oxide at 300° to give the corresponding unsaturated alcohol octadecenol or ducosenol in yields of above 60%.

MADISON, WISCONSIN RECEIVED NOVEMBER 9, 1936

[CONTRIBUTION FROM THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY, THE OHIO STATE UNIVERSITY]

Studies on the Chemistry of the Fatty Acids. I. The Purification of Linoleic Acid by Crystallization Methods[1]

BY J. B. BROWN AND GEORGE G. STONER

On account of the recently discovered nutritional significance of linoleic acid, $C_{18}H_{32}O_2$, interest in its chemistry has increased greatly during the past few years. The acid occurs naturally in large amounts in the semi-drying oils, especially such oils as cottonseed, corn, soy bean and wheat germ. Despite several investigations on the subject there is still some question whether the acid occurs in these oils as a single geometric isomer or whether it occurs along with one or more of the other three possible forms. (We are considering here only the 9–10, 12–13, octadecadienoic acids although the possibility of other dienoic acids is not precluded.) One reason for this uncertainty is the fact that so far only one method has been available for preparing the pure acid, namely, that of Rollett.[2] In this method the mixed fatty acids of an oil are brominated in cold ether, the petroleum ether–insoluble bromides are isolated and purified and the linoleic acid is regenerated by heating with zinc dust. The product is satisfactory and is designated as α-linoleic acid.

Preparation of the acid by Rollett's method is unsatisfactory from the standpoint of yield, since in the bromination process considerable amounts of material are converted into petroleum ether-soluble isomeric bromides which are lost, and from the standpoint of chemical identity, since there is the question of alteration of the acid during bromination and debromination.

The object of the present work was to prepare linoleic acid by direct crystallization methods which did not involve bromination. As raw materials we have employed the fatty acids of refined cottonseed and corn oils containing approximately 50% of linoleic acid, the other acids present being principally palmitic and oleic.

Crystallization of cottonseed fatty acids from methyl alcohol, 95% ethyl alcohol, petroleum ether and acetone at −20° gave separations of saturated and unsaturated acids with an efficiency approaching that of the widely used lead soap–ether method. Fractional crystallization of cottonseed and corn oil fatty acids from acetone gave linoleic acid preparations 80–93% pure. The chief obstacle to the isolation of purer specimens is the fact that in attempting to obtain the more soluble constituent in every case, the product was always contaminated with oleic acid in proportion to the ratio of the solubility of linoleic acid to oleic acid under the experimental conditions. Crystallization of fatty acid mixtures at low temperatures constitutes a valuable method for the separation of these acids.

A large number of soap separations were tried. Of these, crystallization of the lithium soaps of cottonseed oil fatty acids from *n*-butanol at −20° gave 81% linoleic acid in the filtrate.

Experimental

Materials.—The sources of the fatty acids used in this work were commercial refined cottonseed and corn oils.

The following analytical data were obtained on these oils.

	Cottonseed	Corn
	Original oil	
Saponification no.	194.5	194.0
Iodine no.[a]	112.0	129.9
	Fatty acids	
Mean mol. wt.	276	282.9
Iodine no.	119	133.8

[a] All iodine numbers determined by the Wijs method.

Typical analyses of these oils have shown the following fatty acid composition.

	Cottonseed[a]	Corn[b]
Linoleic	45–50	39.1
Oleic	25–30	43.4
Myristic	Small amount	. .
Palmitic	19.8	7.3

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[1] Presented at the September, 1936, meeting of the American Chemical Society.

[2] A. Rollett, *Z. physiol. Chem.*, **62**, 410 (1909).

Stearic	Small amount	3.3
Arachidic	Small amount	0.4
Lignoceric	...	.2

[a] T. P. Hilditch and J. Priestman. *Analyst*, **56**, 358 (1931). [b] G. S. Jamieson and W. F. Baughman, THIS JOURNAL, **43**, 2696 (1921).

The fatty acids were prepared by saponification and acidification. They were distilled at low pressure. Linolenic acid was shown to be absent by the hexabromide test.

Special Equipment.—Through the courtesy of Dr. F. A. Hartman of the Physiology Department we had the use of a room maintained at −20 to −25°. This room was equipped with suction for rapid filtration. For work at temperatures lower than −20° we used the following apparatus. A 200 × 200 mm. specimen jar was boxed and surrounded by rock wool insulation, with a glass plate as a cover. A circular disk was cut from this plate so that when in place it would fit loosely over a 2-liter Erlenmeyer flask, which was set in the jar on a wire tripod to raise it off the bottom. The flask was equipped with a good stirrer and a low temperature thermometer. The jar was partially filled with alcohol. Dry ice was added to the alcohol as needed. By this arrangement temperatures as low as −50 to −60° could be obtained in twenty to thirty minutes, and as low as −75° in a little longer time. This apparatus was used in the laboratory but all low temperature filtrations were carried out by suction in the −20° room in apparatus cooled to that temperature. Practically all of our crystallization mixtures filtered rapidly, so that there was very little "warming" effect before the filtration was complete.

Separation of the Saturated and Unsaturated Acids.—Four hundred and fifty grams of the cottonseed oil acids was dissolved in 4000 cc. of acetone in a 4-liter Erlenmeyer flask and allowed to stand at −20° overnight. They were filtered by suction on a large Büchner funnel previously cooled to −20° and were washed with cold acetone. Acetone was removed from both crystals and filtrate by warming under reduced pressure. The unsaturated acids were distilled before analysis; yield 140 g. of saturated acids and 300 g. of unsaturated acids. The analytical data on these preparations as well as on a number of others prepared under various experimental conditions are given in Table I.

The best separations were from 10% solutions in acetone and in methyl alcohol at −20°. Petroleum ether and ethyl alcohol were almost as efficient. Separation from solutions of higher than 10% concentration was rendered less complete due to the bulk of the precipitate, which makes filtration and washing more difficult. For best results the initial concentration of saturated acids in the acetone solution should not exceed 2.5 to 3.0%. The separation in acetone and methyl alcohol is at least as efficient as the standard lead soap–ether procedure. This is borne out by the fact that lead soaps of the unsaturated acids which were prepared by the acetone method were completely soluble in ether at room temperature, and precipitated no insoluble soaps even at ice box temperature. Further work is in progress, studying the possibility of this method as a substitute for the lead soap–ether procedure.

Assuming the unsaturated acids in experiment 4 to be a mixture of oleic and linoleic acids, and calculating from the iodine number, the mixture contains 70% linoleic acid.

Crystallization of the Unsaturated Fatty Acids of Cottonseed Oil from Organic Solvents at Low Temperatures.—The unsaturated fatty acids, prepared by the −20° treatment in acetone and containing 70% linoleic acid, were crystallized at various temperatures and concentrations from several organic solvents. From a 10% solution in toluene at −70° the acids in the filtrate contained 76% linoleic acid; from the same concentration in ether the crystals contained 53% and the acids of the filtrate 73% linoleic acid. A 10% solution in acetone crystallized at −50° gave crystals of 49% and a filtrate containing 82% linoleic; at −60 and at −70° the separation was less satisfactory, the acids of the filtrate being 78 and 76% linoleic, respectively. A 1% solution in acetone crystallized at −65° gave 79% linoleic acid in the filtrate. Of these data the −50° crystallization in acetone is the most promising. This favorable result is no doubt due in part to removal of further traces of saturated acids which remained unavoidably in the unsaturated acids and in part to the crystallization of an oleic acid rich fraction.

Fractional Crystallization of the Unsaturated Acids.—In order to study further crystallization in certain solvents at various low temperatures, the unsaturated acids of cottonseed oil were fractionally crystallized from methyl alcohol, acetone, ethyl alcohol and 76% acetone. The solution was cooled to a given temperature with fairly rapid

TABLE I

SEPARATION OF SATURATED AND UNSATURATED ACIDS

Expt.	Solvent	Concn. of acids, g./100 cc.	Temp., °C.	Satd. fatty acids (Not distilled)		Unsatd. acids (Distilled)	
				Iod. no.	Mean mol. wt.	Iod. no.	Mean mol. wt.
1	Acetone	12	0	None		....	..
2	Acetone	18	−20	Not determined		153.4	279
3	Acetone	12	−20	33.0	263	154.9	280
4	Acetone	10	−20	7.2	258	153.7	280
5	Petroleum ether	10	−20	5.6	267	151.5	..
6	Alcohol (95%)	10	−20	12.6	264	152.5	..
7	Methyl alcohol	12	0	4.1	263	140.2	280
8	Methyl alcohol	12	−20	3.4	...	149.2[a]	280[a]
9	Methyl alcohol	10	−20	5.8	260	154.4[a]	282[a]

[a] Saponified before analysis to remove any esters which might have been formed.

TABLE II

FRACTIONAL CRYSTALLIZATION OF THE UNSATURATED ACIDS OF COTTONSEED OIL

Solvents	Concn. in solvents, %	Temp., °C.	% total wt.	Iodine number	% linoleic
Methyl alcohol	10	Ppt. −50	25	134.8	50
		Ppt. −55	16	159.2	74
		Ppt. −60	28	157.7	75
		Ppt. −65	15	168.2[a]	86
		Filtrate −65	16	156.5	73
Acetone	13	Ppt. −45	29	128.0	42
		Ppt. −50	16	155.0	72
		Ppt. −55	26	159.0	76
		Ppt. −60	9	167.8[a]	85
		Filtrate −60	20	153.4	70
Alcohol	10	Ppt. −50	44	151.6	68
		Ppt. −60	26	160.6	78
		Ppt. −70	13	161.8	79
		Filtrate −70	17	149.6	65
Acetone 76% by volume	7	Ppt. −40	29	111.0[a]	23
		Ppt. −70	46	160.0[a]	77
		Filtrate −70	25	163.3	81

[a] These specimens were distilled before the iodine number was determined.

stirring. It was filtered rapidly in the −20° room; the filtrate was then cooled further, this process being repeated, as shown in Table II.

The precipitate from methyl alcohol at −65°, *i. e.*, that portion of the total acids which came down by cooling a solution, saturated at −60, to −65° contained 86% linoleic acid; that precipitated at −60° from acetone contained 85% of the desired product. These represent the best products we have been able to obtain from cottonseed oil, but they constitute too small a part of the total acids to be of great value. It should be noted especially that in each instance maximum removal of unwanted acids (product of low iodine number) occurred in the first crop of crystals.

In order to take advantage of this fact the following procedure was carried out. Two thousand grams of refined cottonseed oil was saponified and converted into fatty acids. Four hundred and fifty grams of these acids was placed in a 4-liter Erlenmeyer flask and the flask filled with acetone. After standing overnight at −20°, the contents of the flask were filtered with suction. Four such lots were run (1800 g.), the total filtrate being 12 liters. In batches of 2 liters this filtrate was cooled to −40°, which was slightly below the point of initial appearance of crystals. The crystals were filtered off; the resulting filtrate from the entire lot contained 1010 g. of acids with an iodine number of 160 (77% linoleic acid).

Fractional Crystallization of the Unsaturated Methyl Esters from Methyl Alcohol.—Methyl esters (iodine number 152) were prepared from the unsaturated acids previously described. These esters were fractionally crystallized from a 12% solution in methyl alcohol, successive crops of crystals being removed at −45, −65 and −75°. The iodine numbers of the esters of these products and of the esters in the filtrate were 56.7, 142.3, 156.1 and 147.4, respectively, the yields, calculated as percentage total weight of ester used, were 2, 58, 32 and 8, respectively. The crystals at −75° contained approximately 82% methyl linoleate. Undoubtedly this procedure offers possibilities, but, so far, we have not studied it further.

Fractional Crystallization of Corn Oil Fatty Acids.—The unsaturated fatty acids of corn oil were prepared by the −20° acetone procedure; these were fractionally crystallized from this solvent under a variety of conditions. The details and results are shown in Table III.

We have succeeded here in obtaining satisfactory yields of linoleic acid 85, 86 and 88% pure, and less satisfactory yields of the acid 89 and 93% pure. It is possible then to prepare the acid by these latter procedures in sufficient purity for most chemical studies. We hope to have available shortly data comparing the acid which has been purified in this manner with α-linoleic acid prepared by Rollett's procedure.

Attempts to Purify Linoleic Acid by Crystallization of the Soaps of the Fatty Acids of Cottonseed Oil.—Several

TABLE III

CRYSTALLIZATION OF CORN OIL FATTY ACIDS[a]

Description	Wt. acids used. g.	Vol.	Yield, g.	Mean mol. wt.	Iod. no.	% linoleic
Filtrate at −55°	360	4000	78	...	167.1	85
−55 to −70° fraction of unsatd. acids[b]	320	4000	40	...	171.3	89
Acids, −45 to −70°	150	2000	95	279.6	168.5	86
Acids, −50 to −70°	150	2000	75	279.3	170.2	88
Filtrate (−70°) cooled to −80°; crystals rejected		1700	15	281.3	174.6	93

[a] Iodine no. of corn oil unsatd. acids, 156 (72.5% linoleic). [b] The −55 to −70° fraction was crystallized from 1 liter of acetone at −75°. Data above are on the crystals.

TABLE IV

FRACTIONAL CRYSTALLIZATION OF LITHIUM AND POTASSIUM SOAPS

Soap	Solvent	Temp., °C.	% total wt.	Iod. no.	% linoleic
Lithium	Butyl alcohol	Ppt. 30	3	...	..
		Ppt. 0	41	82.4	..
		Ppt. −20	41	144.0	60
		Filtrate −20	15	164.5	82
Potassium	Ethyl alcohol	Ppt. 30	1	...	..
		Ppt. 0	34	49.2	..
		Ppt. −20	26	134.8	49
		Filtrate −20	39	157.8	74.3

of the more useful methods of fatty acid separation involve differences of soap solubility. Early in this work we were hopeful of finding some suitable soaps of oleic and linoleic acids which would exhibit wide enough difference in solubility to be useful in their separation. Only a few of our more promising results will be reported here. Starting with cottonseed oil fatty acids, crystallization of the potassium soaps from a 10% solution in absolute alcohol at 0° gave 45% insoluble soaps and 55% soluble soaps, containing 68% linoleic acid. The lithium soaps, prepared by neutralizing a solution of the acids in alcohol with lithium hydroxide, removing the alcohol and water under reduced pressure and taking up the soaps in butyl alcohol, were fractionally crystallized from that solvent; also the potassium soaps from absolute alcohol with the results in Table IV.

When the fact is considered that we began here with a mixture of cottonseed oil fatty acids, it is clear that either of the separations in Table IV has possibilities. In the former case, a comparatively small amount of the acids comes out as 82% linoleic, while in the latter a much larger portion came out 74% linoleic acid.

Summary

1. The saturated and unsaturated fatty acids of cottonseed and corn oils may be separated conveniently by crystallization of the mixed acids from a 10% solution in acetone at −20°.

2. Linoleic acid preparations containing up to 93% of the acid were obtained by fractional crystallization of the unsaturated acids (and methyl esters) of these oils from acetone and methyl alcohol at low temperatures.

3. Fractional crystallization of the lithium soaps from *n*-butyl alcohol and of the potassium soaps from absolute alcohol may also be used in obtaining linoleic acid concentrates.

4. Crystallization of unsaturated fatty acids from appropriate solvents at low temperatures is a useful procedure for their separation.

COLUMBUS, OHIO RECEIVED OCTOBER 3, 1936

[CONTRIBUTION FROM THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY, THE OHIO STATE UNIVERSITY]

Studies on the Chemistry of the Fatty Acids. II. The Preparation of Pure Oleic Acid by a Simplified Method[1]

BY J. B. BROWN AND G. Y. SHINOWARA

Although oleic acid (*cis*-9,10-octadecenoic) is perhaps the most common of the naturally occurring fatty acids, the pure acid is still one of the rare chemicals. The reason for this state of affairs is that oleic acid always occurs in fats and oils associated with saturated acids and usually with varying amounts of linoleic acid and other acids of higher unsaturation. The methods for separating these impurities are only semi-quantitative so that purification of oleic acid is extremely tedious. Olive oil is usually employed as a source of the acid. The saturated acids, mostly palmitic, are removed by the lead soap separation in alcohol or ether; the unsaturated acids are then converted into barium soaps which are repeatedly crystallized from moist benzene to remove linoleic acids.

Excellent reviews of work previous to 1925 are given by Lewkowitsch[2] and by Lapworth, Pearson and Mottram.[3]

These investigators prepared the pure acid by a procedure which included the lead salt treatment, distillation, barium soap crystallization and finally distillation. Holde and Gorgas[4] claimed

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[1] Presented at the Pittsburgh Meeting of the American Chemical Society, September, 1936.

(2) J. Lewkowitsch, "Technology of Oils, Fats and Waxes," Macmillan and Co., Ltd., London, Vol. I, 1921, p. 182.

(3) A. Lapworth, L. K. Pearson and E. N. Mottram, *Biochem. J.*, **19**, 7 (1925).

(4) D. Holde and A. Gorgas, *Z. angew. Chem.*, **39**, 1443 (1926).