TABLE III

Estimation of the Linoleic Acid Contents of the Fatty Acids of Several Oils by Bromination in Pentane and by Thiocyanometry

Fatty Acids of	Bromides from 1.0 g. of Acids, g.	Linoleic Acid, %	
		From Pentane Curve	From SCN No.
Olive Oil[a]	0.0091	9.7	12.2
Corn Oil	0.5564	59.7	59.2
	0.5675	60.7	
	0.5419	58.4	
	0.5613	59.9	
Peanut Oil	0.1463	21.7	21.0
	0.1412	21.2	
	0.1213	19.3	
	0.1371	20.7	
Tobacco Seed Oil	0.7403	75.5	75.5
	0.7049	72.5	
	0.7415	75.8	
	0.7433	75.6	
Almond Oil	0.1580	22.0	21.7
	0.1574	21.9	
	0.1566	21.8	
	0.1639	22.4	
Poppyseed Oil	0.6208	65.0	64.6
	0.6211	65.0	
	0.6125	64.0	
	0.6192	64.6	
Sesame Oil	0.4073	44.8	44.7
	0.4063	44.6	
	0.4020	44.4	
	0.4099	45.1	

[a] It is to be noted here that in petroleum ether no tetrabromides would have been obtained. The significantly lower result here by the tetrabromide method is an indication of the presence of isomeric linoleic acids in this oil, as previously reported from this laboratory (3).

at −12.9° to −12.1°. The tetrabromide number of the filtrate acids in heptane, 61.7, show the composition to be 35% iso-acids and 65% linoleic acid.

Tetrabromide Yields

Linoleic acid and known mixtures of linoleic and oleic acids were brominated in n-pentane and n-heptane according to the procedure described by White and Brown (1). The linoleic acid used in this work was the recrystallized specimen described above; the oleic acid was a very pure specimen isolated from corn oil. The results are found in Table I.

In order to compare the data in Table I with the previously reported data (1) in which Mallinckrodt petroleum ether was employed in bromination and washing of the bromides, the percentages of linoleic acid in Table I were plotted against tetrabromide yields and the results at certain round number percentages were estimated by interpolation. These are summarized in Table II.

The linoleic acid contents of the fatty acid mixtures from several oils were next determined from the tetrabromide-pentane curve and the results compared with those obtained by analysis of these mixtures by thiocyanometry. Admittedly this comparison would have been the more valuable if spectrophotometric analyses were included, but this was not done. The oils selected for these comparisons were those which contain no appreciable amounts of linolenic acid. The results are shown in Table III.

Summary

Known mixtures of linoleic and oleic acids were brominated in n-pentane and n-heptane and the yields of insoluble tetrabromides determined under precise conditions. In general, the yields were somewhat higher in both hydrocarbons than in Mallinckrodt petroleum ether. Tetrabromides were obtained in detectable amounts in mixtures containing as little as 10% linoleic acid when pentane is used. Therefore it is recommended that this solvent be employed in applying the procedure in the analysis of fatty acid mixtures. The linoleic acid contents of the fatty acids of several oils were estimated from the tetrabromide-pentane curve and the results found to agree favorably with those obtained by thiocyanometry.

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Carbohydrate Constituents of Soybean "Lecithin"[1]

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THE status of carbohydrate material in plant phosphatides, whether bound or free, has been a point of discussion for many years. As long ago as 1906 Winterstein and Hiestand reported that some of the carbohydrates in various plant phosphatides studied could be removed by washing with water, but that part appeared to be in firm chemical combination, and that it was necessary to boil for several hours with 5% sulfuric acid to split off all carbohydrates (24, 25). In a series of publications beginning in 1929 Rewald has maintained however that the carbohydrates in soybean "lecithin" were free, and he believed that they could be removed from the phosphatides by physical means (18, 19). The work of McKinney, Jamieson, and Holton (15) supported the earlier concept of Winterstein and Hiestand and indicated that some sugar in soybean phosphatides was bound to lecithin by a glycosidic linkage. More recently Woolley (26) and Folch (7) have reported the presence of galactose-containing phosphoinositides. The presence of the free sugars, sucrose and stachyose, has been reported in the whole soybean many times (14). Celmer and Carter (3, 4) have shown that these free sugars, as well as a sugar-containing phosphoinositide, are present in commercial lecithin.

This paper reports the identification of both free and bound sugars by means of paper chromatography along with an estimate of the amounts of each sugar in one sample of phosphatides.

[1] Presented before the American Oil Chemists' Society Meeting, Oct. 8-10, 1951, Chicago, Ill.

[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study in which certain phases were carried on under the Research and Marketing Act of 1946.

TABLE I

Analysis of Phosphatide Preparations

Preparation	% of total weight	Sugar %	Phosphorus %	Nitrogen %	Inositol %	Amino N %	Choline N %
I. Commercial lecithin	100	....	1.97	0.78	2.71	....	0.28
II. Oil-free phosphatides	73	8.77	2.97	1.07	3.30	....	0.39
III. High sugar fraction	8.7	53	0.99	0.63	1.50	0.29	0.13
IV. Last acetone-soluble fraction	0.8	1.03	1.49	0.89	0.06	....	0.41
V. Low sugar phosphatides	63.5	1.63	3.31	1.10	3.97	0.51	0.42
VI. Main alcohol-soluble fraction	28.6	1.05	3.32	1.56	0.04	0.51	0.90
VII. Fraction originally alcohol soluble—would not redissolve	6.1	2.30	2.36	0.89	2.49	....	0.12
VIII. Fraction alcohol soluble at 50°	6.0	0.68	3.10	0.96	3.31	....	0.07
IX. Alcohol-insoluble fraction	22.8	2.94	3.25	0.65	10.4	0.43	0.002

Analytical Methods

Because of the variety of sugar compounds present and differences in their ease of hydrolysis, any hydrolysis condition chosen is at best a compromise between completeness of hydrolysis and some destruction of the more labile sugars. The procedure used in this work, except when otherwise stated, is as follows. The sample was dispersed in 10 ml. of water, and 3 ml. of 2 N H_2SO_4 were added. This dispersion was heated on a steam bath from 7 to 8 hours. The hydrolyzate was filtered and then neutralized with sodium hydroxide. Sugar was determined in the hydrolyzate by the method of Somogyi (22). In results from this laboratory total sugar in phosphatides has ordinarily been calculated as galactose since Woolley (26) had reported that sugar in lipositol. This hydrolysis procedure gives a maximum value with most samples. It however will not completely free the bound sugars of the phosphoinositides. If this material is refluxed with 2 N H_2SO_4, the sugar value is increased markedly. On the other hand, some sugar is destroyed even by the usual hydrolysis, particularly in high sugar fractions. In spite of these inherent difficulties the procedure described has given relative if not absolute sugar values satisfactory for following the progress of our fractionation procedure.

The other analytical data in Table I were obtained by methods previously used (20, 8, 1) except that a modification of Van Slyke's manometric apparatus (23) was used in the determination of amino nitrogen.

Experimental

In preliminary work phosphatides were dissolved in hexane and shaken with various concentrations of aqueous ethanol. Appreciable quantities of the phosphatides were dissolved in the alcohol layer at high concentration levels of ethanol. However when the ethanol was diluted to 55%, the carbohydrates were concentrated in the alcohol layer and nearly all the phosphatides remained in the hexane.

Subsequently acetone-insoluble phosphatides which had been prepared from commercial lecithin were dissolved in hexane (3 ml./gram) and extracted five times with 55% ethanol. The alcohol solution was evaporated to dryness under reduced pressure to give the high-sugar fraction, Preparation III (Table I). Hexane was evaporated under vacuum from the remaining low-sugar phosphatides, and the residue was again extracted twice with acetone. The small amount of acetone-soluble material is Preparation IV, and the remaining low-sugar phosphatides are Preparation V. This and subsequent fractionations are indicated in the flow sheet in Figure 1.

Identification of the sugars present was made by paper chromatography. The procedure followed very closely that used by Jeanes, Wise, and Dimler (13). Preparation III, the high-sugar fraction, dissolved in water to give a cloudy solution. A 10% solution of this fraction was "spotted" on Whatman No. 1 filter paper, and the chromatogram developed, using a descending technique with fusel oil-pyridine-water (1:1.06:1)[3] as solvent.

After the solvent had traveled down the paper, the chromatogram was dried and the sugars were located by spraying with naphthoresorcinol (16), a reagent which produces spots with ketose-containing carbohydrates. Spots were found with R_f values 0.31 and 0.12 corresponding to sucrose and stachyose respectively. In addition, a fainter spot of R_f value 0.21 was found corresponding to raffinose. The presence of these sugars was confirmed by the use of the mixed chromatogram technique. A sample of Preparation III and a sample of a mixed standard containing sucrose, raffinose, and stachyose were spotted together on a chromatogram. The chromatogram was developed twice, using a multiple development procedure (13) in order to obtain greater resolution. This chromatogram is shown in Figure 1. It is seen that the spots from the high-sugar fraction and those from the standards are identical, thereby confirming the identification of these ketose-containing sugars as sucrose, raffinose, and stachyose. When similar chromatograms were sprayed with ammoniacal silver nitrate (9), some additional faint spots were found. Silver nitrate however is reduced by other substances in addition to sugars. These additional spots were not found in chromatograms sprayed with metaphenylene diamine dihydrochloride (5). If they are due to

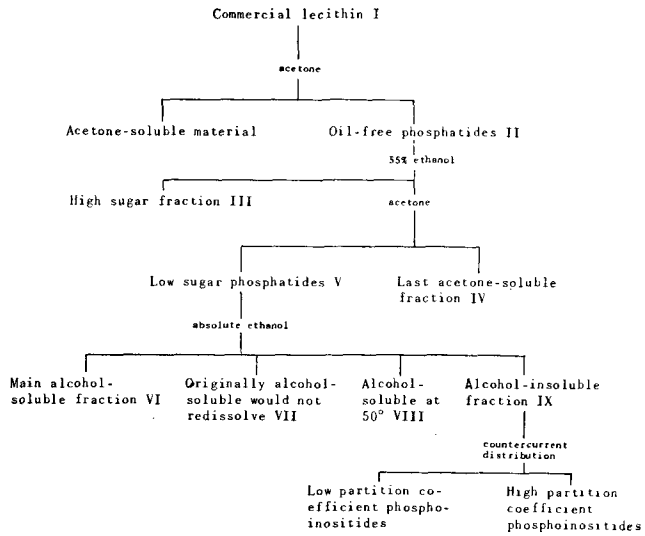

FIG. 1. Preparation of phosphatide fractions.

[3] Jeanes, Wise, and Dimler used this solvent mixture in proportions 1:1:1. With the present sample of fusel oil, 1.06 parts of pyridine were necessary to make the solvent mixture miscible.

sugars, the sugars must be present in extremely small amounts.

A quantitative estimation was made of the sucrose, raffinose, and stachyose present in the high-sugar fraction (III). Spectrophotometric measurements were made of the color formed by the individual sugars with anthrone after they had been separated on a paper chromatogram and eluted from the paper (6). Table II gives those analyses and confirms the indications of qualitative chromatographic evidence that sucrose and stachyose are major components and raffinose a minor component. This table also includes the analysis of a sample of Preparation II, the oil-free phosphatides, which was dissolved in toluene and run in the same way.

TABLE II

Free Sugar Composition of Phosphatide Fractions

	Weight % of fraction	% of total free sugar
Oil-free phosphatides II		
Sucrose	3.1	43
Raffinose	0.7	9
Stachyose	3.4	47
High sugar fraction III		
Sucrose	20.2	48
Raffinose	2.8	7
Stachyose	19.1	45
Alcohol-soluble fraction, A		
Sucrose	4.4	87
Raffinose	0.5	9
Stachyose	0.2	4
Intermediate fraction soluble in alcohol at 60°, B		
Sucrose	5.7	68
Raffinose	1.4	16
Stachyose	1.3	16
Alcohol-insoluble fraction, C		
Sucrose	0.7	9
Raffinose	0.9	12
Stachyose	6.0	79

Previous work has shown that when oil-free phosphatides from which sugars have not been removed are separated into absolute alcohol-soluble and insoluble fractions, sugars are found in both fractions. To identify these sugars a sample of acetone-extracted phosphatides prepared in a way similar to Preparation II, but from a different source, was separated into a fraction A soluble in absolute alcohol at room temperature, an insoluble fraction C, and an intermediate fraction B soluble at 60°. Paper chromatograms showed sucrose, raffinose, and stachyose in all fractions. In the alcohol-soluble fraction A, the sucrose spot was strong while the stachyose spot was rather faint; in the alcohol-insoluble fraction C the stachyose spot was strong while the sucrose spot was rather faint. The results of a quantitative estimation by the anthrone procedure are also shown in Table II and confirm the fact that sucrose tends to be concentrated in the alcohol-soluble fraction A, stachyose in the alcohol-insoluble fraction C, and raffinose is distributed rather evenly. The intermediate fraction B has sucrose as the major sugar component with a relatively high raffinose content.

Since the sugar remaining in Preparation V, the low-sugar phosphatides, could not be removed by further extraction with dilute alcohol, a part of this material was fractionated upon the basis of its solubility in absolute alcohol as was done in previous work (20). The fractions obtained are shown as Preparations VI, VII, VIII, and IX in Table I. A 3.02-g. sample of Preparation IX, the alcohol-insoluble fraction, was distributed between hexane and

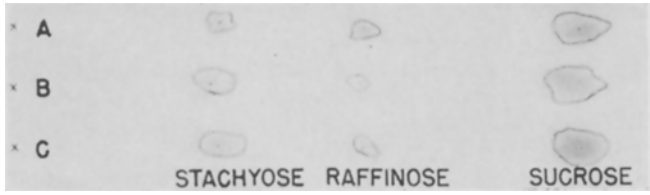

FIG. 2. Chromatogram showing presence of sucrose, raffinose, and stachyose in high sugar fraction III.
A. Combined standards containing stachyose, raffinose, and sucrose.
B. Preparation III, material extracted by 55% alcohol.
C. Mixture of combined standards and Preparation III.

95% methanol in a preparative model Craig apparatus which holds 84 ml. of each solvent in each tube. The results of the distribution are shown in Figure 3. Two peaks in the weight curve are found similar to results of earlier work[4] (20). In the low-partition-coefficient fractions very little sugar is found. For example, tube 0 contains only 1.78% sugar while in

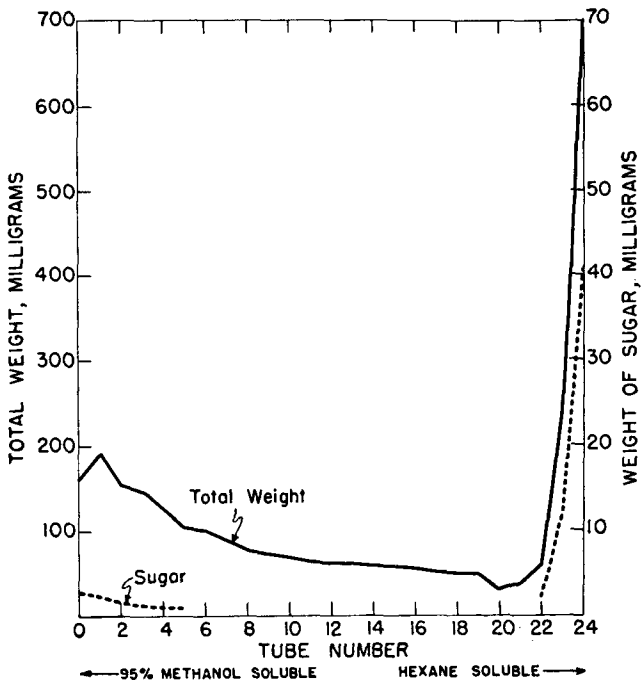

FIG. 3. Countercurrent distribution of alcohol-insoluble fraction.

the previous distribution, with material from which free sugar had not been removed, 19% sugar was found in this tube. On the other hand, in the high-partition-coefficient fractions, tube 24 contains 5.9% sugar as compared with 3.9% in the previous work. If conditions of hydrolysis are made more drastic and the sample from tube 24 is refluxed with 2 N H_2SO_4 for 8 hours, a value of 8.05% is obtained. The high partition coefficient of this fraction indicates that the sugar must be chemically bound, probably to the phosphoinositides as reported by Celmer and Carter (3, 4).

In order to identify these bound sugars a portion of the material from tube 24 was refluxed with 2 N

[4] The material in the peak at the right of the curve was concentrated in the hexane layers and will be referred to subsequently as high-partition-coefficient phosphoinositides. Conversely the fractions at the left, which are concentrated in the 95% methanol layer, will be referred to as low-partition-coefficient phosphoinositides.

H_2SO_4 for 12 hours. After filtration the hydrolyzate was passed through Amberlite IR-4 and IR-105[5] to remove acid and mineral constituents and evaporated to a small volume under reduced pressure. The hydrolyzate was placed on filter paper together with glucose, galactose, fructose, arabinose, and mannose standards and developed with a butanol-pyridine-water (3:2:1.5) solvent mixture. Upon spraying with aniline phthalate (17) two spots were found. One spot corresponds to galactose (R_f 0.29). The other spot is in a position corresponding to fructose, mannose, or arabinose (R_f 0.38). With this solvent combination these three sugars move at essentially the same rate, and it is not possible to distinguish between them by position alone. The presence of arabinose was indicated however by the red-brown color of the spot, which was the same as that of the arabinose standard. Fructose gives only a very faint spot with aniline phthalate, and mannose gives a grey-brown color similar to glucose and galactose. The absence of fructose was shown by similar chromatograms which were sprayed with the ketose reagent, naphthoresorcinol, and later was confirmed with the more sensitive reagent, urea hydrochloride (10). No spot was found corresponding to glucose (R_f 0.34).

Mannose is also present as was shown by the use of a two-dimensional chromatogram. The high resolution of this technique was required since the mannose spot is obscured by the red-brown color of the arabinose in the chromatogram described above. In this procedure the chromatogram was developed first with the butanol-pyridine-water mixture with which mannose and arabinose move at the same rate but with which galactose moves more slowly. The chromatogram was then developed in a direction perpendicular to that of the first development with phenol saturated with water. With this solvent mannose and galactose move at the same rate and more slowly than arabinose. After spraying with aniline phthalate, three spots corresponding to arabinose, galactose, and mannose were found as shown in Figure 4.

Further confirmation of the identity of the sugars was obtained by biological methods. A selective fermentation procedure similar to that of Auernheimer, Wickerham, and Schniepp (2) was used. Samples of high-partition-coefficient phosphoinositides were hydrolyzed by refluxing 24 hours with 2 N H_2SO_4. After filtration the hydrolyzates were passed through Amberlite IR-4. The volumes of the hydrolyzates were reduced by boiling and then were made to 500 ml. Aliquots were treated with the following yeasts: *Saccharomyces bayanus* (NRRL Y-966), which assimilates only glucose, fructose, and mannose; *Saccharomyces carlsbergensis* (NRRL Y-379), which in addition to these sugars assimilates galactose; and *Candida guilliermondii* (NRRL Y-488), which in addition to the above sugars assimilates arabinose. After treatment with the yeasts the amount of sugar remaining was determined by the Somogyi procedure. It was found that the sugar contents of the hydrolyzates were reduced by each treatment, and the amount of sugar assimilated was smallest with NRRL Y-966, larger with NRRL Y-379, and still larger with NRRL Y-488. Although the identity of the sugars was thus confirmed, the quantitative results on duplicate hydrolyzates checked too poorly to permit an estimate of the relative amounts of the sugars.

[5]The mention of these products does not imply that they are endorsed or recommended by the Department of Agriculture over others of similar nature not mentioned.

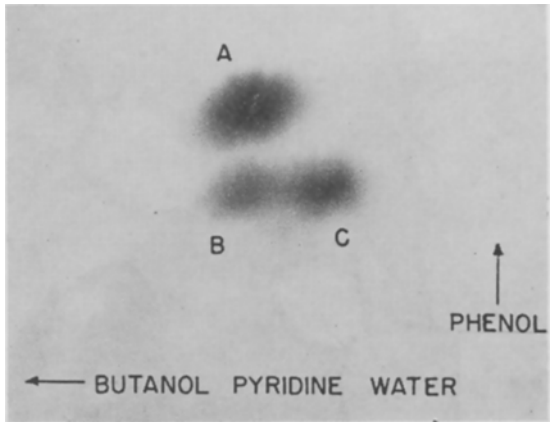

FIG. 4. Two dimensional chromatograms of hydrolyzate from high-partition-coefficient phosphoinositides showing presence of mannose, galactose, and arabinose.
A. Arabinose.
B. Mannose.
C. Galactose.

Hutt, Malkin, Poole, and Watt (11) have isolated a disaccharide and a small amount of arabinose from the alcohol-insoluble fraction of peanut phosphatides after a very mild selective hydrolysis with 0.01 N H_2SO_4. For comparison, a sample of high-partition-coefficient soybean phosphoinositides was hydrolyzed with 0.01 N H_2SO_4. Only one distinct spot, that corresponding to arabinose, was found after spraying chromatograms with either aniline phthalate or silver nitrate.

Since the high-partition-coefficient corn phosphoinositide fraction also contains sugars, it was investigated for comparative purposes. A part of tube 24 from the countercurrent distribution of the alcohol-insoluble fraction of corn phosphatides remaining from previous work (21) was hydrolyzed with 2 N H_2SO_4 and chromatograms run. Galactose, mannose, and arabinose were present as in the corresponding soybean phosphatide fraction. In addition, a very faint spot was found whose R_f value was greater than arabinose when either the butanol-pyridine-water or the phenol-water mixtures were used. The spot is in the region expected for ribose or fucose, but its identity was not investigated further.

Although, as shown in Figure 3, the low-partition coefficient fractions from the countercurrent distribution of the alcohol-insoluble soybean phosphatides (Preparation IX) were quite low in sugar, small amounts were still present as measured by the Somogyi procedure. Chromatograms were therefore run on an unhydrolyzed portion of tube 0; however no sugar could be detected. After hydrolysis, galactose and arabinose were found, but insufficient sample remained to establish the presence or absence of mannose. Similar results were obtained upon the alcohol-soluble preparations VI, VII, and VIII. These unhydrolyzed fractions showed no sugar spots, suggesting that all free sugar had been removed. After hydrolysis glucose and galactose were found, but no fructose, arabinose, or mannose. The sugar in Preparations VII and VIII was predominantly galactose, and the glucose spot was very faint. Again, in comparison with corn phosphatides it is of interest to note that the same two sugars were found in the

alcohol-soluble fraction of corn phosphatides which had been prepared for previous work.

These sugars are probably present as glycosides; however the nature of the aglycone has not been determined. Sterol glucosides are reported to occur in soybean "lecithin" to the extent of 3% (12). Celmer and Carter (3, 4) used a chloroform alcohol treatment to remove them from their phosphoinositide preparations. In order to determine the location of sterol glycosides, qualitative Liebermann-Burchard tests were run on various fractions. Strong positive tests were obtained for the alcohol-soluble fractions VI, VII, and VIII. A very weak positive test was obtained for the phosphoinositide fractions (IX). Thus it appears that in our fractionation procedure the sterol glycosides are found in the alcohol-soluble fractions and account at least in part for the sugars found there upon hydrolysis.

TABLE III

Sugar Content of Soybean Phosphatide Sample

	% of oil-free phosphatides	% of total sugar
Free sugars	7.20	82.5
Sugars in high-partition-coefficient phosphoinositide fraction	.77	8.8
Sugars in low-partition-coefficient phosphoinositide fraction	.10	1.1
Sugars in absolute alcohol-soluble fractions	.66	7.6

Discussion

The carbohydrates present in commercial soybean lecithin consist of two types: the free sugars which can be extracted from a hexane solution of phosphatides with dilute alcohol, and bound sugars which remain with the phosphatides. The free sugars are made up mainly of sucrose and stachyose, both of which have been reported previously in soybeans and soybean phosphatides (14, 3, 4). In addition, a smaller amount of raffinose is present. In one sample these free sugars were found to be made up of 43% sucrose, 47% stachyose, and 9% raffinose. As would be expected, this composition is in approximate agreement with that found for the 55% ethanol extract from the same sample. No other samples of commercial lecithin were examined; however the composition may be expected to differ with variations in source and methods of preparation.

If acetone-insoluble phosphatides from which free sugar has not been removed are fractionated upon the basis of their solubility in absolute alcohol, the sugars found with the alcohol-soluble fraction will be predominately sucrose while that remaining with the alcohol-insoluble phosphatides will be predominately stachyose.

The sugars not extracted by 55% alcohol consist in part of bound mannose, galactose, and arabinose. These are found to be associated primarily with the high-partition-coefficient phosphoinositide fraction. In a 24-distribution countercurrent separation the contents of tube 24 consists of all material which is extremely soluble in hexane compared to 95% methanol. Thus tube 24 probably contains other substances in addition to pure phosphoinositides. However in view of the large amount of phosphoinositide in this fraction it seems likely that the sugar is bound to the phosphoinositide and not to some other substance. This agrees with the conclusion reached by Woolley (26), Folch (7), and Celmer and Carter (3, 4). There is no evidence to indicate whether there is a group of closely related phosphoinositides containing different sugars or whether the sugars are bound together in a single compound and the remainder of the material in tube 24 is sugar-free. It is somewhat surprising that these sugar-containing compounds on countercurrent distribution are highly soluble in hexane while sugar-free phosphoinositides are found in the fractions that are more soluble in methanol.

In view of the presence of galactose, mannose, and arabinose, both in soybean phosphoinositides and in corn phosphoinositides, it seems likely that these sugars may occur generally among plant seed phosphatides. This conclusion is supported by the work of Hutt, Malkin, Poole, and Watt (11), who found arabinose and galactose in the alcohol-insoluble fraction of peanut phosphatides. Their identification was made by single dimension paper chromatography and mannose could easily have been overlooked.

Small amounts of sugars occur in the low-partition-coefficient phosphoinositide fractions and in the fractions soluble in absolute alcohol. These fractions in the unhydrolyzed form do not give sugar spots on chromatograms; hence it seems that this sugar is not free. A part is probably present as sterol glycosides; the remainder may be bound either to the phosphatides or to some other aglycone.

From the information obtained in this investigation an estimate of the sugar composition of the phosphatides was calculated and is presented in Table III. The sugar values might be altered somewhat if different assumptions and approximations were used in the calculations; however it is believed the values presented are a good estimate of the actual composition. Thus it is seen that although a portion of the carbohydrates in commercial soybean lecithin seems to be bound to the lipid constituents, about 85% is free and can be removed by appropriate means.

Summary

The carbohydrates present in commercial soybean lecithin consist of two types: free sugars which can be removed by extraction with 55% alcohol, and bound sugars which remain with the phosphatides. The free sugars consist mainly of sucrose and stachyose with a smaller amount of raffinose. Upon extraction of phosphatides with absolute alcohol, the sucrose is found mainly in the absolute alcohol-soluble fraction, the stachyose in the insoluble fraction, and the raffinose in both fractions.

The greater part of the bound sugars are found with that phosphoinositide fraction which is more hexane soluble. These bound sugars consist of galactose, mannose, and arabinose. The arabinose is easily freed by mild acid hydrolysis and all three sugars are freed by refluxing with 2 N H_2SO_4. The same three sugars together with a smaller amount of an unidentified sugar are found in the corresponding fraction of corn phosphatides. In the absolute alcohol-soluble fractions of both soy and corn phosphatides the bound sugar consists of glucose and galactose.

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Study of Carcass Fats of Beef Animals. I. The Composition of Beef Brisket Fat[1,2]

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IN the grading of commercial inedible fats from animal sources a titer value of 40° is accepted as the characteristic which delineates a tallow (titer of 40° or higher) from a grease (titer of less than 40°). Since the fat from beef animals normally has a titer in excess of 40°, it is commonly accepted that fat from beef animals will be graded as a tallow. An exception to this was noted some months ago in a sample of so-called "tallow" rendered from trimmings of briskets used to make corned beef. In this case the fat had a titer of 38.7°.

Subsequent investigations on other samples of brisket fat revealed a titer of less than 40° in 13 of 17 samples. The color of the brisket fats varied with the grade of the animal. The higher-grade animals produced the lighter-colored fat while the fat from the lowest-grade animals was quite yellow. As a result of these observations it was decided to make a study of beef brisket fats to learn whether any correlation could be made between unsaturated fatty acid composition, iodine value, titer, and color associated with the grade of the animal.

Experimental

Portions of the fatty tissue were rendered by standard laboratory procedures. The iodine value was determined by a rapid Wijs method as described by Hiscox (3). The titer was determined by the A.O.C.S. official method (4). The unsaturated fatty acid composition was determined by the method of Mitchell, Kraybill, and Zscheile (5), using the constants of Beadle and Kraybill (1).

In many cases, the brisket fat of an animal is present in two distinct portions, which are labeled "outside" and "inside" according to location in the brisket. The first two samples obtained were marked only "brisket fat" and were undoubtedly composited from many carcasses without regard to type or grade of animal or portion of brisket used. The next three were from a cow, a steer, and a yearling, respectively, but were not identified as to grade or portion of brisket sampled.

In the next group the samples were from the "inside" portion of steer briskets, and all three were found to be composed of two layers of fat separated by what appeared to be a connective tissue. The outer layer of the "inside" brisket fat was found to be more highly colored. In sample No. 1 the yellow and white layers were composited, but in samples No. 2 and 3 the layers were divided and run separately.

Samples from the last group of briskets to be analyzed were labeled in all respects. In one instance (Choice steer—"Outside") the fat was in two layers, one more yellow in color than the other. These layers were divided and analyzed separately. This last group of brisket fats represents a range in grade of animals from prime steers to canner and cutter cows.

Results and Discussion

A total of 17 determinations have been made, and only four of the fat samples were found to have a titer of 40° or higher. These four were from samples of "inside" brisket fat, three from finished steers and one from a steer of unknown grade. Composite samples, "outside" fats and all other "inside" brisket fats, were found to have a titer of less than 40° as shown in Table I.

It is interesting to note that "outside" fats are the only brisket fats available from the low-grade animals. The fat from the canner and cutter cow was the most yellow, which probably is indicative of the influence of age on the character of the fat. Other characteristics of this fat were found to be very comparable to that from other animals.

The iodine values vary in inverse ratio to the titer values. All fats having a titer of 40° or greater have an I.V. of less than 56.

The fatty acid composition studies reveal only minor variations in polyunsaturated fatty acids, irre-

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