TABLE VII

Relative Intensities of the 1st, 8th and 10th Orders of Diffraction of the Continuous Layer Lines of Palmitic Acid and Its 9- and 10-Keto Derivatives [a]

	F_{001}/f	F_{008}/f	$F_{00.10}/f$	F^2_{001}/f^2	F^2_{008}/f^2	$F^2_{00.10}/f^2$	Relative Intensities		
							001	008	00.10
p.a	—2.044	—1.063	—1.526	4.18	1.13	2.33	151	3.5	4.7
9-keto-p.a	—1.962	—0.265	—2.251	3.85	0.07	5.07	139	0.02	10.1
10-keto-p.a	—1.789	—1.523	—0.725	3.20	2.32	0.53	116	7.2	1.1

[a] Compare with Figure 13.

ACKNOWLEDGMENTS

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REFERENCES

1. Schlenk, H., "Progress in the Chemistry of Fats and other Lipids," Vol. 2, edited by R. T. Holman, W. O. Lundberg, and T. Malkin, Academic Press Inc., New York, 1954, p. 243–267.
2. Schlenk, W. Jr., Ann., *573*, 142–162 (1951).
3. Schlenk, W. Jr., Analyst, *77*, 867–873 (1952).
4. Schlenk, H., and R. T. Holman, J. Am. Chem. Soc., *72*, 5001 (1950).
5. Holman, R. T., and S. Ener, J. Nutr., *53*, 461 (1954).
6. Jancosek, A. T., and J. S. Brown, (Standard Oil Co.), U. S. 2,906,744 (1959).
7. Schlenk, W. Jr., Experientia, *8*, 337 (1952).
8. White, D. M., J. Am. Chem. Soc., *82*, 5678 (1960).
9. Brown, J. F., and D. M. White, *Ibid.*, *82*, 5671 (1960).
10. Hermann, C., and H. U. Lenné, Naturwiss., *39*, 234 (1952).
11. Smith, A. E., Acta Cryst., *5*, 224 (1952).
12. Borchert, W., Heidelberger Beiträge z. Min. u. Petr., *3*, 124 (1952).
13. Laves, F., and N. Nicolaides, Abstract in Proceedings of Am. Cryst. Assoc. Meeting, Tamiment, N. H., 16–17 (1952).
14. The early X-ray studies of Hermann and Renninger were reported in Ref. 2.
15. Lenné, H. U., Acta Cryst., *7*, 1 (1954).
16. For further discussion of the fundamental concepts discussed in this and the following section, the reader is referred to a good text such as Bijvoet, J. M., "X-Ray Analysis of Crystals," Butterworths Scient. Publications, London, 1951.
17. Nicolaides, N., F. Laves, and A. Niggli, J. Am. Chem. Soc., *78* 6415 (1956).
18. Nicolaides, N., and F. Laves, *Ibid.*, *80*, 5752 (1958).
19. This procedure, which is generally unknown to chemists, is quite a familiar one to mineralogists and is described in textbooks in mineralogy.
20. Laves, F., N. Nicolaides, and K. C. Peng, Z. Kristallographie, in press.
21. H-atoms are not considered since they do not diffract X-rays.
22. Teare, P. W., Acta Cryst., *12*, 294 (1959).
23. The generally accepted values for a normal hydrocarbon chain are 1.54 Å for the C–C distance and 111° for the C–C–C bond angles; however, better values are those given in Ref. 22.
24. To be published.
25. We wish to thank Drs. Starn and MacGillavry for the kind permission to present this datum prior to publication.
26. That the planes of the two carboxyl groups need not be identical is strikingly shown in the case of α-pimilic acid: Kay, M. I., and L. Katz, Acta Cryst., *11*, 289 (1959).
27. Aleby, S., and E. von Sydow, *Ibid.*, *13*, 487 (1959).
28. Nicolaides, N., and F. Laves, J. Am. Chem. Soc., *76*, 2596 (1954).
29. Nicolaides, N. and F. Laves, Z. Kristallographie, in press.
30. We wish to thank Drs. O. Isler and R. Rüegg of the research group at Hoffmann LaRoche & Co., Basle, Switzerland, for donating a sample of perhydrosqualene and Sample Nos. 2,5,6,10 and 11 of Table IV. The preparation of Samples 5 and 6 have been described in Ref. 32 and channel length measurements have been used by the authors to establish the stereochemistry of their synthetic products.
31. Nicolaides, N., O. K. Reiss, and R. G. Langdon, J. Am. Chem. Soc., *77*, 1535 (1955).
32. Isler, O., R. Rüegg, L. Chopard-dit-Jean, H. Wagner, and K. Bernhard, Helv. Chim. Acta, *39*, 879 (1956).
33. Nicolaides, N., and R. Foster, J. Am. Oil Chem. Soc., *33*, 404 (1956).
34. Assignments of peak position in n.m.r. spectra for *cis* and *trans*-nerolidol have recently been made by Bates, R. B., D. M. Gale, B. J. Gruner, and P. O. Nicholas, Chem. Ind., 1907 (1961).
35. Dicker, D. W., and M. C. Whiting, J. Chem. Soc., 1994 (1958).
36. Dicker, D. W., and M. C. Whiting, Chem. Ind., 351 (1956).
37. Langdon, R. G., and K. Bloch, J. Biol. Chem., *200*, 135 (1953).
38. International Tables for X-ray crystallography, Vol. II, The Kynoch Press, Birmingham, England, 1959.
39. Heilbron, I. M., E. D. Kamm, and W. M. Owens, J. Chem. Soc., 1630 (1926).

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Acid-Treated Florisil as an Adsorbent for Column Chromatography

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Abstract

Acid-treated Florisil, prepared by the action of hot concentrated hydrochloric acid on Florisil, was found to be a useful adsorbent for separation of phospholipids and other complex lipids by column chromatography. This material gave separations similar to those obtained with commercial silicic acid but its coarse mesh size simplified the technical operations and permitted faster flow rates. Its use is illustrated by separations of model compounds and of lipids extracted from liver and brain.

Introduction

Florisil, a commercially-prepared magnesia silica gel, has been used successfully in our laboratory for several years as an adsorbent for the separation of different classes of neutral lipids by column chromatography (3). The order of elution of neutral lipid classes from Florisil columns is the same as that observed with columns of silicic acid, but Florisil has the advantage of a coarse mesh size which makes for easier handling and more rapid flow rates. In spite of its coarse mesh size, Florisil seems to have as much adsorptive surface as the commonly used fine mesh preparations of silicic acid, and lipid loads of 10 mg or more per gram of adsorbent may be readily separated without evidence of overloading.

Although Florisil proved very satisfactory for the separation of neutral lipid classes, it could not be used to separate and recover phospholipids satisfactorily. Only part of the phospholipid was recovered by eluting the columns with methanol and there seemed to be no clean separation of different phospholipid classes (3). Rouser et al. (18), working with beef brain lipids, also reported difficulties due to trailing of peaks. They found that large volumes of solvents were required for elution of phospholipids and some of the adsorbent (magnesium silicate) was eluted along with the lipids.

Free fatty acids, like phospholipids, are adsorbed more strongly on Florisil columns than on silicic acid columns, but it is possible to recover free fatty acids quantitatively by including acetic acid in the eluting solvent (3). This is a further advantage of Florisil over silicic acid, since the free fatty acid and triglycer-

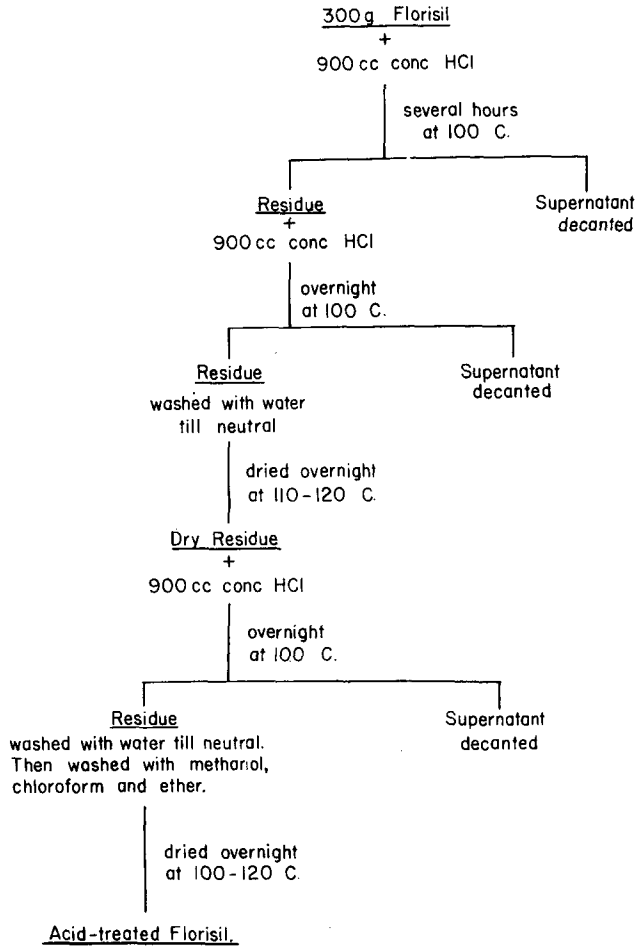
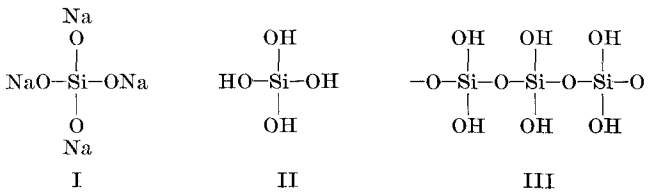


FIG. 1. Flow sheet of the procedure used for preparation of acid-treated Florisil.

ide fractions tend to overlap on silicic acid whereas they are easily separated on Florisil.

It seems likely that the selective retention of ionic lipids on Florisil is due to its magnesium content. Analytical data provided by the supplier (Floridin Company) is: MgO 15.5 ± 0.5%, SiO_2 84.0 ± 0.5%, and Na_2So_4 0.5% average (1.0% maximum). The presence of magnesium is in fact the main distinguishing feature between Florisil and silicic acid and methods used for the preparation of these two materials are rather similar. In each case the starting material is an aqueous solution of sodium silicate (water glass). Silicic acid or silica gel (used synonymously in the literature (21)) is prepared by addition of a strong acid (hydrochloric or sulfuric) which converts the sodium silicate (I) to an acidic form (II), which polymerizes by elimination of the elements of water (9) to form chains consisting of alternating silicon and oxygen atoms (III). The exact mechanism and the nature of the molecular species involved are uncertain and this simplified description should not be taken too literally. Cross-linking between chains can also occur by a similar mechanism, giving a 3-dimensional structure. Water is adsorbed to



these chains, particularly in association with the silanol (Si–OH) groups, so that the whole structure is highly hydrated. The gel which precipitates is separated and dried, usually at elevated temperatures, to remove most, but not all, of the adsorbed water and to fix the gel structure by further elimination of the elements of water from adjacent silanol groups.

Florisil is prepared by addition of magnesium sulfate to an aqueous sodium silicate solution. This produces a hydrated polymer with a structure similar to III except that some of the silanol groups are replaced by –Si–O–Mg–OH or –Si–O–Mg–O–Si– groups. This gel is also dried at an elevated temperature (500F) to remove adsorbed water and to set

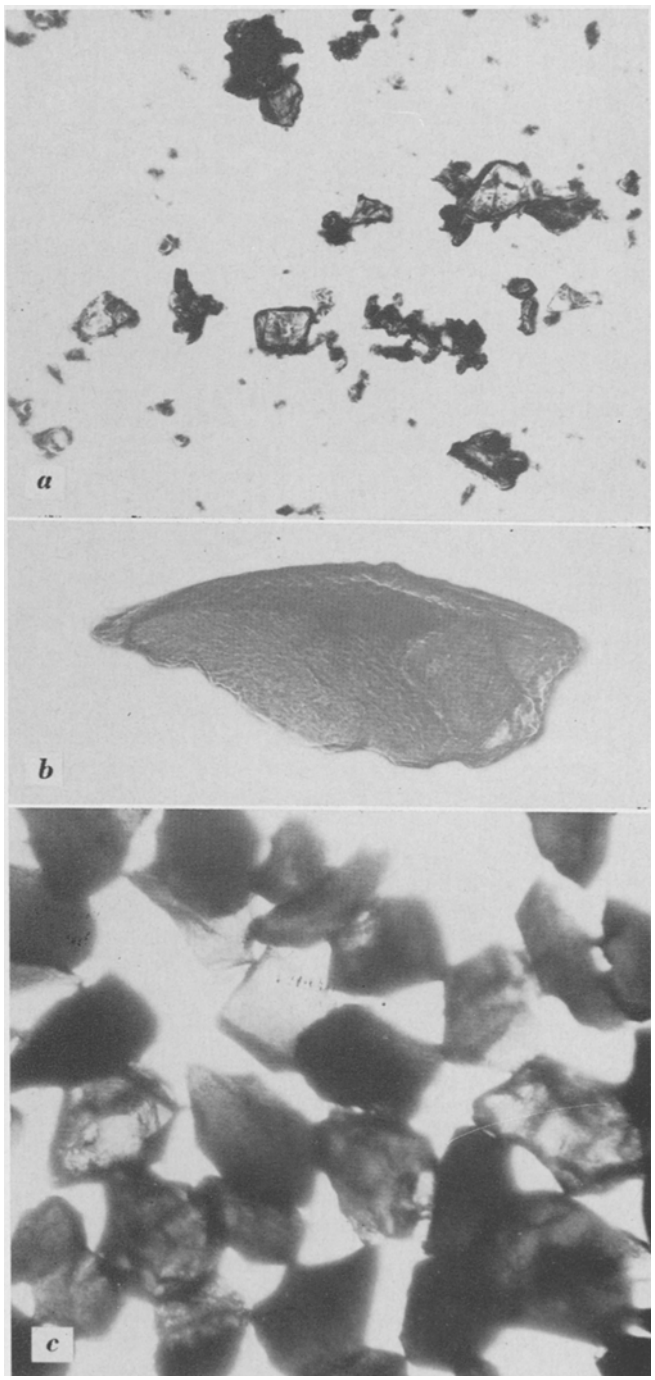


FIG. 2. a) A commercial preparation of silicic acid suitable for chromatography (x 250).
b) A particle of acid-treated Florisil at the same magnification (x 250).
c) Acid-treated Florisil at lower magnification showing the range of particle size (x 100).

the gel structure. Material with greater adsorptive activity is prepared by subsequent heating at 1200F.

From this brief discussion it can be seen that treatment of Florisil with a strong acid to replace Si—O—Mg—OH and Si—O—Mg—O—Si groups with Si—OH groups should yield a silicic acid polymer similar to commercial silicic acid. Treatment of Florisil with dilute hydrochloric acid at room temperature did in fact give a product whose chromatographic properties with respect to free fatty acids more nearly resembled those of commercial silicic acid (4). However, this material was not entirely satisfactory as an adsorbent because the ease of elution of free fatty acids and of phospholipids depended to some extent on the column load. A small load of an individual component was more difficult to elute from the column than a larger load and required either more eluting solvent or an eluting solvent of greater polarity. This behaviour was attributed to the presence in the adsorbent of residues of magnesium or other metals sufficient to retard small loads of ionic lipids. The difficulty was overcome by using repeated treatment with concentrated acid at elevated temperatures, and the resultant product appears to be a useful adsorbent for the chromatography of phospholipids and other complex lipids such as cerebrosides.

Procedures

Preparation of Acid-Treated Florisil

Florisil (300 g of 60–100 mesh material activated at 1200F) was mixed with 3 vol of concentrated hydrochloric acid and heated on a steam bath for several hours. The hot supernatant was decanted, the residue was washed with a little concentrated hydrochloric acid, and then heated overnight with another 3 vol of concentrated acid. The following day the hot supernatant was again decanted and the residue was washed with water—first in the flask by decantation, and then on a Buchner funnel until the washings were neutral. The residue was sucked dry and then heated for 24 hr in an oven at 110–120C. Finally this material was again heated overnight with 3 vol of hydrochloric acid and again the hot acid was decanted and the residue washed with water until neutral. In this instance the washing was continued with ca. 400 ml each of methanol, methanol-chloroform (1:1), chloroform and finally ether. The treated Florisil was allowed to air-dry and was activated by heating overnight at 110–120C. It was then ready for use and was stored in a stoppered flask at room temperature.

A flow-sheet of the procedure is shown in Figure 1. The quantities used provide enough material for about 20 twelve-gram columns. Larger batches could presumably be prepared and it is also possible that the proportions of hydrochloric acid to Florisil could be reduced. The first treatment with acid probably removes most of the magnesium and the second and third treatments were included to eliminate residual traces. Heat activation between the second and third acid treatments was included to remove most of the adsorbed water from the gel structure so that on the third treatment acid could penetrate more readily to remove metal ions from relatively inaccessible sites. The final washing with organic solvents removes a certain amount of colored material which otherwise is eluted during the running of chromatograms. Some of this material is probably derived from impurities in the hydrochloric acid, and it seems desirable to use a good grade of water-clear acid for this procedure.

Acid-treated Florisil prepared in this way has approximately the same particle size as the Florisil prior to treatment. Figure 2 illustrates the difference in particle size between this preparation and a commercial brand of silicic acid commonly used for column chromatography.

Chromatographic Methods

The column chromatograms were carried out with 12 g lots of adsorbent packed in chromatographic tubes 1.2 cm in diam. This amount of acid-treated Florisil gives a column 20 cm in height as compared with 15 cm for Florisil. In packing columns, it was found more convenient to prepare a slurry of acid-treated Florisil in solvent for addition to the column rather than adding the dry material to a column filled with solvent as was done in the case of Florisil. For chromatography of neutral lipids, columns were packed in Skellysolve B (a mixture of hydrocarbons, mainly n-hexane) and eluted with solvent mixtures used previously with Florisil columns (3). For chromatography of phospholipids and glycolipids, columns were packed in chloroform and eluted with 75 ml of that solvent, followed by 75 ml each of solvent mixtures containing 5,10,25, and 50% methanol in chloroform and finally with 75 ml of methanol.

For thin-layer chromatograms (TLC), thin glass plates (3¼″× 4″, thickness 1 mm) were coated with Silica Gel G (prepared according to Stahl, E. Merck, A. G. Darmstadt, Germany) by means of a Desaga applicator and dried in an oven at 120C for 2 hr.

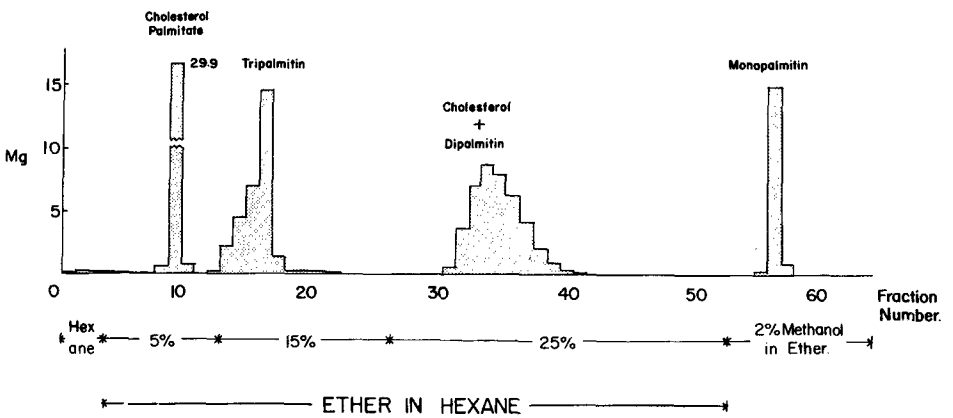


FIG. 3. Separation of neutral lipids on a 12 g column of acid-treated Florisil. Five ml fractions were collected. The column load consisted of 30 mg each of cholesterol palmitate, tripalmitin and cholesterol and 15 mg each of dipalmitin and monopalmitin. The elution schedule was the same as that used previously for Florisil columns (3).

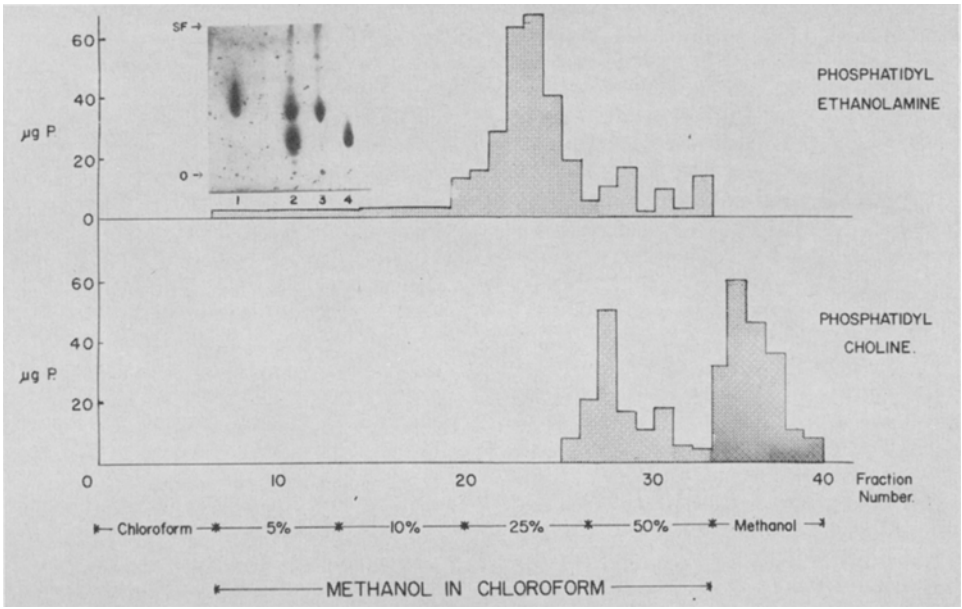


FIG. 4. Chromatography of commercial synthetic phospholipids on a 12 g column of acid-treated Florisil. Elution was carried out with 75 ml of each of the solvent mixtures indicated. Eleven ml fractions were collected. The thin-layer chromatogram was run on Silica Gel G. Developing solvent: chloroform-methanol-water 65:20:3. SF—solvent front, O—origin. From left to right:
Lane 1) material eluted with 25% methanol in the upper chromatogram
2) commercial phosphatidyl choline (dipalmitoyl)
3) material eluted with 50% methanol in the lower chromatogram
4) material eluted with methanol in the lower chromatogram

After the plates were developed with either hexane-ether-acetic acid (60:40:1) or chloroform-methanol-water (65:20:3), they were sprayed with concentrated sulfuric acid and heated on a hot plate until the charred spots appeared and the residual sulfuric acid evaporated.

Separation of Model Compounds

The pattern of elution of neutral lipids from acid-treated Florisil is shown in Figure 3. This pattern is similar to that observed previously with Florisil (3), except that tripalmitin and dipalmitin were eluted earlier in relation to cholesterol palmitate and free cholesterol. Because of this, the cholesterol and dipalmitin peaks overlapped on acid-treated Florisil. In earlier studies it was found that the positions of tripalmitin and dipalmitin on Florisil chromatograms were more sensitive to the moisture content of the adsorbent than were those of cholesterol palmitate and cholesterol, and this may also hold true for acid-treated Florisil. The separations obtained may thus depend on the temperature used for activation of the acid-treated Florisil.

In contrast to the above compounds, palmitic acid behaved very differently on acid-treated Florisil. Whereas it was retained on Florisil throughout the elution procedure shown in Figure 3, it was eluted from acid-treated Florisil immediately after tripalmitin, and partially overlapped the tripalmitin peak. This is analogous to the behaviour of free fatty acids on silicic acid columns (10). The behaviour of phospholipids on acid-treated Florisil was also much the same as on silicic acid. When a commercial sample of synthetic dipalmitoyl-L-α-glycerylphosphorylethanolamine was chromatographed with increasing amounts of methanol in chloroform, the major peak was found in the 25% methanol fraction (Fig. 4). A commercial sample of synthetic dipalmitoyl-L-α-glycerylphosphorylcholine gave two major phospholipid peaks, in the 50% methanol and pure methanol fractions respectively. Investigation of the original sample with TLC showed that it contained two main components which were separated on the column of acid-treated Florisil. Comparison of these two substances with samples of liver lecithin and egg lecithin and with a pure sample of dimyristoyl-L-α-glycerylphosphorylcholine on TLC indicated that the material eluted from the column with methanol was probably phosphatidyl choline. The other component was not identified.

Separation of Liver Lipids

Normal rat liver lipids prepared by the method of Folch et al. (7) gave the pattern shown in Figure 5 when chromatographed on acid-treated Florisil with chloroform and methanol as eluting solvents. TLC indicated that neutral lipids, which run near the solvent front in the system used, were eluted mainly with chloroform. The 5% methanol fraction also appeared to contain small amounts of neutral lipids, but in addition there were several compounds with lower mobility. The 10% methanol fraction gave one main spot on the TLC. The material in these two fractions was not identified further but may contain phosphatidic acids and polyglycerophosphatides (8, 21). Phosphatidyl ethanolamine was eluted with 25% methanol in chloroform and this fraction may have contained phosphatidyl serine. The elution of phosphatidyl choline began in the 50% methanol fraction and continued in the pure methanol fraction. The latter also contained sphingomyelin. The location of phosphatidyl inositol has not been determined.

Separation of Brain Lipids

Lipids of white matter of normal human brain were prepared by a scaled-down version of the procedure described by Carter et al. (5), using as extracting solvents acetone and ether, both at room temperature, followed by hot ethanol. Chromatography of the acetone, ether, and ethanol extracts on acid-treated Florisil gave the patterns shown in Fig-

ure 6. TLC gave some indication of the complexity of these fractions and the degree of separation achieved on acid-treated Florisil.

The acetone-soluble material consisted largely of cholesterol, which was eluted in the chloroform fraction, but appreciable amounts of other lipids were also present and were eluted in later fractions. The ether and ethanol extracts gave very little material in the chloroform eluent. The material eluted with 10% methanol in chloroform seemed to be mainly cerebrosides as judged by the TLC and by sugar and phosphorus analysis (Table I). Gas-liquid chromatography of the fatty acid components of this fraction gave patterns which were also characteristic of cerebrosides. This was the largest fraction in the hot ethanol extract, making up about 40% of the total.

The material eluted with 25% methanol in chloroform made up 55 and 25% respectively of the ether and hot ethanol extracts. This fraction of the ether extract appeared to consist mainly of phospholipids while the corresponding fraction from the ethanol extract had a fairly high sugar content and a rather low phosphorus content. The TLC indicated that this fraction from both the ether and ethanol extracts was a mixture of a number of different compounds. The third major peak in the ether and ethanol extracts was eluted with methanol and consisted of a mixture of lecithin and sphingomyelin in each case, as judged by TLC.

Discussion

The separations observed on acid-treated Florisil were analogous to those obtained with columns of commercial silicic acid (6,8,12,15,19,20,21), but the coarse mesh of the Florisil preparations simplified the technical operations involved in packing and running columns. It has been reported that better separations can be achieved by using material of finer mesh size

TABLE I
Analysis of Brain Lipids from Columns of Acid-Treated Florisil

	Fraction from Column			
	10% Methanol	25% Methanol	Methanol	
Sugar (%)[a]	22.1	2.3	0.9	Ether Soluble Lipid
Phosphorus (%)[b]	0.6	4.1	4.5	
Sugar (%)[a]	24.8	12.3	0.6	Hot Ethanol Soluble Lipid
Phosphorus (%)[b]	0.1	1.5	4.1	

[a] Sugar calculated as galactose and determined by a direct anthrone method (2). Values less than 1% are probably due to non-specific colors.
[b] Phosphorus determined by the method of King (11).

(10,14), and this may be a factor to consider in attempting separations of substances with very similar chromatographic properties, but for a preliminary fractionation of lipid classes from a crude extract the simplicity of operation of acid-treated Florisil columns appears to outweigh possible losses in resolving power. Recoveries of material were in general better than 90%, whether calculated in terms of weight or of lipid phosphorus applied.

Borgstrom (1) showed that neutral lipids and phospholipids could be separated on silicic acid by eluting the former with chloroform and the latter with methanol and subsequently, Hirsch and Ahrens (10) used a single silicic acid column to separate individual classes of neutral lipids and some phospholipids. However, this involves a lengthy elution time, and since phospholipids are eluted last, they are subjected to prolonged contact with column packing material, which may result in some decomposition. Further the use of methanol alone as eluting solvent, as in the Hirsch and Ahrens procedure, is not well-suited to the separation of complex mixtures of phospholipids.

Our approach has been to elute the neutral lipids from acid-treated Florisil as a group with chloroform, and then to separate different phospholipid and gly-

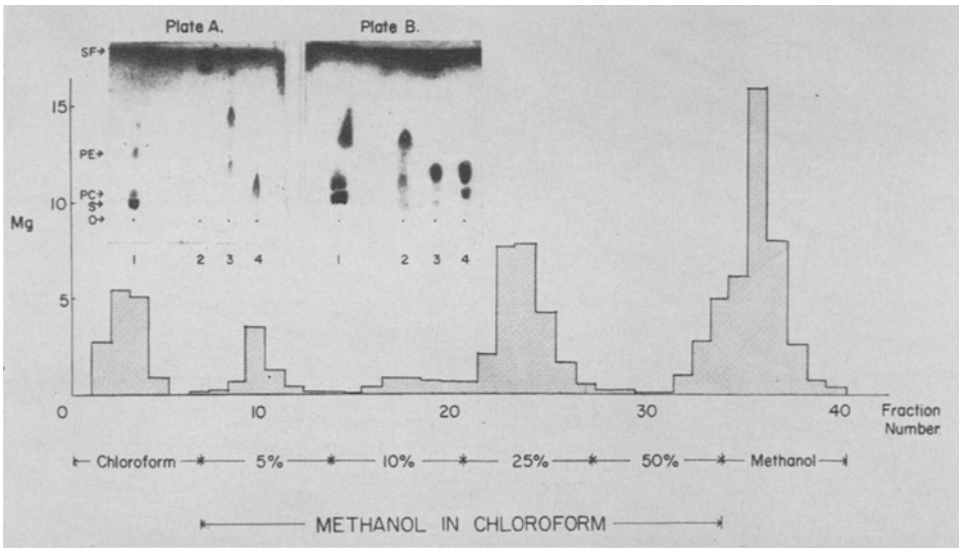


FIG. 5. Separation of rat liver lipids on a 12 g column of acid-treated Florisil. The column load was approximately 100 mg. The elution schedule was the same as in Figure 4 and 11 ml fractions were collected.

The thin-layer chromatograms were run on Silica Gel G and developed with chloroform-methanol-water 65:20.3. SF—solvent front, O—origin.

From left to right: Plate A
Lane 1) standard mixture of phosphatidyl ethanolamine (PE) (impure), phosphatidyl choline (PC) and sphingomyelin (S)
2) chloroform fraction from the column chromatogram
3) 5% methanol fraction
4) 10% methanol fraction

Plate B
Lane 1) standard mixture of PE, PC and S (spotted more heavily)
2) 25% methanol fraction from column chromatogram
3) 50% methanol fraction
4) methanol fraction

colipid classes on the same column by stepwise increases in the methanol content of the eluting solvent. In some instances the neutral lipids in the chloroform eluent from our columns were subsequently fractionated on a Florisil column by the procedure described previously (3). Florisil is still preferred for this separation, mainly because it gives a clean-cut separation of triglycerides and free fatty acids, which tend to overlap on either acid-treated Florisil or commercial silicic acid.

One advantage of carrying out these separations on two columns is that each of the columns can be conveniently packed and eluted during a working day. The elution time for chromatograms such as those shown in Figures 5 and 6 was 4–5 hr, and, since flow rate is not a limiting factor, the separations could perhaps be done in shorter times by increasing the rate of flow. The effect on resolution of altering flow rate was not investigated with acid-treated Florisil, but in earlier studies with Florisil columns an increase in flow-rate up to 180 ml per hr gave no apparent loss in resolving power.

A useful discussion of the forces involved in binding lipids to silicic acid has been provided by Rouser et al. (17,18), who pointed out that silicic acid columns may fail to give reproducible results because of variations in the water content of the system or because of differences in the silicate and free salt content of the silicic acid. Lack of reproducibility due to these factors may be minimized in acid-treated Florisil, since treatment with hot concentrated acid followed by washing with water should eliminate most of the silicate and free salt from Florisil, and the water content of the product can be controlled by the temperature used for the final activation. When Florisil columns were eluted with solvent mixtures containing appreciable amounts of methanol, the eluent contained extraneous material which was thought to be free salt present in the absorbent and magnesium silicate itself (3,17,19). This was not a problem with

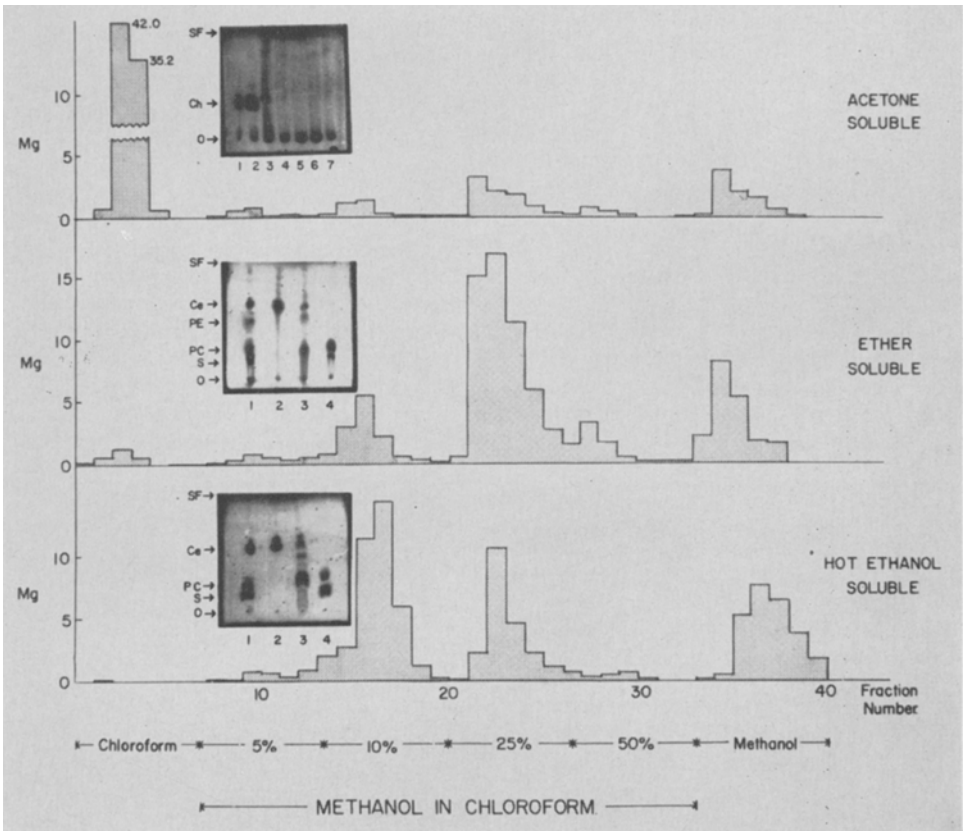


FIG. 6. Separation of lipids from white matter of human brain on 12 g columns of acid-treated Florisil. The column load was approximately 100 mg in each case. The elution schedule was the same as in Figure 4, and 11 ml fractions were collected.
The thin-layer chromatograms were run on Silica Gel G.

Top Plate: developing solvent: hexane-ether-acetic acid 60:40:1; From left to right:
Lane 1) cholesterol (Ch) standard
2) chloroform fraction from chromatogram of acetone soluble material
3) 5% methanol fraction
4) 10% methanol fraction
5) 25% methanol fraction
6) 50% methanol fraction
7) methanol fraction

Center Plate: developing solvent: chloroform-methanol-water 65:20:3.
Lane 1) standard mixture of cerebrosides (Ce), phosphatidyl ethanolamine (PE), phosphatidyl choline (PC) and sphingomyelin (S) in order of decreasing R_f values.
2) 10% methanol fraction from chromatogram of ether soluble material
3) 25% methanol fraction
4) methanol fraction

Lower Plate: developing solvent: chloroform-methanol-water 65:20:3.
Lane 1) standard mixture of cerebrosides (Ce), phosphatidyl choline (PC) and sphingomyelin (S)
2) 10% methanol fraction from chromatogram of hot ethanol soluble material
3) 25% methanol fraction
4) methanol fraction

acid-treated Florisil, which indicates that the interfering material had been removed by the procedure used for preparation.

One drawback of silicic acid chromatography is its failure to give clean separations of some classes of lipids such as lecithin and sphingomyelin. Acidic lipids also overlap with non-acidic lipid fractions (13, 16,17,18). Acid-treated Florisil undoubtedly shares these deficiencies, but it seems almost inevitable that any type of column used for preliminary fractionation of complex naturally-occurring mixtures will give some overlap of different lipid classes. The simplicity of operation and rapid flow rates of Florisil and acid-treated Florisil columns, coupled with the fact that they do provide separation of a number of different lipid classes, suggests their use for certain types of fractionation.

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R9FERENCES

1. Borgstrom, B., Acta Physiol. Scand., *25*, 101–110 (1952).
2. Carroll, K. K., J. Lipid Research, *1*, 171–178 (1960).
3. Carroll, K. K, *Ibid.*, *2*, 135–141 (1961).
4. Carroll, K. K., *Ibid.*, *3*, 388–390 (1962).
5. Carter, H. E., W. J. Haines, W. E. Ledyard, and W. P. Norris, J. Biol. Chem., *169*, 77–82 (1947).
6. Faillace, L. A., and S. Bogoch, Biochem. J., *82*, 527–530 (1962).
7. Folch, J., M. Lees, and G. H. Sloane Stanley, J. Biol. Chem., *226*, 497–509 (1957).
8. Hanahan, D. J., J. C. Dittmer, and E. Warashina, *Ibid.*, *228*, 685–700 (1957).
9. Hauser, E. A., "Silicic Science," D. Van Nostrand Co., Inc., N. Y., 1955, p. 54.
10. Hirsch, J., and E. H. Ahrens, Jr., J. Biol. Chem., *233*, 311–320 (1958).
11. King, E. J., Biochem. J., *26*, 292–297 (1932).
12. Long, C., and D. A. Staples, Biochem. J., *78*, 179–185 (1961).
13. Long, C., B. Shapiro, and D. A. Staples, *Ibid.*, *85*, 251–256 (1962).
14. Malmberg, E. W., Anal. Chem., *27*, 840–842 (1955).
15. Marinetti, G. V., J. Lipid Research, *3*, 1–20 (1962).
16. Rathbone, L., Biochem. J., *85*, 461–466 (1962).
17. Rouser, G., "Cerebral Sphingolipidoses," edited by S. M. Aronson and B. W. Volk, Academic Press, N. Y., 1962, p. 215.
18. Rouser, G., A. J. Bauman, G. Kritchevsky, D. Heller, and J. S. O'Brien, JAOCS, *38*, 544–555 (1961).
19. Rouser, G., J. O'Brien, and D. J. Heller, *Ibid.*, *38*, 14–19 (1961).
20. Weiss, B., Biol. Chem., *233*, 523–534 (1956).
21. Wren, J. J., J. Chromatog., *4*, 173–195 (1960).

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Special Methods of Purifying Fatty Acids

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Abstract

Preparative gas chromatography and thin-layer chromatography have been used successfully in several purification applications. Both methods are very good when small (10 mg or less) samples are involved. In fact, the low capacity of the methods is actually an advantage in such cases and is a major reason for using the methods. When larger samples are to be prepared by gas chromatography, the low capacity becomes a problem. However, by the use of conventional purification procedures to concentrate the desired component as much as possible, and the use of large columns (up to 1 in. diameter) gram quantities of rare fatty acids have been successfully prepared. Gas Liquid Chromatography has been an invaluable tool where other purification methods have failed.

Introduction

PREPARATIVE gas-liquid chromatography (GLC) and thin-layer chromatography (TLC) have become important methods in purifying fatty acids and their derivatives. Separations either impossible or unduly difficult in the past have yielded to the new methods. However, even though the techniques appear very attractive, and indeed do have great potential, the practical application of them in the purification of lipids is often very difficult.

Typical applications will be described here. It is intended that these examples should illustrate the the scope of the methods and, especially in the case of relatively large scale GLC preparations, the examples will indicate detailed techniques useful in actually applying the methods. Auxiliary methods such as distillation and crystallization will also be set in proper perspective.

The first section describes concentration steps that should be taken prior to preparative chromatography. In the second section the preparation of uniformly carbon-14 tagged fatty acids is used to illustrate GLC purification of relatively small amounts of methyl esters. This is followed by an example of the use of thin-layer chromatography to prepare small amounts of radioactive cholesteryl palmitate. The last section covers in some detail the use of GLC to prepare larger amounts (1–10 g) of methyl esters.

Procedures

Concentration of Fatty Acids from Natural Sources

A desired fatty acid is often present in very low concentration in a very complex mixture. If any appreciable amount of the component is needed (such as a gram) chromatography techniques are at a disadvantage because of their inherently low capacity. It is usually worthwhile, therefore, to use conventional separation procedures to concentrate the component into a smaller sample. Every effort is made to purify to the greatest possible extent in this way—preferable to about 90%.

Although it is impossible to make general rules for the concentration procedure, certain guides have been listed below:

1) Select a source of the desired fatty acid which contains a reasonable concentration of the acid. The source selected will not necessarily be that which contains the highest concentration. Two factors which often lead to some other source are a) availability, and b) interference from components which are hard to separate.

2) Analyze by GLC, preferably using both polar and non-polar stationary phases.

3) Try to determine which impurities will be hard to separate or at least what their characteristics are. This may be done entirely by careful GLC with standards, or it may require attempted separations