Unsaturated Fatty Acids. I. Low Temperature Urea Complexes of Polyunsaturated Acids from Bovine Tissue[1, 2]

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A MIXTURE OF unsaturated fatty acids was obtained by the extraction of autolyzed saline extracts of bovine testicular tissue. The hemolytic properties of this mixture appeared to differ from those of oleic and linoleic acids, and an attempt was made to identify and separate the constituents of the mixture. Crystallization of the urea complexes at temperatures as low as —75°C. yielded fractions which contained high concentrations of hexaenoic and tetraenoic acids.

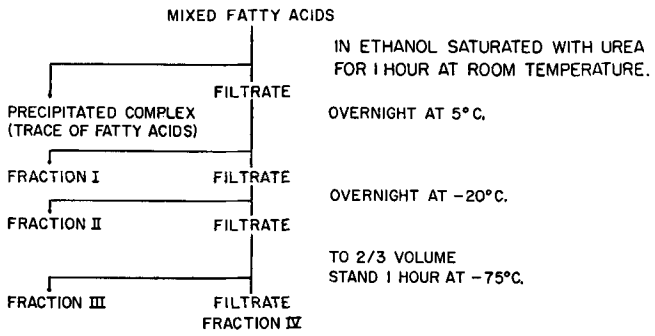
Since Bengen (3) in 1940 reported the preparation of urea complexes of straight chain compounds, the method has been used in the separation of a variety of fatty acids. Swern and Parker (8) prepared methyl oleate and oleic acid of 97 to 99% purity by fractional precipitation of the urea complexes. Later (9) they applied the method to the separation of linolenic acid from vegetable oils and found that the final concentration appeared to be dependent on the fatty acids present in the original mixture. Perilla oil yielded a concentrate containing 87 to 98% linolenic acid while linseed oil yielded only a 66% concentration. Schlenk and Holman (7), and Abu-Nasr, Potts, and Holman (2), using the formation of urea complexes as a method of separation, concluded that the reaction between urea and unsaturated fatty acids was favored by low temperatures and that the urea complex protected the acid against auto-oxidation. Abu-Nasr and Holman (1) used the fractional crystallization of the urea complexes as a preliminary step in the preparation of docosahexaenoic acid from cod liver oil acids.

Procedure

The starting mixture was obtained from autolyzed saline extracts of bovine testicular tissue by a method previously described (6). This mixture of fatty acids had an iodine number (Wijs) of 257 and a neutral equivalent of 305. Low temperature crystallization from acetone removed the major portion of the saturated acids and yielded a light yellow oil with an iodine number of 313 and neutral equivalent of 330.

Sufficient reagent grade urea to saturate the solution was dissolved with slight warming in an ethanolic solution of the fatty acid mixture. The fractionation was carried out according to the scheme outlined in Figure 1. The insoluble complexes were removed by filtration on a Buchner funnel with suction, and the fatty acids were freed from urea by treatment with dilute hydrochloric acid and extraction with ether. The ether solution was washed several times with water, and the ether was evaporated under reduced pressure. The alcohol from the filtrates was evaporated under vacuum, and the fatty acids were recovered in the same manner as from the insoluble complexes.

The urea complexes of fractions II and III were recrystallized several times from alcohol.



FLOW SHEET FOR SEPARATION OF FATTY ACIDS BY LOW TEMPERATURE-UREA COMPLEX PROCEDURE.

FIG. 1

Results and Discussion

Examination of the analytical results recorded in Table I shows that, as the temperature is lowered, the acids precipitating as solid urea complexes increase in unsaturation and decrease in chain length. Recrystallization of Fractions II and III yielded products of higher iodine value than the original precipitates. Infrared and ultraviolet spectra showed no indication of either *trans* or conjugated isomers.

TABLE I

Characteristics of Fractions Described in Figure 1

	Amt. conc. obtained	I.V.	Neut. equiv.	Refractive index
From 1.6 g. acids		313	330	
Fraction I	0.49 g.	245	364	
Fraction II	0.13 g.	316	341	
Fraction III	0.19 g.	329	338	
Fraction IV	0.32 g.	416	301	1.4989
From 4.3 g. Acids				
Fraction I	0.48 g.	303		
Fraction II	0.53 g.	323		
(Fraction II[a])	(0.23 g.)	(367)	(331)	(1.4939)
Fraction III	1.14 g.	316		
(Fraction III[a])	(0.32 g.)	(323)	(306)	(1.4821)
Fraction IV	0.87 g.	367		

[a] Recrystallized from Ethanol at —75°C.

Conjugation of the double bonds and examination of the ultraviolet spectrum of the conjugated isomers has been recommended as a method of determining individual constituents in mixtures of unsaturated fatty acids. A method frequently used for the more highly unsaturated fatty acids is that of Herb and Riemenschneider (5). It consists of heating the sample for 15 min. at 180°C. in ethylene glycol containing 21% KOH. This method was used in order to identify the individual acids in the fractions obtained from the urea complexes.

Table II shows the specific extinction coefficients at the wavelengths representing conjugation of 2, 3, 4, 5, and 6 double bonds. As can be seen from the data, the hexaenoic acids present in the original mixture

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TABLE II

Spectral Absorption after Conjugation of Fractions Described in Figure 1

Fraction	Specific Extinction Coefficient ($E^{0.1\%}_{1\,cm.}$)				
	3750A	3460A	3150A	2680A	2330A
Original Mixture	7.5	13.8	35.4	35.1	36.6
Fraction II[a]	14.8	23.9	45.3	44.6	45.3
Fraction III[a]	3.6	8.9	38.4	39.8	45.6
Fraction IV	18.7	30.0	47.1	44.3	46.8

[a] Recrystallized from Ethanol at −75°C.

are concentrated in Fractions II and IV with only a small quantity in the intermediate Fraction III. This difference in solubility of the urea complexes suggests that two hexaenoic acids were present in the original mixture.

Hammond and Lundberg (4) have reported a specific extinction coefficient of 28.1 at 3,750 Å for a sample of methyl docosahexaenoate of high purity. Conjugation was accomplished under approximately the same conditions as those used in our laboratory. Comparison of this figure with the 14.2 obtained for Fraction II leads to the conclusion that approximately 50% of the acids crystallizing as urea complexes at −20°C. was docosahexaenoic acid.

The specific extinction coefficients at 3,750, 3,460, and 3,150 Å show a low content of hexaenoic acid and a high percentage of tetraenoic acid in Fraction III. This indicates that the major portion of the docosahexaenoic acid had been removed as a urea complex at the higher temperature and that arachidonic with a shorter chain length constituted the main constituent precipitating at −75°C.

Fraction IV yielded a higher specific extinction coefficient at 3,750 Å than Fraction II, indicating greater percentage of hexaenoic acid. However the high iodine value, low neutral equivalent, and high refractive index all indicate that Fraction IV contains a high percentage of an acid with six double bonds but a shorter chain length than the docosahexaenoic of Fraction II. It is probably an eicosahexaenoic acid.

The eicosahexaenoic acid was present in the mixture of free acids liberated by enzymic action taking place during autolysis of the saline suspensions. It could not be detected in the acids obtained by alkaline saponification of either the neutral esters or phosphatides extracted from fresh tissue.

Summary

A mixture of fatty acids obtained from autolyzed saline extracts of beef testicular tissue was fractionated by crystallization of the urea complexes at 5°, −20°, and −75°C. Fractions rich in docosahexaenoic and in arachidonic acids were obtained as solid complexes. The filtrate remaining after precipitation of the solid urea complexes contained a high percentage of hexaenoic acid of shorter chain length than docosahexaenoic, probably eicosahexaenoic acid.

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The Effect of Various Antioxidants on the Keeping Quality of Yellow Grease[1]

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RECENTLY there has been considerable interest in the addition of fats to animal feeds. This interest was probably brought about mainly by the price situation in the lower grades of fats which gave a favorable comparison from a nutritive standpoint with wheat, corn, and other carbohydrates normally used in animal feeds. Work at various experiment stations and research centers has shown that most animals as well as poultry will show a definite gain in weight as well as over-all physical appearance when fat is used in proportions of 5-10% of the total feed formula. Matsushima and Dowe (3) of the Nebraska Experiment Station have shown that cattle when placed on a diet containing approximately 5% fat gained weight more economically than when fed grain alone.

Rice *et al.* (6) have shown that there is definitely a need for fats in animal feeds. They have shown that, in addition to the nutritive value of the fat, it controls dustiness and the physical appearance of the feed and makes it much easier to handle.

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Armstrong (1) of the American Meat Institute Foundation reports that at the beginning of 1954 approximately 200 million pounds of tallows and greases were used in mixed feeds annually. During the past year there has been a continued increase in the use of fats in animal feeds, and apparently many customers are now asking for mixed feeds which contain from 3-6% added fats.

One of the reasons that many feed users have held back on the use of fats in feed is the fact that most animals as well as poultry will not eat feed containing fats when they have become rancid except to prevent starvation. In addition to this, Quackenbush (5) of Purdue University has reported that fats which are oxidized will bring about a more rapid breakdown of the fat-soluble dietary essentials such as vitamin A, vitamin D, and vitamin K. He also reports that cases of dermatitis and failure of the reproductive organs have been reportedly caused by rancid dietary fat.

Since our previous work on the stabilization of the higher grades of fat, such as lard, tallow, etc., has shown that various synergistic mixtures of butylated