THE DIRECT ALCOHOLYSIS OF SUPRARENAL PHOSPHATIDES

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Abstract

Previous attempts to directly prepare methyl esters from phosphatides failed apparently because not enough HCl was present as catalyst. In the present work suprarenal phosphatides were directly converted into esters by alcoholysis with methyl, ethyl, propyl, butyl and amyl alcohols using 5.0% HCl and 7.5 and 12.0% H_2SO_4 as catalysts. The process of esterification is greatly simplified by this procedure.

IN THE course of a study, now under way in this laboratory, on the chemistry of arachidonic acid it was necessary to prepare the methyl esters of beef suprarenal phosphatides in considerable quantity. The method previously used by Brown and Ault[1] involved saponification with strong alcoholic KOH, acidification and extraction of the fatty acids with butyl alcohol. The alcohol was removed under reduced pressure and the resultant acids were then esterfied with methyl alcohol. In isolating the esters a second treatment with butyl alcohol was necessary to break emulsions with water and to prevent foaming in removing the last traces of water. Although this procedure was tedious and troublesome, it gave a satisfactory product.

It occurred to us that if direct alcoholysis were possible, the procedure would be greatly simplified. Alcoholysis, whereby it is possible to prepare esters directly from fats and oils, has been known since 1846[2], but was especially developed by Haller in 1907[3]. Since then the method has been widely employed by investigators in the field of fats and oils. In 1909 Rollet[4] attempted alcoholysis of egg lecithin with some success. Rollet, however, employed tin or zinc as a means of preventing resinification. Several attempts had been made previously in this laboratory to directly esterify suprarenal phosphatide without success. It was suggested that a possible cause of this failure might have been that not enough acid was used as catalyst (1-2% HCl). It seemed possible that at least one product of the reaction, choline, might combine with enough of the acid to seriously lower its effective concentration.

With this suggestion in mind, we tried the use of larger amounts of alcohol and a higher concentration of acid (5%). By the use of this modification it has been possible to prepare directly in good yield methyl, ethyl, propyl, butyl and n-amyl esters, thus removing the necessity of a preliminary alkaline saponification. Also, we have found it possible to use sulphuric acid in 7.5 and 12.0% concentration in place of HCl. Methyl esters, thus prepared with H_2SO_4, contain as much arachidonic acid as those prepared with HCl.

In connection with this work we wish to report bromine analyses and melting points of three new ester octabromides of arachidonic acid.

TABLE 1

Data on Esters Prepared by Direct Alcoholysis From Beef Suprarenal Phosphatides.

Catalyst	Ester	Weight Phosphatide. Gms.	Weight Ester Grms.	Yield % *	Mean Mol. Wt.	Iod. No.	Polybromide No.	Per cent Methyl Arachidonate
5% Dry HCl	Methyl	500	232	46.4	297.6	110.2	21.0	24.2
	"	5,000	2,300	46.0	296.4	100.5	18.2	21.0
	"	2,000	905	45.3	296.8	103.6	19.5	22.6
	"	4,000	1,864	46.3	296.6	102.9	19.2	22.2
	Ethyl	250	120	48.0	301.3	98.2	19.0	....
	n-Propyl	250	125	50.0	318.1	96.7	18.5	....
	n-Butyl	500	248	49.6	332.5	93.2	16.2	....
	n-Amyl	250	130	52.0	346.9	88.6	15.9	....
Conc. H_2SO_4 7.5%	Methyl	500	268	53.6	296.6	103.0	19.0	21.9
12.0%	Methyl	4,000	2,015	50.4	297.2	108.2	19.9	23.0

*The yield is based on the original weight of the phosphatide. The theoretical yield would be much higher since phosphatides contain only about 60-65% of fatty acids and since the phosphatides contain 7-10% unremoved volatile solvent.

EXPERIMENTAL PART

Raw Material

The phosphatide which we have employed in these experiments was kindly furnished us by Dr. Oliver Kamm of Parke Davis and Company. It was obtained by acetone precipitation of the lipids of beef adrenals. It was selected because its fatty acids contain about 20-22% of arachidonic acid.

Direct Alcoholysis of Phosphatides

The following general procedure was followed: One kilogram of the phosphatide was refluxed with 2 liters of methyl alcohol until all had dissolved. The solution was strained through cheese cloth to remove extraneous material. Two liters of alcohol containing 10% dry HCl was then added and the refluxing continued for 36 hours. At the end of this period about half of the alcohol was removed by distillation on the water bath, vacuum finally being employed. Six parts of the warm water were then added to the contents of the flask, also one liter of butyl alcohol to break the emulsion and facilitate separation. The butyl alcohol-ester layer was separated and the washing repeated twice. The ester layer was then heated under reduced pressure to remove the alcohol with minimal butyl ester formation. The residual esters were transferred to a 2 liter Claissen flask and distilled. Several preparations of methyl ester are described in Table I. Two runs employing 7.5 and 12% final concentations of H_2SO_4 instead of HCl were made. Special care in washing the esters before distillation is necessary with H_2SO_4, since the presence of traces of this acid is liable to cause decomposition of the esters, with formation of sulphurous acid. Less trouble from foaming was encountered with H_2SO_4.

Ethyl esters were made by the same procedure as described above. With propyl, butyl and amylalcohols, it was unnecessary to add butyl alcohol to separate the water layer, the esterifying alcohol itself being sufficient to cause separation. With the butyl and amyl alcohols a small amount of ether was added to the esterification mixture to favor refluxing, the boiling points of the alcohols being too high to boil on the water bath.

The various ester runs are summarized in Table I.

In this table the percent of methyl arachidonate in the various methyl ester preparations was calculated

TABLE II

Melting Points and Bromine Analyses of Bromides.

Ester octabromide	M. P. C. ° *	Bromine content per cent Theory	Found
Methyl	228.5-229.5	66.70	66.78
Ethyl	226.0-227.0	65.8	65.67
n-propyl	223.0-224.0	64.9	64.50
n-butyl	222.0-223.0	63.9	64.05
n-amyl	221.5-222.5	63.10	63.24

*Uncorrected.

from the equation of Ault and Brown[5] as follows:

$$\text{Percent methyl arachidonate} = \frac{\text{Polybromide No. Esters} \times 100}{86.5}$$

It is to be noted that the content of arachidonate is essentially the same for esters prepared either with HCl or H_2SO_4.

Melting Points and Bromine Analyses of Octabromides

In Table II are to be found the melting points of the various ester bromides; also their bromine content determined by the peroxide bomb method. The propyl, butyl and amyl compounds have not been previously described.

SUMMARY

(1) Direct alcoholysis of beef suprarenal phosphatides has been accomplished with methyl, ethyl, n-propyl, n-butyl and n-amyl alcohols, using 5% hydrochloric acid and 7.5 and 12.0 % sulphuric acid as catalysts.

(2) Propyl, butyl and amyl octabromo-arachidates have been prepared and described.

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REVIEW OF SCIENTIFIC LITERATURE ON FATS AND OILS FOR 1937

PART III

By M. M. PISKUR

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New data on butter included the butyric acid index of Indian butters which was reported by N. N. Godbole [*Milch. Zentr.* **66**, 93] and A and B values of Norwegian butter taken at different seasons of the year which was recorded by S. Schmidt-Nielsen and A. Asted [*Kgl. Norske Vegenskab. Selskabs Skrifter No.* **7**, 30 pp.]. The latter authors recommend the A and B determinationsas tests for adulteration. This recommendation was criticized by T. Sunberg [*Swensh. Kem. Tids* **49**, 185-94] who praised the Polenske value. A semimicro method for the butyric acid value determination was devised by T. T. van Voorst [*Chem. Weekblad* **33**, 742-3]. A new characteristic for butterfat proposed by V. Venkatachalam [*Current Sci.* **5**, 477-8] was computed by the equation: R-M. value + (n—1.4000) 1000 + (n—1.4440) 1000 where n equals the Abbe refractometer reading at 45°. The values for butters were between 84 and 86.

Methods for determining diacetyl and acetylmethylcarbinol in butter were described by H. Schmalfuss and H. Werner [*Fette u. Seifen* **44**, 509-14], R. Dehove and L. Dessirier [*Ann. fals.* **30**, 288-91] and A. F. Langlykke and W. H. Petersen [*Ind. Eng. Chem. Anal. Ed.* **29**, 163-6]. J. D. Wildman [*J. Assoc. Off. Agr. Chem.* **20**, 93-100] described microscopic and macroscopic methods for determining mold in butter. For the microscope, one gram of butter was heated with 7 cc. of 0.75 per cent solution of carob-bean gum containing 2 per cent formaldehyde and the liquid was decanted. Estimation of the mold was by the Howard method. For macroscopic determniation, 5 grams samples of butter, 15 cc. of hot methyl blue-borax solution and ten methylene blue tablets were agitated and poured through a perforated funnel cone. The mold may be measured in the filter cone.

Committees of the Deutsche Gesellschaft fur Fettforschung and the American Oil Chemists' Society prepared progress reports on collaborative work on analytical methods. These were published in their respective official journals, Fette und Seifen and Oil & Soap. A new committee of the American Oil Chemists' Society intends to collect available data on a few oils from time to time and then analyze a few type samples as completely as possible. Work of the Association of Official Agricultural Chemists was reported by G. S. Jamieson [*J. Assoc. Off. Agr. Chem.* **20**, 418-21]. It was recommended that the Fitelson method for determining tea seed oil in olive oil and the refractometric method for determining oil in flax be made official. Slight changes in some analytical procedures were made. It was suggested that a collaborative study on the Kaufmann thiocyanogen method be made.

The color reaction of oils with acetic anhydride and with arsenic trichloride was the subject of a polemical discussion between H. Heller [*Angew. Chem.* **50**, 752-3] and H. Jesser and E. Thomae [*Agnew. Chem.* **50**, 573]. Heller reported a change from green to emerald green to deep green on treating soybean oil with acetic anhydride, and a green to violet to red reaction with arsenic trichloride. Jesser and Thomae reaffirmed an earlier report that the color change was a development of blue which changed to greenish-brown with acetic anhydride and red to green with arsenic trichloride. The Carr-Price color reaction for determining vitamin A was suggested as a means of identifying certain oils by F. Provvedi [*Olii, minerali, olii e grassi, colori vernici* **16**, 103-4] and S. H. Bertram [*Öle, Fette, Wachse, Seife, Kosmetik* **1937**, No. 8, 102]. Provvedi reported that the color reaction with cottonseed oil was dark reddish-brown, olive oil light green, sesame oil light pink-yellow, peanut oil pink, etc. The results indicated that rancid olive oil can be distinguished from the nonrancid oil because the reagent caused a development of opalescence in the rancid samples. In nonrancid oils the test could be used to identify cottonseed oil. Bertram published similar data. He suggested that the test could be used for the detection of adultering peanut oil with soybean oil. According to A. E. Gillam *et al* [*Nature* **140**, 233] fresh water fish liver oils and marine fish liver oils can be distinguished by the absorption spectra of their Carr-Price reaction. The former have a considerably larger absorption at 693 *mu*.

H. Thaler [*Fette u. Seifen* **44**, 38-42] described chromographs obtained by passing samples of several oils dissolved in benzene through a column of aluminum oxide and "clarite" (an acidic bleaching clay). The chromographs of fats and of their natural coloring matter were easily distinguished from synthetic coloring matter, thus giving a method for detection of artificial coloring matter. H. A. Boekenoogen [*Verfkroniek* **10**, 143-6] applied the above method to linseed oil. The presence of α- and β-carotene in the linseed oil was identified by passing a petroleum ether solution of its unsaponifiable fraction through the aluminum oxide column; a broad yellow band