Summary

The causes of the considerably better keeping quality of elaïdinated oils and fats have been investigated through the rate of peroxide formation. They are physical as well as chemical in nature. The more solid composition of elaïdinated fats is the physical cause.

Traces of selenium have been proved to behave as a strong antioxidation agent. The elimination of traces of metals in the course of the process of elaïdination with Se also has a very favorable influence.

The elaïdo configuration of the unsaturated acids, however, has been shown to be the principal factor in retarding oxidation.

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REFERENCES

1. S. H. Bertram. Chem. Weekblad *33*, 3, 216, 637 (1936).
Oele, Fette, Wachse No. 6, 1 (1936); No. 7, 1 (1938).
Rec. Trav. Chim. *59*, 650 (1940).
2. R. Kuhn and K, Meyer. Zeitschr. physiol. Chem. *185*, 193 (1929).
K. Täufel and B. Spiegelberg. Chem. Umschau *37*, 281 (1930).

The Application of the Polybromide Yield in the Estimation of Methyl Arachidonate in Methyl Ester Mixtures

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IN a report from this laboratory in 1928 (1) it was noted that when a specimen of methyl arachidonate (methyl 5,8,11,14-eicosatetrenoate), prepared from liver lipids, was brominated in cold ether, only a small fraction of the predicted amount of insoluble polybromides resulted. The polybromide number, i.e., the per cent yield of polybromides, of the ester in question was 77.6 instead of the predicted theoretical value, 301. A similar observation with respect to esters of the highly unsaturated fatty acids of fish oils had been made by Brown and Beal in 1923 (2). On the basis of this behavior generally of acids and esters of this type and of this specific finding for methyl arachidonate it was proposed that the methyl arachidonate (MA) content of methyl ester mixtures could be calculated from the following equation:

$$\text{MA per cent} = \frac{\text{PN} \times 100}{77.6}$$

where PN is the polybromide number of the ester mixture in question. The following year one of us (J.B.B.) discussed this method of estimation further (3) and suggested a possible application of the method to linolenic acid (and its methyl ester). As improvements were made later in the method of preparation of MA and as purer specimens become available for study, the yield of polybromides was found to be somewhat higher. Thus Ault and Brown (4) reported a PN of 86.5 for a specimen of the ester which was prepared by debromination. Shinowara and Brown prepared specimens of MA by a combination of crystallization and distillation procedures, thus avoiding the possibility of isomerization during bromination and debromination. Although the purity of the products as calculated from their iodine numbers did not exceed 95%, the average PN of their preparations, calculated to 100% purity, was 90.7. Mowry, Brode, and Brown (6) reported a PN of 92.7 for their best product prepared by physical methods. In the meanwhile Smedley-Maclean and Nunn (7) had brominated arachidonic acid in 2% solution in several solvents, their results, expressed as PN's being the following: in 50-60° boiling petroleum ether, 256; in ether, 86-90; in carbon tetrachloride, 89; and in benzene, 100. They noted that about half of the polybromides insoluble in benzene and two-thirds of the bromides insoluble in petroleum ether were soluble in ethyl ether.

In 1930 A. K. Silberman (8) in this laboratory found the solubility of methyl octabromoarachidate in ether to be 0.0127 g./100 cc. of solution at 2-3°. The corresponding ethyl and butyl esters were about 10 times as soluble. Stoneburner (9) in this laboratory in 1940 found a somewhat higher solubility for the methyl ester in ether, namely 0.0409 g./100 cc. He brominated amounts of MA ranging from 2.119 g. to 0.0704 g. and obtained PN's (with five ether washings of product) of 98.3 to 49.7, respectively. However, when he attempted to correct these values (PN's) for dissolved bromides in the wash ether, anomalous values, namely PN's of 98.9 to 153.7, resulted. Thus in this laboratory we have realized for several years that the use of an empirical equation for the estimation of MA, or of other polyethenoic acids, based on polybromide (hexabromide or tetrabromide) numbers will result in considerable error in ester or acid mixtures of low contents of the ester or acids in question. In such mixtures the solubility error is excessive and solubility corrections are apparently not constant, probably because we are dealing with complex mixtures of isomeric polybromides.

Cramer (10) in this laboratory in 1942 attempted to correct for these errors in a large series of ester mixtures from human body fats by interpolation from a curve, the data for which were obtained by brominating 20, 30, 50, and 70 mg. of MA and determining the polybromide yields by essentially the method we have described below. The resulting yields of bromides were 8.9, 15.3, 28.7, and 43.5 mg., respectively, corresponding to polybromide numbers of 44, 51, 56, and 62. In his original work Cramer felt that the arachidonate content of an ester mixture could be found by interpolation from a curve in which he plotted arachidonate percentages against polybromide numbers based on the examples cited above.

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However, it is to be noted from the data in Table IV that the polybromide number of a given ester mixture is dependent on the weight of the sample so that Cramer's curve would be valid only when the sample brominated amounted to exactly 1 g., or when the sample was approximately this amount and was corrected to exactly 1 g. To illustrate, when a 3.0 g. sample of adrenal esters was brominated, the polybromide number found was 26.6; on the other hand, a 0.25 g. specimen gave a PN of 19.1. Obviously, the bromide yield is related to the amount of arachidonate brominated and is affected by solubility relationships and perhaps other factors, but, as we have shown later (Table II), it is not appreciably affected by the presence of other soluble bromides.

In view of these considerations we propose to determine the methyl arachidonate content of ester mixtures by the use of a curve drawn from data in which amounts of MA were brominated, ranging from 2.0 g. to 3 mg., and the respective amounts of polybromides determined by a precise analytical procedure. In this curve polybromide yields are plotted against amounts of MA brominated.

Five specimens of MA were employed in the present study. Two of these, MA-III and MA-V, we believe to be the purest specimens of the ester so far prepared. Iodine numbers and molecular weights are very close to the theoretical values for methyl eicosatetrenoate. MA-V has been taken as a standard because the bromide yields were highest from this specimen. This criterion is believed to be the most specific indication of the highest purity although, as brought out later, MA-V still contains about 1.6% of conjugated material and an unknown amount of isomeric eicosatetrenoate. Thus if the total impurity in the ester is 5%, estimation by the proposed interpolation method would give values too high in that proportion.

By the use of the curve described it has been possible to compare the purities of all five specimens. Thus MA-III, the purity of which is equal to that of MA-V if evaluated from the iodine number and molecular weight, is only 99.3% pure on the basis of the bromide yield (Table III).

Some of the factors affecting bromide yield were studied. Bromination at 15°, instead of 2-3°, had little effect on the results. Addition of 1 cc. of glacial acetic acid to the bromination mixture raised the yield of bromides appreciably, but 3 and 5 cc. portions of this acid reduced the yield. Addition of 2 cc. of the methyl esters of olive oil to varying amounts of pure ester had a negligible effect on the yield.

The polybromide method of estimating MA in ester mixtures is especially valuable when used along with the spectroscopic method. Within its admitted limitations the former method is highly specific while, on the other hand, the spectroscopic method will give accurate results for total tetrenoic ester. If the results by the two methods are at variance to an important degree, and especially if results by the latter method are higher than those by the bromide method, they may be interpreted as evidence for the presence of isomeric tetrenoic esters.

Experimental

The Procedure for Determining the Polybromide Yield. Since the proposed method of estimating MA is dependent on the polybromide yield, which in turn is affected by solubility relationships and other factors, it is to be emphasized that details of the method used in determining the yield of bromides must be followed exactly. The procedure follows: A sample of the ester is weighed into a 50 cc. centrifuge tube and 35 cc. of cold, anhydrous ether is added. The tube with contents is set in a water-ice mixture. The ether solution is stirred with an electric stirrer fitted with a bent tip, glass rod of about 4-5 mm. diameter. Bromine is added dropwise from a small separatory funnel until its color persists. The stirrer is removed, washed with 2-3 cc. of cold ether, the centrifuge tube stoppered and kept at 2-3° for at least four hours. After centrifugation the supernatant ether is decanted, 35 cc. of fresh cold ether added, stirred about 5 minutes, and again centrifuged. Three such washings with 35-cc. portions of fresh ether are made, making a total of four centrifugations and decantations. After the final washing, the tube containing residual, white polybromides, is placed on its side and kept at 50-60° overnight. During this evaporation of solvent the tube is covered with a sheet of filter paper to avoid contamination with dust. It is finally cooled and weighed. All the precautions of a gravimetric analysis should be followed. Thus it is desirable, although not absolutely necessary, that the original centrifuge tube be cleaned and dried in a desiccator before the determination is begun, and cooled and dried in a desiccator before final weighing. However, if laboratory humidity is relatively constant, checks can be obtained by simple air-drying before and after. Precautions should also be taken to avoid oxidation of sample during the original weighing. We usually employ a small nitrogen-filled dropper weighing bottle in weighing the sample. Specimens larger than 350 mg. are weighed directly. With specimens of 350 mg. or less, a volumetric solution of the ester in ether is usually employed, the required specimens being measured by pipette, and the volume made up to 35 cc. with ether when necessary. Our arachidonate specimens are kept in evacuated ampules at —20° when not in use.

Description of Specimens of Methyl Arachidonate. Five specimens of MA were used in this work. The first two of these, MA-I and MA-II were prepared several years ago, the former by debromination and the latter by a combination of crystallization and distillation technics. Both were redistilled before they were used. Although both gave iodine numbers of 306, indicating a purity of about 95%, it was subsequently found by application of the method here proposed that their purities were not greater than 92.5 and 88.5%, respectively.

Specimens MA-III, MA-IV, and MA-V were prepared by the following method: 600 g. of the methyl esters of beef adrenal phosphatides were brominated in 3 l. of cold ether in a 5-l. R.B. flask. The bromine was added slowly, the temperature at no time rising above 15°. After standing overnight, the upper ether layer was siphoned off and enough fresh cold ether added to fill the flask. After shaking, the ether was again allowed to settle and was removed. Three additional ether washings were made; a total of four washings subsequent to the original separation. To the layer of ether and polybromides remaining in the flask was added a liter of C.P. methanol. The ether was then largely removed by distilling through a column under such conditions that most of the alcohol was returned to the flask. To the resulting suspension of polybromides in alcohol was added, with continuous refluxing and in small portions, 170 g. of zinc powder. During the early stages of the debromination small amounts of conc. HCl were added from time to time through the top of the condenser to catalyze the reaction, the total HCl added being about 25 cc. After a reaction period of 18 hrs., the residual zinc and other insoluble reaction products ($ZnBr_2$) were allowed to settle, the upper clear methanol layer poured off through a Büchner suction filter, the residue washed several times with fresh hot acidified methanol, and the combined methanol filtrates were reduced to 400-500 cc. under reduced pressure. About a liter of water was then added. The layer of MA which rose to the top (under H_2 gas) was quantitatively removed by ether extraction, the ether solution transferred to a small distillation flask, the ether distilled off and the product finally distilled under reduced pressure. The yields, with analytical data on the products, are shown in Table I.

TABLE I

Yields and Analytical Data on MA-III, MA-IV, and MA-V

Esters Brominated, g.	Yield MA, g.	Mol. Wt.	Iod. No.	Thiocyanogen No.[1]			
				M.B.B. (11)	L.and D. (12)	AOCS (13)	P.N.[2]
600	34.6 (MA-III)	318.2	321.1	180.8	179.1	194.5	90.4
613	35.3 (MA-IV)	321.0	304.5[3]	....	169.4	....	66.5[2]
596	38.0 (MA-V)	316.5	321.6	180.3	178.8	....	90.3

[1] Thiocyanogen number determinations were made by the three procedures cited.
[2] P.N. on 1 g. specimens.
[3] MA-IV is seriously isomerized.

The adrenal esters, used in Table I, contained, as shown later, 29.4% of arachidonate. Thus from 600 g. of these esters the theoretical yield would be about 180 g. of the desired product whereas in the preparation of MA-V only 38 g. was obtained, or 21% of the theory. The principal loss occurred not in the debromination reaction but in the low yield of ether-insoluble bromides. In comparison, it is to be noted that by the crystallization-distillation method it would be possible theoretically to get a much higher yield of product, but the resulting product by this purely physical procedure has so far not been more than 95% pure (5, 6) with respect to total eicosatetrenoate.

All three specimens of arachidonate, described in Table I, were examined for us spectroscopically by B. W. Beadle, of the American Meat Institute Laboratory of the University of Chicago. MA-III and MA-V are being used as spectroscopic standards and will be described in detail by him elsewhere. His findings, before alkali isomerization, are of special interest and are as follows:

	MA-III	MA-IV	MA-V
Diene Conjugation, percent	0.84	6.16	1.2
Triene Conjugation, percent	0.16	1.05	0.35
Tetraene Conjugation, per cent	tr	tr	0.04

Spectroscopically MA-III, MA-IV, and MA-V are shown to be 99, 92.8, and 98.4% pure, respectively, if the only impurities are considered to be conjugated materials. MA-IV, assayed by the proposed bromide method (see Table III), is only 71.5% pure. The remaining 28.5% must be composed in part of 7.2% of conjugated esters (diene and triene conjugation) and of 21.3% of material of unknown nature. The molecular weight of MA-IV is 321 (Table I), very close to that of eicosatetrenoate (318.6). Its iodine number (304.5), on the other hand, is seriously low for tetrenoate. It seems likely, therefore, that the 21.3% impurity in question will include both isomerized (non-conjugated, cis-trans) eicosatetrenoate and other more fundamentally altered C_{20} esters.

Previous determinations of the thiocyanogen number of MA were made by Shinowara and Brown (5); who reported 161.1, and by Cramer (10), who found a value of 167.3. These data were obtained on arachidonate preparations which were not as pure as MA-III or MA-V. Thiocyanogen numbers, reported in Table I, were determined by the procedures of Matthews, Brode, and Brown (11), Lambou and Dollear (12), and by the official procedure of the American Oil Chemists' Society. In all cases the results (Table I) are substantially higher than previously reported, average values for MA-III being 180.8, 179.1, and 184.5 by the three methods, respectively.

Bromination Experiments on MA-V. In the preliminary work leading up to the very complete study of the arachidonate-polybromide relationships on MA-V we used specimens MA-I and MA-II although at the time we realized they were not more than 95% pure. Our data, however, were corrected to 100% purity, and the results gave us workable curves of reasonable accuracy. Since in later work MA-V was found to give the highest yields of polybromides of any of our preparations, we assumed this to be the purest specimen and have used it to obtain our final reference data. In Table II are included results on polybromide yields from amounts of MA-V varying from 3 mg. to 2.0 g. Certain data are also included in which the methyl esters of olive oil, which contain no polyethenoic acids, were added.

The data in Table II show that the use of a constant factor in calculating the methyl arachidonate

TABLE II

Polybromide Yields From Varying Amounts of MA-V, Alone and With Added Olive Oil Esters

Amount MA-V Brominated, mg.	Olive Oil Esters Added, cc.	Polybromides, mg.	P.N.
2002.2	0	1964.7[1]	98.1
1504.5	0	1452.6	96.5
1501.1	0	1448.0	96.4
1001.6	0	922.8	92.1
995.7	0	916.0	92.0
1000.7	1.0	926.5[1]	92.6
492.9	0	417.3	84.7
350	0	276.9(4)[2]	79.2
350	2.0	280.3(4)[1]	80.1
100	0	64.2(4)	64.2
100	2.0	65.9(4)[1]	65.9
50	0	30.5(4)	61.0
50	2.0	31.4(4)[1]	62.8
10	0	4.6(4)	46
10	2.0	5.0(4)[1]	50
5	0	1.4(6)	......
5	2.0	1.7(6)[1]	......
3	0	1.1(6)	......
3	2.0	1.4(6)[1]	......

[1] See text for suggested use of these data in analyzing ester mixtures.
[2] Figures in parentheses indicated that the result is an average of this number of determinations.

(MA) content of ester mixtures, as has been suggested by previous reports from this laboratory, results in serious errors, especially in mixtures which contain only small amounts of this highly unsaturated ester. This is evident from the fact that the polybromide numbers ranged from 98.1 when a 2-g. sample of MA was brominated to 46 with a 10-mg. sample, clearly demonstrating that the polybromide yield is in part a function of the amount of MA present. Cramer's proposal, therefore, to plot arachidonate (per cent) against polybromide numbers of known ester mixtures would be valid only if the amount of ester specimen brominated were close to 1.0 g. The data in Table II can be used to estimate the MA contents of ester mixtures by drawing a standard reference curve in which weights of arachidonate are plotted against yields of polybromides over the range of values cited. The results either with or without added olive oil esters can be used in the composition of this curve. Because added olive oil esters have little effect on the results, we suggest the use of the data obtained without these esters. The curve is drawn in two sections on coordinate paper 10 lines to the inch. The first section of the curve is drawn from arachidonate-polybromide data over the range 100-2,000 mg. MA, ten mg. per line. The accuracy with which the results can be read over this section is about ± 1 mg. The second section of the curve covers the range 5 mg. to 100 mg. of MA, 2 lines per mg.; it is possible to read to ± 0.05 mg. In making a determination, it is suggested that a 2-g. sample of mixed methyl esters be brominated under the conditions described previously and the bromide yield determined. The amount of MA in the sample may be found by interpolation from the curve. Larger or smaller samples, however, can be used, as shown in Table IV.

To compare the results with the old and the proposed procedures, two examples are cited: 1. A 2-g. sample of esters gives 31.4 mg. of bromide, or a PN of 1.57. By the old method we would obtain

$$\frac{1.57 \times 100}{92.7} = 1.7\%\ \text{MA}$$

By the proposed method we would find that 31.4 mg. of bromide is equivalent to 50 mg. of MA, or 2.5%. 2. 2-g. sample yields 0.9265 g. of bromide (PN, 46.3). By both methods, in this instance the MA content is found to be 50%.

The observation that the addition of olive oil esters had little effect on bromide yield surprised us. The yield of petroleum ether-insoluble tetrabromides from linoleic acid, on the other hand, is seriously affected by the presence of other fatty acids. Linolenic acid is intermediate in its behavior. Olive oil is very rich in oleic acid and contains approximately 10% of linoleic acid. Apparently, the presence of methyl dibromo stearate and of tetrabromo stearate exerts no important solubilizing effect on the methyl octobromoarachidate, the slightly higher values obtained with added olive oil esters being explained, we believe, by simple failure to wash out this large amount of added material under the bromination conditions we have employed.

Comparison of Four Methyl Arachidonate Specimens by the Use of the MA-V Polybromide Curve. We thought it would be of considerable interest to determine and compare the purities of MA-I, MA-II, MA-III, and MA-IV by the use of the standard curve derived from data on MA-V. In Table III are recorded the results of bromination of varying amounts of the four specimens, the MA contents of each specimen being read in each case from the standard curve.

The data in Table III show that satisfactorily accurate determinations of purity of the several specimens of arachidonate are possible even with quantities of esters as small as 50-100 mg. Further, the purities agree reasonably well over a wide range of values with MA-I and MA-II and over a more restricted range as found with MA-III and MA-IV. MA-III is 99.3% pure on the basis of the bromide yield. The remaining preparations, MA-I, MA-II, and MA-IV, are 94-95% pure, as evaluated from their iodine numbers, but, based on bromide yield, their purities are 92.5, 88.5, and 71.5%, respectively. As mentioned previously, the low purity of MA-IV is likely due to the presence of considerable amounts of the cis-trans isomers of methyl arachidonate.

TABLE III

Purities of MA-I, MA-II, MA-III, and MA-IV as Determined by Use of the Standard Curve

Specimen	Amount Brominated, mg.	Polybromides, mg.	Methyl Arachidonate (MA) from Curve	
			mg.	% Purity
MA-I	1566.2	1397.0	1453.0	92.7
MA-I	1489.7	1318.4	1378.0	92.5
MA-I	1000.6	855.9	937.0	93.6
MA-I	747.9	603.8	684.0	91.4
MA-I	700.6	565.4	644.0	91.9
MA-I	355.3	256.6(2)[1]	327.0	92.0
MA-I	101.5	61.1(3)[1]	92.5	93.7
MA-I	50.8	29.3(3)[1]	48.0	94.5
				Avg. 92.5
MA-I	10.2	4.6(3)[1]	10.0	98.0[2]
MA-I	5.1	1.2(3)[1]	4.0	80.0[2]
MA-II	2192.6	1920.2	1959.0	89.4
MA-II	1387.6	1151.9	1222.0	88.1
MA-II	1007.0	806.8	887.0	88.1
MA-II	1001.0	803.6	884.0	88.3
MA-II	764.9	592.9	672.0	87.9
	109.0	63.2	98.4	90.3
				Avg. 88.5
MA-II	5.5	1.7(2)[1]	5.5	100.0[2]
MA-III	1006.8	910.5	993.0	98.6
MA-III	256.5	196.3	256.0	99.8
MA-III	96.5	62.3	97.0	100.4
				Avg. 99.3
MA-IV	910.8	564.0	643.0	70.6
MA-IV	496.3	284.7	360.0	72.5
				Avg. 71.5

[1] Figures in parentheses indicate the result is the average of this number of determinations.

[2] Error is too large to be included in the average.

Factors Affecting the Bromide Yield. As shown in Table IV, we have brominated samples of the original adrenal esters, from which MA-III, MA-IV, and MA-V were prepared, by the standard procedure and with certain variations. The results of applying these bromide yields to the standard curve have been used first to test the validity of the method on an ester mixture over a range of esters brominated and second to show the effect of certain variations in procedure on the results.

The use of the standard curve in estimating the MA content of adrenal methyl esters gave consistent results over a weight range of samples from 3.0 to 0.25 gm. Addition of 2 cc. of 95% ethanol to the bromination mixture lowered the observed finding about 1.0%, while a like amount of glacial acetic acid increased the result about 2.5%. Larger amounts of

TABLE IV

Polybromide Yields of Methyl Esters of Adrenal Phosphatides Under Various Conditions

Adrenal Esters Brominated, g.	Bromide Yield, g.	Methyl Arachidonate From Curve		Variation in Analytical Procedure
		g.	Per cent	
3.1090	0.8291	0.910	29.3	Method as described
3.0085	0.7998	0.880	29.2	Method as described
2.0874	0.5310	0.610	29.2	Method as described
2.0304	0.5195	0.598	29.4	Method as described
1.0959	0.2413	0.310	28.3	Method as described
1.0522	0.2431	0.312	29.5	Method as described
0.5026	0.1085	0.150	29.8	Method as described
0.4952	0.1061	0.148	30.1	Method as described
0.2563	0.0490	0.076	29.6	Method as described
0.2540	0.0486	0.076	29.6	Method as described
			Avg. 29.4	
2.0058	0.4942	0.570	28.4	2 cc. alcohol added to bromination mixture
1.9950	0.4942	0.570	28.5	
2.0042	0.5630	0.640	32.0	1 cc. glacial acetic added
1.9996	0.5589	0.640	32.0	
2.0000	0.4813	0.557	27.9	3 cc. glacial acetic added
2.0000	0.4818	0.557	27.9	
2.0000	0.4666	0.542	27.1	5 cc. glacial acetic added
2.0000	0.4633	0.540	27.0	
2.0069	0.5107	0.586	29.1	15° bromination temp.
2.0048	0.5080	0.584	29.2	15° bromination temp.
2.0000	0.4339	0.510	25.5	Brominated at room temp.
2.0000	0.4369	0.512	25.6	Brominated at room temp.

acetic acid had the opposite effect. Raising the bromination temperature to 15° was without effect on the result, but a temperature of about 25° seriously lowered the findings. In another experiment, not reported in Table IV, the esters were brominated and washed with petroleum ether, in place of ethyl ether. The yield of insoluble bromides was increased almost 100%. These bromides may include, however, both tetra- and hexabromides, or possibly petroleum ether insoluble octabromides.

The procedure for estimating bromide yield, described above, will detect 3 mg. of methyl arachidonate (MA) in the presence of 2 cc. of olive oil methyl esters. Two mg. of arachidonate under these conditions fails to yield detectable precipitated bromides. The method may be made more sensitive qualitatively by the use of only 5 cc. of ether in the original bromination and of similar portions in the washings. Under these conditions, as little as 0.2 mg. can be detected, and there is no reason why, if a more sensitive balance were used and other conditions altered so as to observe the more rigorous requirements of a micro-analysis, the method could not be used quantitatively for amounts of arachidonate from 10 mg. to as little as 0.2-0.5 mg.

Discussion of Results. Since the polybromide-arachidonate relationships are empirical, which we have known for many years, and as has been recently demonstrated for hexabromide-linolenic acid by Kass, Roy, and Burr (14) it is obvious that the accuracy of the proposed method is dependent on following the exact analytical procedure, which has been described. The ultimate accuracy of the method is further dependent on the assumption that the methyl arachidonate used in setting up the standard curve is a chemically pure substance. This assumption has already been shown not to be entirely valid because MA-V contained 1.6% of conjugated unsaturation, which presumably will not yield precipitable bromide. Then there is the added question as to the amount of isomeric eicosatetrenoates present. Debromination linoleic and linolenic acids have already been shown to contain 8 to 15% of dienoic and trienoic acids which do not yield petroleum ether-insoluble tetrabromides and ether-insoluble hexabromides, respeectively (15). However, it was possible to remove most of the contaminating cis-trans isomers from linoleic and linolenic acids by repeated low temperature crystallization (15). As yet, such a separation has not been successful with methyl arachidonate because the ester is a glass-like non-crystalline solid at the temperature of dry ice. To the extent therefore that MA-V contains conjugated esters and other isomers which do not yield ether-insoluble polybromides, the results found in examining ester mixtures by the use of the standard curve will be too high. This has actually been found to be the case with three specimens of the adrenal phosphatide esters, similar to those from which MA-III, MA-IV, and MA-V were prepared. Analyses by the curve were higher than those by spectroscopic examination. We are at present endeavoring to find the reason for this anomaly. Other than the reason postulated above for such results, there is the possibility that the trienoic and dienoic esters in the mixture obtained from the phosphatide, as were unexpectedly shown to be present spectroscopically, may have enhanced the yield of insoluble bromides.

In view of the above considerations, we believe that the bromide method, as we have described it, will give a highly specific estimate of methyl arachidonate in ester mixtures which do not contain linolenate or excessive amounts of linoleate, in contradistinction to the spectroscopic method which will detect total tetraene unsaturation. Incidentally, arachidonate as determined by the bromide method is presumably the nutritionally essential methyl arachidonate; that found spectroscopically is not necessarily this biologically important material.

We are now engaged in attempting to further purify methyl arachidonate by two types of procedures, namely, chromatographic adsorption and crystallization at temperatures below that of dry ice.

Summary

1. The preparation of and analytical data on three specimens of methyl arachidonate are described; these data include determinations of thiocyanogen number.

2. By a standard procedure polybromide yields are reported on five specimens of methyl arachidonate over a wide range of amounts of arachidonate brominated.

3. The empirical nature of the polybromide yield is demonstrated.

4. It is proposed to estimate methyl arachidonate in methyl ester mixtures by the interpolation from a standard curve which is drawn from bromide- arachidonate data over a wide range of values.

5. The use of the proposed method along with the spectroscopic procedure is discussed.

REFERENCES

1. Brown, J. B., J. Biol. Chem., *80*, 455 (1928).

2. Brown, J. B., and Beal, G. D., J. Am. Chem. Soc., *45*, 1289 (1923).

3. Brown, J. B., J. Biol. Chem. *83*, 777 (1929).

4. Ault, W. C., and Brown, J. B., *ibid.*, *107*, 615 (1934).

5. Shinowara, G. Y., and Brown, J. B., *ibid.*, *134*, 331 (1940).
6. Mowry, D. T., Brode, W. R., and Brown, J. B., *ibid.*, *142*, 671 (1942).
7. Smedley-Maclean, I., and Nunn, L. C. A., Biochem. J., *34*, 884 (1940).
8. Silberman, A. K., M.S. Thesis, The Ohio State University (1930).
9. Stoneburner, L. N., Unpublished results from this laboratory.
10. Cramer, D. L., Ph.D. Thesis, The Ohio State University (1942).
11. Matthews, N. R., Brode, W. R., and Brown, J. B., Oil & Soap, *18*, 182 (1941).
12. Lambou, M. G., and Dollear, F. G., *ibid*, *22*, 226 (1945).
13. Official Procedure, American Oil Chemists' Society.
14. Kass, J. P., Roy, W. R., and Brown, G. D., Anal. Chem., *19*, 21 (1947).
15. Matthews, N. R., Brode, W. R., and Brown, J. B., J. Am. Chem. Soc. *63*, 1064 (1941).

☆ ☆ ☆ ABSTRACTS ☆ ☆ ☆

Oils and Fats

Edited by
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MILK FAT IN MILK CHOCOLATE. L. W. Ferris (Food and Drug Admin. Federal Security Agency, Buffalo, N. Y.). *J. Assoc. of Agr. Chem. 31,* 728-31(1948). Straight extraction of milk chocolate as in the A. O. A. C. method leaves unextracted a small amount of fat, which is principally milk fat. A newly proposed method is based on repeated shakings with ether, centrifuging, and decanting solvent oil layer.

ESTERS OF NATURALLY OCCURRING FATTY ACIDS. Carl W. Bonhurst, Paul M. Althouse, and Howard O. Triebold. Ind. Eng. Chem. *40,* 2379-84(1948). An apparatus is described and the operating procedure is given for the determination of a complete vapor pressure curve on one or two drops of a pure liquid. The apparatus was calibrated, and the vapor pressure curve for each of the methyl, propyl, and isopropyl esters of the naturally occurring C_6 to C_{18} saturated fatty acids was determined. Decomposition of the esters was found to be progressive above 205°C. and occurred over a wide temperature range rather than at a specific temperature. The relationships of the densities and viscosities of the esters to temperature were studied by determining these constants at 20°, 37.8°, 60°, and 98.9°C. Relationships between the vapor pressures, densities, and viscosities indicate that the forces governing these three properties have some factor or factors in common.

THE RAMAN SPECTROGRAMS AND THE STRUCTURE OF OLEIC AND ELAIDIC ACIDS. A. van den Hende (Univ. Ghent, Belg.). *Bull. soc. chim. Belges 56,* 328-38 (1947). Useful Raman spectrograms have been obtained for oleic acid, ethyl oleate, and their ethylene stereoisomers, elaidic acid, and ethyl elaidate. The spectra show that the frequencies due to the double union between the 2 doubly bound C atoms have a lower value for the oleic acid and the oleate than for the elaidic acid and the elaidate. It had already been observed that in many groups of unsaturated compounds the mention frequencies are smaller for the cis than for the trans isomers. The Raman spectra indicate the cis structure for oleic acid and the trans structure for elaidic acid. (*Chem. Abs. 42,* 6667-8.)

WATER INSOLUBLE FATTY ACIDS IN CREAM AND BUTTER. F. Hillig and S. W. Ahlmann (Food and Drug Admin., Washington, D. C.). *J. Assoc. Agr. Chem. 31,* 739-49(1948). Data are presented on the determination of WIA in 90 authentic churns of butter. When decomposed cream was present in the churn more WIA were found in the butter, in most cases, than in the butter churned from cream containing no decomposed material. It was shown that WIA in cream, for the most part, are retained in the butter on churning. Individual cans of cream classified as being decomposed usually contained much larger quantities of WIA than cans classified as satisfactory for butter making. There was in general little or no increase in WIA in butters stored at 0°F. for 5 months. Except in those cases in which visible mold appeared, there was no significant change in WIA in butters stored for 5 months at 40°F.

VOLATILE ACIDS IN CREAM AND BUTTER. PART I. THE DEVELOPMENT OF BUTYRIC ACID DURING THE PROGRESSIVE DECOMPOSITION OF CREAM. F. Hillig (Food and Drug Admin., Washington, D. C.). *J. Assoc. of Agr. Chem. 31,* 750-60 (1948). Progressive decomposition experiments on cream show that butyric acid is frequently produced in cream when it reaches that stage of deterioration where it is unfit for human consumption. Results indicate that the butyric acid probably results from the breakdown of the lactose, through lactic acid as an intermediate step, and not from the hydrolysis of the fat and accompanying liberation of the combined butyric acid in the glycerides. When the acid is present in cream, some is usually carried over into the butter made therefrom.

DETERMINATION OF WATER INSOLUBLE FATTY ACIDS IN DRIED EGGS. F. Hillig (Food and Drug Admin., Washington, D. C.). *J. Assoc. Agr. Chem. 31,* 731-4 (1948). An adaptation of the method for determination of water-insoluble fatty acids (WIA) in cream and butter is proposed for the determination of these acids in dried eggs. It has been shown that small amounts of WIA are found in authentic dried edible eggs, but that when liquid eggs are allowed to become sour before drying, the WIA in the dried product are increased several fold. Commercial dried eggs of acceptable quality were found to contain WIA in the range found for authentic dried edible eggs.

EFFECTS OF AUTOXIDATION ON ANTIACRODYNIC POTENCY OF FATS AND LINOLEIC ESTERS. F. A. Kummerow, Ta-Kung Chu, and P. Randolph (Kansas Agr. Exper. Sta., Manhattan). *J. Nutr. 36,* 523-36 (1948). The ability of small quantities of oxidized fats to cure acrodynia was used as a means of testing their nutritive value. The results indicated that rancid Me linoleate or the fat extracted from turkeys which had been subjected to cold storage for 2 years alleviated the symptoms but were not as effective as the freshly distilled ester or fresh turkey fat. Toco-