A Study of the Hexabromide Number and Hexabromide Yield of Pure Linolenic Acid With a Suggested Interpolation Method for Its Estimation

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ACCURATE thiocyanometric and spectroscopic procedures for the estimation of the linolenic acid content of the drying and semi-drying oils have become available in recent years. However, neither of the technics is specific for this important fatty acid, the accuracy of the findings depending on the validity of the assumption that the only triene unsaturation present is actually linolenic acid (cis-cis-cis 9,12,15-octadecatrienoic acid). In the light of present knowledge the assumption seems to be valid in most instances so that both procedures have been of great value in the examination of the mixtures of fatty acids found in the lipids.

Only two specific tests for linolenic acid are now recognized, namely the isolation of linusic acid, following oxidation with cold permanganate, and the formation of the characteristic hexabromostearic acid by treatment of fatty acid mixtures in cold ether with bromine. The former test is of definite value, but for various reasons has never been proposed as a quantitative technic. The hexabromide test was used as early as 1920 by Steele and Washburn (1) as a method of detecting linolenic acid in drying oils. In early practice it was the custom to weigh the precipitated bromides and calculate the linolenic acid content of the original mixture from the 37.3% of linolenic acid in these bromides. Such a calculation, however, introduces an enormous error because of the recognized fact that upon bromination linolenic acid yields ether-insoluble bromides amounting to only about one-fourth to one-third of the theoretical yield, corresponding to a hexabromide number (per cent yield of hexabromides) of 70 to 90 (theory 272) (2, 3). Shinowara and Brown in 1938 (3) found the hexabromide number of a specimen of debromination linolenic acid to be 75, and the average of several specimens prepared by low temperature crystallization to be 92, and suggested the use of the equation,

$$\text{linolenic acid, per cent} = \frac{\text{HN} \times 100}{92}$$

where HN is the hexabromide number of the specimen of fatty acids under investigation. They proposed this equation as a method of specifically estimating linolenic acid in fatty acid mixtures in spite of the fact that "the hexabromide number is dependent on the concentration of acids being brominated and especially because it varies in different preparations of linolenic acid." Later, Matthews, Brode, and Brown (4) successfully resolved debromination linolenic acid by repeated low temperature crystallization. Their final crystal fraction, probably the purest specimen of linolenic acid so far prepared, had an iodine number of 273.8 and a hexabromide number of 96.0. The substitution of this value in place of 92 in the equation above is logically indicated. However, we have realized for many years that because of solubility of hexabromides in ether and of other factors the hexabromide yield, either from the pure acid or from fatty acid mixtures, is in general a function of the amount of linolenic acid present. Errors as a result of use of the corrected equation will increase, therefore, with decreasing amounts of linolenic acid in the sample. It has been our experience, further, both with methyl arachidonate (5) and with linolenic acid that correction of the bromide yield for solubility of the bromide in ether is not the answer to the problem.

In 1947 Kass *et al.* (6) reported a study of the hexabromide number and again emphasized the empirical nature of this value. Upon brominating specimens of linseed oil fatty acids, ranging from 1.07 to 0.101 g., the hexabromide numbers were found to be 49.5 to 19 respectively. When these values were corrected for solubility of hexabromides in ether, the results ranged from 51.5 to 41.8. Their hexabromide number procedure involved a much more elaborate series of ether washings than we use in the method described later. By their method, without the solubility correction, the average hexabromide number of linseed oil acids was 32 over the range of samples studied.

Work reported in the present paper was completed before publication of the results by Kass and coworkers. Our findings are not intended as criticism of their work. It should be pointed out, however, that if the linolenic acid-hexabromide relationships are empirical, it is time lost to go through the extensive additional washings they have used unless it can be shown that the extra washings are essential to the attainment of a constant result. The data in Tables I and III show that by the method we have used satisfactory checks can be attained.

In the present work we have used Matthew's specimen of recrystallized debromination linolenic acid (4), from which presumably most of the isomeric linolenic acids, yielding no ether-insoluble hexabromides, had been removed. Specimens of this pure acid alone and with added olive oil fatty acids were brominated and the bromide yields determined by an empirical procedure. When linolenic acid was brominated alone, hexabromide numbers ranged from 96.0 with a 1.5 g. sample of acid to 40.2 with 100 mg.; with 25 mg. of acid no bromide precipitation occurred. The addition of olive oil fatty acids to the sample before bromination resulted in a small but appreciable decrease in hexabromide yields. The limit of sensitivity of the test is between 25 and 35 mg. of linolenic acid; in a sample of mixed fatty acids amounting to 2.0 g. this amounts to about 1.5%.

We propose to determine linolenic acid in fatty acid mixtures by interpolation from a curve in which are plotted amounts of linolenic acid brominated against hexabromide yields, the latter being determined in the presence of 2 cc. of olive oil fatty

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* Supported in part from funds granted by the Ohio State University Research Foundation to the University for aid in fundamental research.

acids by the exact procedure described below. Since this curve takes into account the empirical nature of the hexabromide yield over the entire range of values, it should be useful in quantitative estimations to the lower limit of accurate analytical weighings. The errors will be inordinately large when the mixture brominated contains less than 50 mg. of linolenic acid.

A specimen of soybean oil fatty acids has been assayed by the proposed method and shown to contain 8.8% of linolenic acid. Bromination of these acids at 15° instead of 0° and addition of 2.5 cc. of alcohol to the ether solution before adding bromine lowered the observed linolenic acid content. Addition of 1 cc. of acetic acid, on the other hand, appreciably enhanced the observed value.

Generally speaking, our results on linolenic acid are similar to those we have previously reported for methyl arachidonate (5). In the near future we hope to report results of applying our interpolation curve for estimating linolenic acid in fatty acid mixtures in comparison with spectroscopic and thiocyanometric values.

Experimental

Description of Procedure for Determining the Hexabromide Yields. This method was described in detail previously (5) in our study of bromination of methyl arachidonate. Briefly, it includes bromination of the sample in 35 cc. of anhydrous reagent ethyl ether at 0 to 5° in a tared 50-cc. centrifuge tube. An excess of bromine is added slowly while cooling in an ice-water mixture. After standing at least 4 hours the tube and contents are centrifuged and the ether supernatant poured off. The residual bromides are washed three times with 35-cc. portions of cold ether and efficient stirring and centrifugation each time. After the last washing the bromides in the centrifuge tube are dried overnight at about 60°C. and weighed. Precise analytical conditions must be followed at all times. Specimens of acid larger than 350 mg. were weighed directly under nitrogen from a dropper weighing bottle. With smaller amounts known weights of the acid were dissolved in ether and aliquots taken for samples.

Description of Linolenic Acid. We were fortunate to have available a reserve specimen of Matthew's purest acid (4). The reported hexabromide number of this acid, namely 96.0, agrees exactly with the value reported below (Table I) for 1.5 g. specimens of the acid. This we interpret as indicating no deterioration during six years' storage in sealed evacuated ampules at —25°. Our data below have been necessarily limited by the amount of pure linolenic acid available. Incidentally, another of Matthew's specimens, which he reported to have a hexabromide number of 94.0, was at our disposal; a 1.5-g. sample gave a hexabromide number of 94.8; purity by our curve was 98.6%.

The Hexabromide Yields From Varying Amounts of Linolenic Acid With and Without Addition of Olive Oil Fatty Acids. The method described above for determining hexabromide yield was applied to amounts of linolenic acid varying from 1.5 g. to 25 mg. Because of the predicted solubilizing effects of other fatty acids and especially of bromo-acids resulting from addition of bromine to oleic and linoleic acids, a series of brominations was carried out in the presence of 1-2 cc. of olive oil fatty acids. It would perhaps have been preferable to have used cottonseed or corn oil fatty acids for this purpose, but we were anxious to employ a fatty acid mixture composed mostly of unsaturated acids and to avoid the possibility of using one which would contain appreciable amounts of linolenic acid. The detailed results are shown in Table I.

TABLE I

The Hexabromide Yields From Various Amounts of Linolenic Acid, With or Without Added Olive Oil Fatty Acids

Linolenic acid, mg.	Hexabromide yield, mg.	Hexabromide number	Linolenic acid, mg.	Hexabromide yield, mg.	Hexabromide number
1493.6	1435.7	96.1	50	15.6	31.2
1495.1	1435.0	96.0	50	14.8	29.6
1516.8	1457.4	96.0	50	14.1	28.2
		Ave. 96.0	50	13.2	26.4
			50	13.1	26.2
					Avg. 28.1
1004.4	944.4	94.1			
1012.9	950.6	93.8	50[1]	10.1[1]	20.0[1]
1010.8	951.4	94.1	50[1]	10.1[1]	20.2[1]
		Avg. 94.0	50[1]	10.7[1]	21.4[1]
					Avg. 20.5[1]
1016.6[1]	913.1[1]	89.8[1]			
			40[1]	4.3[1]	10.8[1]
350	297.4	84.2	40[1]	3.7[1]	9.3[1]
350	292.4	83.5			Avg. 10.0[1]
		Avg. 83.8			
350[1]	276.3[1]	78.9[1]	35[1]	1.7[1]	4.9[1]
350[1]	278.8[1]	79.7[1]	35[1]	2.0[1]	5.7[1]
		Avg. 79.3[1]			Avg. 5.4[1]
100	43.6	43.6	30[1]	1.0[1]	3.3[1]
100	44.1	44.1	30[1]	0.5[1]	1.7[1]
100	42.2	42.2			Avg. 2.5[1]
100	44.5	44.5			
100	45.0	45.0	25	2.5	10.0
		Avg. 43.8	25	1.6	6.4
					Avg. 8.2
100[1]	39.9[1]	39.9[1]			
100[1]	40.4[1]	40.4[1]	25[1]	0.0[1]	......
		Avg. 40.2[1]	25[1]	0.3[1]	......
			25[1]	0.1[1]	......
100[2]	39.3[2]	39.3[2]	25[1]	0.0[1]	......

[1] Two cc. of olive oil fatty acids added.
[2] Two cc. of corn oil fatty acids added.

The empirical relationship between the amounts of linolenic acid brominated and the hexabromide yield are shown in the variation of hexabromide numbers in Table I; these fall from a value of 96.0 when a 1.5-g. sample of the acid is brominated to practically 0 with a 25-mg. sample. On the other hand, with 350 mg. of the acid the hexabromide number is still as high as 83.8; it is lowered only to 79.3 in the presence of 2 cc. of olive oil acids. Likewise it is fully obvious that use of a constant factor, i.e., 96, in calculating the linolenic acid content of an acid mixture will result in increasing errors as the amount of linolenic acid in the mixture under investigation decreases. A 2-gram sample of acids containing only 25 mg. of linolenic acid will fail to give detectable hexabromides. Whatever the weight of the sample, it must contain over 25 mg. of linolenic acid for its detection and as much as 50 mg. to secure even a semi-quantitative estimation. If the method is to be applied, therefore, to mixtures containing as low as 1-2% of the acid, the mixture must first be concentrated by low temperature crystallization.

The hexabromide method can be employed as a specific and reasonably accurate quantitative estimation by employing the average values in Table I in the construction of a curve showing the relationship between amounts of linolenic acid brominated and the hexabromide yield. This curve can be based on data either with linolenic acid alone or with linolenic acid brominated in the presence of olive oil acids. In Table II we have listed these values.

The curve derived from values in Table II with olive oil acids was used to determine the linolenic acid content of a specimen of soybean oil fatty acids.

TABLE II
Resumé of Linolenic Acid-Hexabromide Data

Linolenic acid, mg.	Hexabromides, mg.	
	Alone	With olive oil acids
1500	1440	1416[1]
1000	940	906[2]
350	293.3	277.8[3]
100	43.8	40.2[3]
50	14.1	10.3[3]
35	....	1.8[3]
25	tr.	0 [3]

[1] With 0.5 cc. olive oil acids.
[2] With 1.0 cc. olive oil acids; with 2.0 cc., this value is 898.
[3] With 2.0 cc. olive oil acids.

TABLE III
The Linolenic Acid Content of a Specimen of Soybean Oil Fatty Acids, as Evaluated From the Standard Curve. Effect of Altering the Bromination Conditions

Soybean acids, mg.	Hexabromides, mg.	Linolenic acid, mg.[1]	Linolenic acid, per cent	Modification of method
2014.6	112.3	178.3	8.85	None
2003.2	111.9	177.9	8.88	None
2002.6	111.8	177.0	8.84	None
1997.7	42.3	102.3	5.12	2.5 cc. of alcohol added before bromination
2006.0	118.5	184.6	9.20	1 cc. glacial acetic acid
1989.8	102.9	167.1	8.04	Brominated at 15°

[1] From curve.

In Table III they are compared with results of the same specimen as observed when the conditions of bromination are altered.

Summary

1. Varying amounts of a specimen of pure linolenic acid have been brominated and the hexabromide yields have been determined by an empirical procedure. The acid was brominated alone and in the presence of olive oil fatty acids.
2. It is suggested that linolenic acid can be specifically and quantitatively determined in fatty acid mixtures by interpolation from a curve showing the linolenic acid-hexabromide yield relationships over the range cited.
3. The empirical nature of the hexabromide yield is confirmed.

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The Study of Aluminum Soap-Hydrocarbon Systems: Effect of Additives and Metathesis[1]

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THE behavior of systems containing only an aluminum soap, such as di-laurate, and a hydrocarbon has been accounted for by the existence of two phases, each stable over a range of temperature and composition: a *gel* of swollen lumps of soap, and a homogeneous isotropic phase called *jelly* or *sol* depending on its rigidity, which varies continuously from a thin liquid when hot and dilute to an elastic semi-solid when cold and concentrated. The transition between these two phases has been described (4) as well as the effect of time and temperature on the viscosity of the jelly (5).

Napalm, a thickener of gasoline for flame warfare purposes, is essentially a mixture of aluminum disoaps and shows the same type of behavior as a pure soap (6). At room temperature it rapidly forms a jelly in gasoline.

Impurities or added materials are known to have a great influence on the viscosity and stability of these jellies and have also been shown to influence the swelling of the soap and the temperature of transition from gel to jelly (4).

In the present paper we describe a few experiments showing the variety of effects produced by different additives on a given soap, and by the same additive on similar soaps, and show that one of the possible mechanisms of action of additives is metathesis between acidic additives and the soap.

Variety of Effects

Experimental. In most of these experiments jellies of 4.00% weight/volume of a Napalm in commercial gasoline containing M/8 or M/40 additive were used. Soap samples of approximately 1 gram were placed in 100-cc. screw-top bottles and brought to the same moisture content by being stored overnight together in an evacuated desiccator. Their exact weights were then determined and the necessary volume of gasoline containing additive added. The systems were gently shaken until no visible settling occurred ("stir-time") and the "set-time," when the gel held a definite shape, was noted. After a day or more, the apparent relative viscosity of the undisturbed jelly in each bottle was estimated by measuring the velocity of fall of steel balls with the help of a traveling microscope and a stopwatch. In Newtonian liquids the rate of fall of spheres is proportional to the square of their radii and the value tr^2/l is a constant. In pseudo-plastic jellies it generally decreases, but variations of tr^2/l with r give an indication of the tendency of the jelly towards pseudo-plasticity if

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[1] Presented before the Division of Colloid Chemistry at the 113th meeting of the American Chemical Society, April 19, 1948, in Chicago, Illinois. Study conducted under Contract OEM-1057 between Stanford University and the Office of Emergency Management, recommended by Division 11.3 of the National Research Council, and supervised by Prof. J. W. McBain.
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