long-chain fatty acids having 20 to 24 carbon atoms and 3 to 6 double bonds. On the other hand, such oils also contain abundant amounts of the shorter chain, more saturated fatty acids. The heterogeneous make-up of marine oils is exemplified by the analysis of North Atlantic menhaden oil reported by Armstrong and Allen (1). They found 23.5% of saturated fatty acids varying from 14 to 18 carbons in chain length. The unsaturated constituents were composed of 15.5% C_{16} acids having one double bond, 30% C_{18} acids having an average of two double bonds, and 31% C_{20} and C_{22} acids, each series having an average of five double bonds per molecule.

This broad mixture of saturated and unsaturated fatty acids in marine oils makes necessary a preliminary separation before the oils can be used for some purposes, particularly those requiring oils with good drying properties. Fractional crystallization at low temperatures and fractionation with propane by the Solexol process are examples of methods used to remove the more saturated components from oils.

The demonstration by Bengen (2, 3) that urea forms crystalline complexes with saturated fatty acids, monoenoic fatty acids, and less readily with more highly unsaturated fatty acids opened the way for the development of a simple and rapid means of concentrating the more unsaturated fatty acids occurring in marine oils. In 1950 papers by Schlenk and Holman (4) and Newey *et al.* (5) called attention to the possibilities of the urea method for segregating the more highly unsaturated fatty acids or their esters from a number of commercial oils including linseed oil, soybean oil, corn oil, and olive oil. Newey and his co-workers also applied urea crystallization to long-chain fatty alcohols prepared from linseed oil and to nitriles prepared from soybean-oil fatty acids. A recent review by Schlenk (6) thoroughly discusses the urea inclusion compounds of fatty acids.

[1] Presented at the Regional American Chemical Society Meeting, Richland, Wash., June 11–12, 1954.
[2] Smith-Mundt Fellow at the University of Washington, 1952–1953.
[3] One of the laboratories of the Branch of Commercial Fisheries, Fish and Wildlife Service, U. S. Department of the Interior.

The fact that marine oils contain considerable quantities of polyunsaturated fatty acids suggests that urea crystallization may be especially useful for preparing concentrates of these fatty acids. The present report describes the preparation and properties of fatty acid fractions obtained by applying urea crystallization to the fatty acids prepared from a number of common marine oils. Two series of experiments were run. In the first series, samples of fatty acids of menhaden oil were complexed at 1°C. with different mole ratios of urea. In the second series, fatty acids from menhaden oil, herring oil, tuna-body oil, seal oil, salmon-egg oil, and salmon-head-and-viscera oil were fractionated with urea at temperatures ranging from 25°C. to —30°C.

Experimental

Preparation of Fatty Acids. The oil samples used for preparing the fatty acids were taken from the normal production of reduction plants, with the exception of the salmon-egg and salmon-head-and-viscera oils. These two oils were prepared from fresh, ground salmon waste. The waste was cooked in a 10% brine solution for a short period of time, and the oil that collected on the surface of the mixture was decanted and centrifuged until clear.

Saponification of the various oils was carried out by dissolving 200-g. samples in 400 ml. of petroleum ether and then slowly adding, with stirring, sodium ethylate reagent in 15% excess. The amount of sodium ethylate required was calculated from the saponification values of the oils reported in the literature. Sodium ethylate was made by dissolving sodium chips in 95% ethanol until the concentration reached 1 mole of sodium per 300 ml. of alcohol. Upon the addition of this reagent, saponification began immediately and proceeded with the production of a moderate amount of heat.

After 24 hrs. the reaction mixture was dissolved in 2 l. of water, and the unsaponified matter was removed by three extractions with 500-ml. aliquots of 1:1 mixture of petroleum ether and ethyl ether. The aqueous solution of soaps was acidified with sulfuric acid, and the fatty acids were extracted with the 1:1 solvent mixture. The fatty acid extract was dried overnight with calcium chloride and then was filtered. After the solvent was distilled off, the fatty acids were dried for 3 hrs. in a vacuum oven at 60°C. The Hanus iodine value and the refractive index of each sample of fatty acids were then determined.

Fractionation of Menhaden Oil Fatty Acids with Urea at Various Mole Ratios

These experiments were set up to provide data concerning the degree of unsaturation of fatty acid fractions obtained by urea crystallization at 1°C. with the mole ratio of urea to fatty acid varied from 4.6:1 to 23.0:1. The experiments were carried out in triplicate. First, 50-g. samples of menhaden-oil fatty acids were weighed into Erlenmeyer flasks and dissolved in 100 ml. of methanol. Methanol saturated with urea was then added to the flasks in volumes of 300, 600, 900, 1,200, and 1,500 ml., which correspond to the following mole ratios of urea to fatty acid: 4.6:1, 9.2:1, 13.8:1, 18.4:1, and 23.0:1. The mole ratios were calculated from the concentration of urea in methanol solution, which was 16.6 g. urea per 100 ml. methanol, and from the saponification equivalent of the menhaden-oil fatty acids, which was 277.8.

The mixtures were warmed until complete solutions were obtained. The flasks were placed in a 1°C. cold room for 48 hrs., and the crystalline, urea-complex precipitates were filtered with suction. Mother liquor was used to rinse each flask free of precipitate. The precipitate was washed twice with small volumes of methanol saturated with urea. The fatty acids were liberated by dissolving the precipitate in 1 l. of water acidified with hydrochloric acid. The fatty acids were extracted with three 200-ml. volumes of 1:1 mixture of petroleum ether and ethyl ether. The extract was dried with calcium chloride, the solvent was distilled, and the fatty acids were placed in a vacuum oven for 3 hrs. at 50°C.

The filtrate containing the fatty acids not precipitated with urea was diluted with two volumes of water and acidified with hydrochloric acid. Three 300-ml. volumes of the mixed solvents were used to extract the fatty acids, which were then recovered from the solvents in the same manner as before.

The weights and Hanus iodine values of the precipitated and unprecipitated fatty acid fractions were determined. The results of these experiments are given in Table I.

TABLE I

The Fractionation of Menhaden Oil Fatty Acids[a] with Different Mole Ratios of Urea in Methanol at 1°C.[b]

Mol. ratio of urea to fatty acids	Yield of fatty acids from complexes (%)	Hanus I. V. of fatty acids from precipitate	Yield of fatty acids from filtrate (%)	Hanus I. V. of fatty acids from filtrate	Unrecovered fatty acids (%)
4.6:1	11.6	12.8	80.8	192.7	7.6
9.1:1	29.8	22.1	61.6	243.4	8.6
13.8:1	49.4	48.1	41.6	308.9	9.0
18.4:1	61.0	54.5	36.4	330.7	2.6
23.0:1	63.0	72.7	34.2	341.6	2.8

[a] The Hanus I. V. for the menhaden oil fatty acids from which these fatty acids were prepared was 159.5.
[b] All values represent averages obtained by treating triplicate 50-g. samples of fatty acids.

Effect of Temperature on the Fractions Obtained by Urea Precipitation of Marine-Oil Fatty Acids

In this series of experiments each sample of fatty acids was fractionated with urea successively at four different temperatures: 25°C., 1°C., —18°C., and —30°C. The experiments were carried out at a mole ratio of urea to fatty acids of approximately 9:1.

The 50-g. samples of fatty acids were placed in Erlenmeyer flasks, and each sample was dissolved in 100 ml. of ethanol. The fractionations were made on duplicate samples. Six hundred ml. of urea-saturated methanol solution were added to each flask, and the solutions were warmed until clear. The flasks were placed in a 25°C. water bath overnight. Light yellow crystalline precipitates were formed. They were filtered quickly with the aid of suction and washed with a small quantity of urea-saturated methanol.

The filtrate was placed overnight in a 1°C. cold room and then filtered exactly as before. The filtrate remaining after the 1°C. precipitation was treated in the same way at —18°C. and finally at —30°C. The precipitates collected at the four temperatures were allowed to dry at room temperature and then were weighed. They were dissolved in warm water, and the fatty acids were extracted with the mixed petroleum ether-ethyl ether solvent. The filtrate remaining after

the final precipitation at —30°C. was diluted with two volumes of water, acidified with HCl, and the fatty acids were extracted. All fatty acid fractions were weighed, and the Hanus iodine value and refractive index were determined. The results are shown in Table II.

Discussion

From a consideration of the data in Table I showing the effect of varying the mole ratio of urea to menhaden-oil fatty acids, it is evident that there is considerable selectivity in the fatty acids precipitated when the moles of urea present are less than the quantity required for maximum precipitation. Thus, at a mole ratio of 4.6, the fatty acids obtained from the precipitates are highly saturated, but at higher mole ratios more unsaturated fatty acids are complexed by urea.

From the analysis of menhaden oil and the results in Table I it would appear that virtually all the saturated and monoenoic fatty acids are precipitated at a mole ratio in the region of 12:1 to 13:1. When the mole ratio is further increased, more of the less stable dienoic fatty acid complexes are precipitated. This observation is in agreement with the results of Redlich and co-workers (7), who observed that substances forming unstable urea complexes were capable of precipitation from mixtures of reactants. Furthermore the data indicate that fatty acids with more than two double bonds are not present in the precipitates in other than minute quantities.

The results of these experiments show that fatty acid fractions of almost any desired iodine value ranging to above 300, a degree of unsaturation corresponding to three to four double bonds per molecule, can be prepared readily from menhaden oil or from other oils of similar characteristics.

The experiments dealing with the effect of temperature on the precipitates bear out the previous results. In addition, from the data in Table II it is evident that menhaden oil has a considerably higher content of saturated fatty acids than any of the other oils used in this work. This fact is borne out by published analyses of the oils.

The fractions precipitated at —18°C. and the very small yields obtained at —30°C. contain appreciable amounts of the dienoic fatty acids whereas the 25°C. and 1°C. fractions contained no measurable quantities of these acids. An especially high proportion of dienoic acids is apparently present in the complexes obtained from the salmon oils.

TABLE II

Characteristics of Fractions Obtained from Marine-Oil Fatty Acids by Urea[a] Crystallization at Different Temperatures

Fatty acids	Weight (g.)	Hanus I. V.	Refractive index[b]
From Menhaden Oil			
Total fatty acids	50.0	159.5	1.4705
25°C. fraction	13.9	19.4	1.4431
1°C. fraction	8.7	46.0	1.4548
—18°C. fraction	3.0	82.7	1.4601
—30°C. fraction	0.1	......	1.4901
Unprecipitated fatty acids in filtrate	24.2	255.0	1.4891
From Herring Oil			
Total fatty acids	50.0	136.4	1.4736
25°C. fraction	12.2	47.7	1.4570
1°C. fraction	9.1	55.2	1.4575
—18°C. fraction	4.7	75.8	1.4667
—30°C. fraction	1.9	92.9	1.4687
Unprecipitated fatty acids in filtrate	21.2	246.0	1.4899
From Tuna-Body Oil[c]			
Total fatty acids	50.0	149.6	1.4707
25°C. fraction	6.9	27.7	1.4336
1°C. fraction	9.2	42.4	1.4567
—18°C. fraction	3.9	61.9	1.4594
—30°C. fraction	0.1	......	1.4865
Unprecipitated fatty acids in filtrate	24.8	243.1	1.4867
From Seal Oil[d]			
Total fatty acids	50.0	129.0	1.4784
25°C. fraction	5.4	52.3	1.4584
1°C. fraction	10.1	63.3	1.4592
—18°C. fraction	3.4	75.5	1.4623
—30°C. fraction	2.6	116.5	1.4692
Unprecipitated fatty acids in filtrate	24.7	210.5	1.4775
From Salmon-Egg Oil			
Total fatty acids	50.0	206.8	1.4757
25°C. fraction	10.7	46.7	1.4567
1°C. fraction	9.2	82.9	1.4595
—18°C. fraction	5.0	153.0	1.4711
—30°C. fraction	0.2	......	1.5174
Unprecipitated fatty acids in filtrate	24.5	290.0	1.4953
From Salmon-Head-and-Viscera Oil			
Total fatty acids	50.0	144.6	1.4729
25°C. fraction	6.0	43.7	1.4573
1°C. fraction	12.0	59.5	1.4592
—18°C. fraction	5.5	119.5	1.4599
—30°C. fraction	0.1	......	1.4760
Unprecipitated fatty acids in filtrate	17.1	241.6	1.4867

[a] Fractionations were made in methanol solution at a mole ratio of urea to fatty acids of approximately 9:1.

[b] All refractive index values are calculated to 20°C. by use of the factor —0.000365 per degree.

[c] The sample of tuna-body oil contained a considerable amount of oxidized fatty acids.

[d] The seal-oil fatty acids contained some waxy acidic substance that was only moderately soluble in methanol.

Summary

In two series of experiments, marine-animal-oil fatty acids were fractionated with urea using methanol as solvent.

In the first series, menhaden-oil fatty acids were fractionated at 1°C. Almost all the saturated and monoenoic fatty acids were removed at mole ratios of 12:1 to 13:1. At higher ratios increasing amounts of the less stable dienoic fatty acids were precipitated. By the use of the appropriate ratio, fractions having iodine values above 300 were prepared.

In the second series, fatty acids from the oils of menhaden, herring, tuna, seal, salmon eggs, and salmon heads and viscera were fractionated at a mole ratio of urea to fatty acid of 9.2:1. At 25° and 1° the complexes were composed almost entirely of saturated and monoenoic fatty acids, but as the temperature was lowered to —30°, the content of dienoic fatty acids in the precipitates increased.

Acknowledgment

The authors are grateful to Clarence Haynes, who made some of the determinations, and to the various concerns who supplied the samples.

REFERENCES

1. Armstrong, E. F., and Allen, J., J. Soc. Chem. Ind., 43, 207–218T (1924).
2. Bengen, F., Ger. Patent Application O. F. 12438 (March 18, 1940).
3. Bengen, F., and Schlenk, W. Jr., Experientia, 5, 200 (1949).
4. Schlenk, H., and Holman, R. T., J. Am. Chem. Soc., 72, 5001–5004 (1950).
5. Newey, H. A., Shokal, E. C., Mueller, A. C., Bradley, T. F., and Fetterly, L. C., Ind. Eng. Chem., 42, 2538–2541 (1950).
6. Schlenk, H., "Progress in the Chemistry of Fats and Other Lipids," Academic Press Inc., New York, 1954, vol. 2, pp. 243–267.
7. Redlich, O., Gable, C. M., Dunlop, A. K., and Millar, R. W., J. Am. Chem. Soc., 72, 4153–4160 (1950).
8. Fisheries Research Board of Canada, "Marine Oils with Particular Reference to Those of Canada," Bull. 89 (1952).

[Received August 2, 1954]

CORRECTION

From the Netherlands J. P. Spruyt writes that the word squalene which appeared in his paper in the April 1955 issue (vol. 32, pp. 197–200) should have been squalane instead. His correction on the galley proof apparently was overlooked by the printer and editorial staff.