concentrated nitric) and the mixture heated on a water-bath for five minutes, after which it was poured on crushed ice and extracted with ether. The ether solution was washed with water, dried over anhydrous sodium sulfate, and evaporated on a water-bath. The crude nitro compound was recrystallized from dilute alcohol, or used without purification for preparation of the corresponding amine.

Derivative AS: *m*-Aminophenyl *s*-Isoamyl Sulfone.—The reduction of nitrophenyl *s*-isoamyl sulfone was made according to a modified method of Waldron and Reid.[11] The crude nitrophenyl sulfone obtained from 1 cc. of the sulfide was mixed with ten times its weight of iron powder (reduced by hydrogen), 10 cc. of water, and one drop of dilute sulfuric acid. With frequent stirring and adding of water to replace that lost by evaporation and the addition of a drop or two more of the acid, the mixture was heated on a water-bath for one-half to one hour. Sodium bicarbonate was added to the reaction mixture, and the aminosulfone was taken up in ether. The ether extract was treated with dilute hydrochloric acid. The free amine was then isolated by rendering the acid solution basic and extracting with ether. The ether solution was dried over anhydrous potassium carbonate and evaporated on a water-bath. The residue was recrystallized from a mixture of ether and heptane.

Summary

The addition of thiophenol to aliphatic olefins proceeds contrary to Markownikoff's rule. Propene, butene-1, isobutene, pentene-1, isopropylethylene, and trimethylethylene react with thiophenol in the absence of acid catalysts to yield *n*-propyl-, *n*-butyl-, *i*-butyl-, *n*-amyl-, *i*-amyl and *s*-isoamyl phenyl sulfides, respectively. The presence of 90% phosphoric acid scarcely affects the course of the reaction since the olefins react more readily with thiophenol than with the acid.

In the presence of sulfuric acid (diluted with water or acetic acid) the reaction proceeds in accordance with Markownikoff's rule: isobutene and trimethylethylene yield *t*-butyl- and *t*-amyl phenyl sulfides, respectively.

Isomerization occurs during the reaction of isopropylethylene with thiophenol in the presence of sulfuric acid. The product is *t*-amyl phenyl sulfide and not the expected *s*-isoamyl phenyl sulfide.

The physical properties of a number of alkyl phenyl sulfides are described. Several of these were characterized by preparation of solid derivatives.

t-Amyl phenyl sulfone cannot be prepared by the metathesis of *t*-amyl halide and sodium benzene sulfinate, as reported by Posner.

RIVERSIDE, ILLINOIS RECEIVED JUNE 16, 1938

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[11] Waldron and Reid, THIS JOURNAL, **45**, 2399 (1923).

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

Studies on the Chemistry of the Fatty Acids. IV. The Purification of Linolenic Acid by Fractional Crystallization of the Fatty Acids of Linseed and Perilla Oils, with Observations on the Properties of this Acid Prepared by Crystallization and by Debromination[*]

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

Linolenic acid is the principal unsaturated fatty acid of the vegetable drying oils; it occurs in small amounts in some of the semi-drying oils. Although it is rarely, if ever, found in animal lipids, unless the animal has eaten vegetable oils containing it, linolenic acid has been shown to replace linoleic as an essential fatty acid.[1] No new methods for the isolation of linolenic acid have been offered for over twenty-five years. The zinc salt purification proposed by Erdmann and Bedford[2] apparently has not been successful in other hands. The hexabromide reduction method[3] has been the one universally employed. This method has the advantage of starting with a pure solid derivative of the acid and gives a water-white product with an iodine number and molecular weight which are close to the theoretical. As in the case of linoleic acid, the method involves bromination–debromination procedures, which greatly reduce the yield, and the product may or may not be the same as the linolenic acid present in the original oil.

In the present work we have attempted to apply the crystallization procedures described by Brown and Stoner, and Brown and Frankel,

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(*) Presented at the fall meeting of the American Chemical Society Milwaukee, Wis., 1938.

[1] Burr, Burr and Miller, *J. Biol. Chem.*, **97**, 1 (1932).

[2] Erdmann and Bedford, *Ber.*, **42**, 3128 (1909).

[3] Hazura, *J. Soc. Chem. Ind.*, 506 (1888); Hehner and Mitchell, *Analyst*, 313 (1898); Rollett, *Z. physiol. Chem.*, **62**, 410 (1909); **70**, 404 (1910).

which were effective in purifying linoleic acid,[4] to the fatty acids of linseed and perilla oils. Fractional crystallization of the acids of these oils from acetone at −23, −45, −60 and −75° gave a final filtrate which contained 75% linolenic acid. Further crystallization from acetone was effective in raising the purity only slightly but when the 75% acid was crystallized from petroleum ether the precipitate contained 83–88% of the acid, assuming the impurity to be only linoleic acid. Four of the crystallization preparations have been compared with the pure so-called α-acid prepared by debromination.

Specimens of the acid prepared by crystallization gave hexabromide numbers ranging from 72–83. Calculating these to the basis of 100% acid, these values would range from 84.5 to 99. The average value for the four crystallization specimens was 92 as compared to 70–75 for the α-acid. The theoretical is 272. Rollett[3] found the yield of hexabromide to be 23%. From these results and from a comparison of other data it seems likely that the acids prepared by crystallization and by debromination are not identical; they may be different mixtures of isomers, either of the *cis–trans* or double bond position type.

In a previous paper[4b] a method for calculating the amount of linoleic acid in a fatty acid mixture was proposed, the method being based on a comparison of the tetrabromide number of the mixture with the tetrabromide number of pure α-linoleic acid. However, the fact that α-linolenic acid prepared by debromination gives a considerably lower hexabromide number than the acid prepared by crystallization makes us hesitate to apply the same principle to linolenic acid. It is suggested, however, that a similar formula, based on the hexabromide number of a fatty acid mixture in ether, will give much more accurate results than any of the procedures now available for calculating the linolenic acid content. Such a formula is proposed and discussed.

Experimental Part

Raw Materials.—The linseed and perilla oils employed in this work were authentic specimens furnished us through the courtesy of E. E. Ware of the Sherwin–Williams Company. The oils were saponified and the acids liberated from the resultant soaps with hydrochloric acid. The acids were distilled *in vacuo*. The constants of the oils and fatty acids appear in Table I. Iodine numbers were determined by the Wijs method with one hour reaction time. Hexabromide numbers were determined as described by Brown and Frankel for linoleic acid, except that dry ether was used as a solvent. Further, it is important, in view of the results below, to note that not more than 2–2.5-g. samples were used in 35–40 cc. ether. The resultant hexabromides were washed four times with ether cooled to 0° in 50-cc. tared centrifuge tubes.

TABLE I

CONSTANTS OF LINSEED AND PERILLA OILS AND OF THE CONSTITUENT FATTY ACIDS

	Linseed	Perilla
Original Oils		
Iodine no.	189.2	208.4
Sap. no.	196.2	201.1
Fatty Acids		
Iodine no.	191.7	214.6
Mean mol. wt.	278.0	277.9
Hexabromide no.	49.7	58.7
Linolenic acid, %[a]	54.0	63.8

[a] Calculated from the hexabromide number, as explained later.

Fractional Crystallization of the Fatty Acids from Acetone. Acetone was added to 500 g. of the acids to a total volume of 6 l. The solution was allowed to stand overnight in a cold room at −20° and was then filtered with suction on a Büchner funnel. The crystals were washed with cold acetone (−20°) and are designated as fraction I. The filtrate was cooled to −45° in a dry-ice–alcohol bath and a second crop of crystals removed (II). The filtrate was further cooled to −60 and −75°, the crystal crops representing fractions III and IV. The acids in the final filtrate constitute fraction V. The solid acids in (I) were recovered by simply drying in air to constant weight. Those in (II) to (V) were recovered by removing the acetone under reduced pressure and distilling the residue at 2 mm. pressure. The analytical data on the fractions are given in Table II.

The iodine numbers of the several fractions progressively increase, indicating a concentration of linolenic acid in the successive filtrates as the temperature was lowered. This bears out our prediction that linolenic acid would be the most soluble acid present. Fraction I with both oils contains most of the saturated acids. The separation into saturated and unsaturated acids is especially efficient in the case of linseed oil. Oleic and linoleic acids in part come out and are removed in the intermediate fractions. The final filtrate acids, fraction V, gave iodine numbers of 252.9 and 249.1, respectively. Assuming these acids to be binary mixtures of linoleic and linolenic acids, and calculating from the iodine numbers, the linolenic acid contents were 77 and 74%, respectively, for linseed and perilla oil acids. Fractions IV of these oils contained 66 and 67%, respectively, of the acid. Values for linolenic acid, thus calculated from the iodine number, are low because small but unknown amounts of oleic acid and traces of saturated acids unavoidably are present in each instance.

In another experiment a similar crystallization was carried out, except that the later fractions were taken off

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[4] (a) Brown and Stoner, THIS JOURNAL, 59, 3 (1937); (b) Brown and Frankel, *ibid.*, 60, 54 (1938).

TABLE II

RESULTS OF FRACTIONAL CRYSTALLIZATION OF THE FATTY ACIDS OF LINSEED AND PERILLA OILS

Fraction	Linseed Yield, g.	Iod. no.	Mean mol. wt.	Hexabrom. no.	Perilla Yield, g.	Iod. no.	Mean mol. wt.	Hexabrom. no.
I (−23°)	65	7.8	...	1.5	40	63.9	...	9.5
II (−45°)	124	165.2	...	35.4	20	142.3	...	30.1
III (−60°)	137	217.6	277.5	56.0	235	220.3	278.4	33.1
IV (−75°)	118	242.1	278.4	66.3	130	242.8	276.0	60.5
V Filtrate	56[a]	252.9	278.3	73.0	65[b]	249.1	277.6	61.5

[a] Yield 11.2% of acids used. [b] Yield 13.0%.

at −55 and −65°. The yields and analytical data appear in Table III.

TABLE III

RESULTS OF FRACTIONATION OF THE FATTY ACIDS OF LINSEED OIL

Fraction		Yield, g.	Iod. no.	Linolenic acid, %	Hexabrom. no.
I	−17°	68	6.8		0
II	−45°	102	162.0		24.8
III	−55°	70	198.0		40.2
IV	−65°	120	225.0	45[a]	52.5
V	Filtrate	140	249.0	73[a]	63.0

[a] Calculated from iodine number.

It is obvious that this procedure has resulted in a much higher yield (28%) of a product of slightly lower purity (73%). Numerous other experiments were conducted in the hope of increasing the yield and the purity. Apparently the degree of success so far as the purity is concerned is limited to the relative solubilities of oleic, linoleic and linolenic acids in acetone.

An attempt to improve the procedure by using petroleum ether as a solvent was unsuccessful, the final filtrate giving an iodine number of only 216. This would indicate that linoleic and linolenic acids are more equally soluble in petroleum ether than in acetone and perhaps explains the success in further purification by recrystallizing the 75% product from petroleum ether described below.

The Further Purification of Linolenic Acid by Crystallization from Petroleum Ether.—Ninety grams of crude linolenic acid (iod. no. 250.4), prepared by crystallization from acetone, was added to 1200 cc. of petroleum ether and cooled to −50°. The acids which crystallized were recovered: yield 66 g.; iodine number 258.1; that of the filtrate was 244.7. This procedure therefore resulted in a considerable improvement in purity (83.5%). The preparation is further described as (1) in Table IV.

Several fractions of iodine number 218–220 were combined and 544 g. of this mixture was made up to 6 liters with acetone. After crystallizing at −60°, the acids in the filtrate (234 g.) were recovered and made up to 3 liters with petroleum ether. Crystal crops were obtained at −55 and −65°; these were combined and distilled. The first 30 g. of distillate (iodine no. 247) was rejected. The next 114 g. was found to have an iodine number of 260 (85%) (see Table IV).

Repeated crystallization from petroleum ether was investigated, starting with 250 g. of acid of iodine number 245 (69%) made up to 4 liters. Six successive crystallizations were made at this volume at −60°, the crystal crop each time being employed. The final yield was 60 g.; iodine number 262; purity 88% (see (3) Table IV).

Still another preparation was made from perilla oil. One kg. of the fatty acids of this oil was made up to 12 liters with acetone and cooled directly to −65°. The precipitate was again dissolved in acetone (8 l.) and cooled to this temperature. The acids of the combined filtrates amounted to 500 g., iodine number 245, purity 69%. This was crystallized five times from petroleum ether at −60°, the crystals each time being used. The product after distilling at low pressure amounted to 130 g., iodine number 259, purity 83% (see (4) Table IV).

Preparation of α-Linolenic Acid by Debromination.—Bromine was added slowly to 2 kg. of the mixed acids of perilla oil in 20% solution in ether, the temperature being kept below 10° with an ice-bath. The resultant bromides were washed very thoroughly seven times with 8 liters of ether, the supernatant wash ether being removed each time by suction. The yield of dried hexabromostearic acid was 640 g., m. p. 179.5–180°.

The hexabromide number as obtained here is only 32, which is much lower than reported above (see Table I). However, when the bromination experiment was repeated starting with 420 g. of the same batch of original acids and making up to only 5% with ether, a much higher yield of insoluble bromides resulted, 256 g. The hexabromide number was 60.9, which is in close agreement with the value obtained under analytical conditions, −58.7. It is apparent from these and other results that the concentration of acids during bromination is an important factor in the yield of bromides obtained.

Six hundred and thirty grams of hexabromostearic acid was suspended in 1500 cc. of hot 95% ethyl alcohol and 630 g. of zinc dust added very slowly in small portions since the reaction is quite vigorous. A few drops of concentrated hydrochloric acid were added occasionally. When all of the zinc had been added, the mixture was refluxed for one hour, the alcohol solution was separated and acidified with hydrochloric acid. About half of the alcohol was removed under reduced pressure. The partly esterified linolenic acid was recovered and distilled and, to remove ethyl ester, it was saponified, the acid recovered and again distilled: yield, 138 g., water-white acid (see (5) Table IV).

Discussion of Results

The analytical data on four crystallization preparations are compared in Table IV with the data on α-linolenic acid. By α-linolenic acid we refer to the debrominated product which may

TABLE IV

ANALYTICAL CONSTANTS OF LINOLENIC ACID PREPARED BY CRYSTALLIZATION AND BY BROMINATION

Specimen and method of prepn.	Purity, %	Mol. wt.	M. p., °C.	n^{20}	Iod. no.	Thiocyanogen no.	Hexabromide no.	Br., %	M. p. bromide, °C.
(1) Crystal.	83.5	279.0		1.4775	258.1	150.1	75.5		
(2) Crystal.	85.1	279.2		1.4775	260.0	152.6	72.0		
(3) Crystal.	88.0	278.9	−11.2–.0	1.4780	262.0	154.1	83.0	63.27	
(4) Crystal.	84.0	278.6	−11.6–.5	1.4795	259.0	151.3	83.2	63.38	180.4– .5
(5) α-Acid	100.0	278.8	−14.5–.4	1.4795	269.5	161.0	70.1[a]	63.13	178.5–9.5
Calcd.	100	278.2			273.7	182.5	272.3	63.32	

[a] This value was slightly higher, *i. e.*, 75, when a large amount of the acid was brominated in 5% solution. Hexabromide numbers reported above were averages of at least two determinations. It is worthy of note that redeterminations of these values eight months later, the specimens having been kept under inert gas at −20°, did not differ appreciably from the above values.

or may not be a mixture of isomeric linolenic acids.

Specimens (1), (2) and (3), described in Table IV, were derived from linseed oil while (4) and (5) originated from perilla oil. With regard to (5), the α-acid, it is assumed that this would be the same acid or mixture of isomeric acids no matter which oil was used in its preparation, since it is derived from ether-insoluble hexabromides. On the other hand, it seems possible that the crystallization acids may be mixtures of isomeric linolenic acids, either of the *cis–trans* or of the double bond position type. Mixtures, resulting from fractional crystallization, may not be identical either as a result of differences in the component linolenic acids of the original oil or as a result of differences in physical properties (solubilities) of these isomers so that certain of the isomers are concentrated by the fractionation. Some of the data support this idea. Specimen (3), 88.0% pure, melted at −11.2°, whereas specimen (4), 84.0% pure, melted at −11.6°. On the other hand, the nearly pure α-acid melted at −14.5°, lower than either. Further, the n^{20} of the 84% acid from perilla oil was actually higher than of the 88% acid from linseed oil and as high as the α-acid. The hexabromide numbers of the 88 and 84% acids were about equal and much higher than that of the α-acid. For these reasons we are inclined to the belief that the crystallization acids prepared from the two oils do not represent identical mixtures of isomeric linolenic acids and, further, with greater emphasis, the crystallization acids as a group are even more different from α-linolenic acid. No evidence for similar differences has been noted in our previous work with linoleic acid. We do not wish at this time to enter into the unanswered question of the occurrence of one or several linolenic acids in the original oils. Nevertheless, we believe that the above evidence seems to favor the multiple nature of "linolenic acid" despite the fact that the melting points of (3), (4) and (5) were quite sharp. The melting point of linolenic acid has not been reported previously.

Three methods have been available previously for the estimation of linolenic acid in fatty acid mixtures: (1) calculation from the iodine number; (2) the thiocyanogen method of Kaufmann[5] which is based on calculation from simultaneous equations using iodine and thiocyanogen numbers; and (3) the hexabromide number. The first of these methods is rarely practical since linolenic acid always occurs with unknown amounts of oleic and linoleic acids which cannot be separated quantitatively by any known procedure. The second method is the best of the three, but fails in being specific since it does not differentiate possible isomeric linolenic acids and also because thiocyanogen does not react quantitatively with two of the double bonds. The thiocyanogen number of the pure α-acid above fails by 21.5 units to approach the theory as expected from reaction with two double bonds. This point has been verified likewise by Kimura.[6] The third method is the oldest of the three and is based on calculation of the amount of linolenic acid present in a mixture from the amount of ether-insoluble hexabromides assuming the formation of such bromides to be quantitative. With one exception, however,[2] no one ever has claimed to have isolated a linolenic acid which would give the theoretical hexabromide number. Both Erdmann's and Rollett's linolenic acids, prepared by debromination, gave yields of hexabromides which were only 23–25% of the theoretical, which agrees well

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(5) Kaufmann and Keller, *Z. angew. Chem.*, **42**, 70, 73 (1929); Kaufmann, *Untersuch. Lebensmittl.*, **51**, 15 (1936).

(6) Kimura, *J. Soc. Chem. Ind.* (*Japan*), **32**, 451 (1929).

with our results for the α-acid. The hexabromide numbers of the five preparations in Table V calculated to the basis of 100% purity are as follows:

Preparation	Purity, %	Hexabrom. no.	Hexabrom. no. calcd. to 100%
(1)	83.5	75.5	91.6
(2)	85.2	72.0	84.5
(3)	88.0	83.0	94.3
(4)	84.0	83.2	99.0
(5)	100.0	70–75	70–75*

In previous work on arachidonic acid[7] an equation was suggested for the determination of the amount of this acid present in fatty acid mixtures based on the polybromide numbers of pure arachidonic acid and of the fatty acid mixture. A similar equation was proposed for linoleic acid, based on tetrabromide numbers.[4b] We hesitate to apply this principle to linolenic acid other than as an approximation, because obviously, from the above data, the hexabromide number is dependent on the concentration of acids being brominated and especially because it varies in different preparations of linolenic acid. The average hexabromide number for the four crystallization specimens, calculated to 100% purity, is 92.3 or in round numbers 92. From this an approximate value for the content of linolenic acid in a mixture could be found from the equation

$$\%\ \text{linolenic acid} = \frac{\text{H. N.} \times 100}{92}$$

where H. N. is the hexabromide number of the fatty acid mixture and 92 is the average hexabromide number for crystallization linolenic acid. In calculating the average, the H. N. for the α-acid is omitted for the reason of its obvious difference from the crystallization acids. It also should be pointed out that the hexabromide numbers calculated to 100% are slightly high because the percentage purities were calculated from the iodine numbers as a mixture of linoleic and linolenic acids. In spite of these objections to the method, we believe it to constitute a decided advance over any other now available both in accuracy and in specificity.

Summary

1. Low temperature crystallization procedures for the isolation of linolenic acid have been applied to the fatty acids of linseed and perilla oils.
2. By fractional crystallization of the fatty acids of these oils in acetone, 75% linolenic acid remained in the filtrate at −75°.
3. Crystallization of the 75% acid from petroleum ether resulted in preparations of 83.5 to 88% purity.
4. The constants of the crystallization acids are compared with those of α-linolenic acid, prepared by debromination.
5. The possibility of the multiple nature of linolenic acid is discussed; also the application of the hexabromide number as a method of estimating the amount of linolenic acid present in fatty acid mixtures.

COLUMBUS, OHIO RECEIVED AUGUST 15, 1938

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[7] Ault and Brown, *J. Biol. Chem.*, **107**, 615 (1934).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF CORNELL UNIVERSITY]

The Colloid Chemistry of Leaf and Flower Pigments. I. The Precursors of the Anthocyanins[1]

BY WILDER D. BANCROFT AND JOHN E. RUTZLER, JR.

Introduction

Most of the research work on leaf and flower pigments has been done by organic chemists who were interested primarily in preparing and analyzing pure compounds, or by biologists who were interested primarily in genetics. It has seemed worth while to us to present to some extent the viewpoint of those colloid chemists who are interested especially in the changes of color with changing conditions.

Some points are much simpler when considered as colloid chemistry. Chlorophyll is soluble in benzene; but benzene will take scarcely any chlorophyll out of the green leaf.[2] Tswett[3] has shown that this is due to marked selective adsorption of the pigment of the leaf tissue. The phenomena of co-pigments or intensifying agents, studied by the Robinsons,[4] become more simple when considered as due to adsorption.

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[1] Original manuscript received December 13, 1935.

[2] Willstätter and Hug, *Ann.*, **380**, 177 (1911).

[3] Tswett, *Ber. deutsch. bot. Ges.*, **24**, 318 (1906).

[4] Robinson and Robinson, *Biochem. J.*, **26**, 1663 (1932).