An Apparatus for the Rapid Preparation of Fatty Acid Esters from Lipids for Gas Chromatographic Analysis

IN APPLYING THE METHOD of Metcalf, Schmitz and Pelka (1), I have found the apparatus shown in Figure 1 useful, especially when only a few milligrams of lipid are available for analysis. The apparatus (all glass) consists of a ground glass joint, a capillary and a bulb. The ground glass joint is a type 10/30, the capillary has an inside diameter of 3 mm and a length of 15 mm. The volume of the bulb can be varied to meet the needs of the individual researcher, but I prefer 5 ml. The overall length of the flask is 90 mm. It was designed to fit into a No. U 6878, International Clinical Centrifuge.

Because of the capillary, it is necessary to introduce all samples and reagents directly into the bulb with a syringe (1 or 5 ml) fitted with 10 cm long needles. It is desirable to blunt the tips of the needles.

The reagents as described in (1) were used, except 0.5 N methanolic potassium hydroxide was used instead of 0.5 N sodium hydroxide. Approximately 25 mg of fatty material is introduced on to the bottom of the flask using the 1 ml syringe. One milliliter of 0.5 N methanolic potassium hydroxide is then introduced using the 5 ml syringe. The mixture is heated on a steam bath until the fatty globules go into solution. This step takes about 2 min. Two milliliters of boron-triflouride reagent is added, again using a syringe, and the mixture boiled for 2 min. During both the saponification and esterification steps, a condenser can be attached to the top of the flask to return low boiling fatty material. After esterifying with boron-triflouride sufficient saturated sodium chloride solution is added to the flask to raise the level of the liquid to about halfway up into the capillary. Again, the syringe is used. Addition of the saturated salt solution too rapidly will cause an air bubble to form in the capillary which will be difficult to remove. The flask is then centrifuged for 2 min.

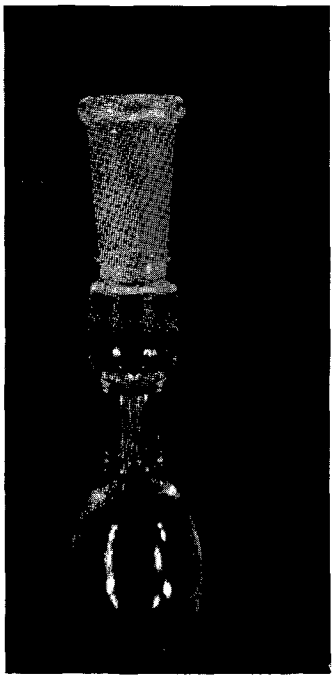

FIG. 1. Apparatus for the preparation of fatty acid esters.

The methyl esters float to the surface and are concentrated in the capillary, providing easy availability of the material.

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REFERENCES

1. Metcalf, L. O., A. A. Schmitz and J. R. Pelka, Anal. Chem. *38*, 514 (1966).

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The Determination of Organically Combined Sulfuric Anhydride In Sulfated Tall Oil Fatty Acid Esters

In the determination of organically combined sulfuric anhydride by the AOCS method (F2a-44) the procedure calls for heating under reflux for 1.5 hr or until the layers are clear. Use of this method for sulfated tall oil fatty esters gives poor results. We have found that a 4- to 6-hr reflux period is required with sulfated tall oil fatty acid esters before the oil and water layers are clear. With longer periods under reflux the combined sulfuric anhydride assay increases. Sulfated tall oil fatty acids, on the other hand, are clear after boiling for one-half to one hour and give a very satisfactory analysis.

A study of the time under reflux versus SO_3 content was made using sulfated tall oil fatty acid n-butyl ester to determine the time necessary for complete removal of combined SO_3. The results are shown in Figure 1. Both layers were clear after 4 hr of boiling, corresponding to 6.18% SO_3; however, the maximum value of 6.54% was obtained after 8 hr.

A variety of other sulfated materials was analyzed using a 2- to 24-hr period to determine the minimum time required for complete hydrolysis. The results are shown in Table I.

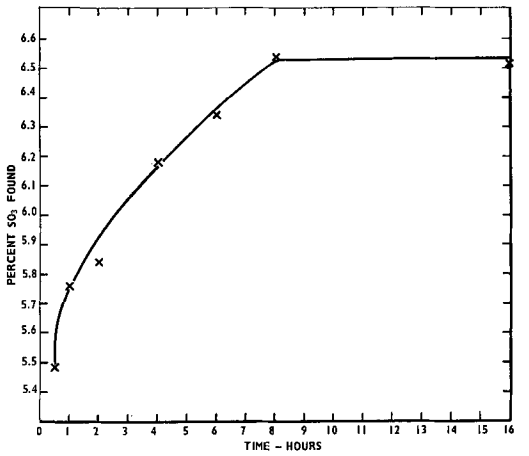

FIG. 1. Study of time under reflux vs. SO_3 content.

Based on the results obtained, the reflux period required for the determination of organically combined SO_3 in sulfated fatty acid esters should be extended at least 2 hr after both layers are clear. In practice, we have found it convenient to carry out the hydrolysis by heating the mixture under reflux overnight (ca. 16 hr) and titrating the samples the following day. Using this method, duplicate runs, by one operator, normally do not vary by more than 0.02% SO_3.

TABLE I

Analysis of Sulfated Materials for Combined SO_3 at Various Reflux Periods

Time under reflux (Hr)	Combined SO_3 (%)[a] 2	6	16	24	
Material sulfated					
n-Propyl tallate	7.32	7.86	7.92	7.92	(4)[b]
Iso-octyl tallate	5.98	6.30	6.34	6.34	(6)[b]
Propyl oleate	7.60	8.06	8.16	8.14	(4)[b]
Tall oil fatty acid	7.12	7.12	7.12	7.12	(1)[b]
Ricinoleic acid	4.34	4.42	4.50	4.50	(3)[b]
Castor oil	5.70	5.78	5.78		(2)[b]

[a] Per cent SO_3 was determined on material as is.
[b] Time (hr) required for both layers to be clear.

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• *Letter to the Editor*

Validity of Gunstone's Acyl Group Distribution Theory in Vegetable Fats Containing Appreciable GS_3

GUNSTONE (1) presented a theory of acyl group distribution of vegetable fats which he stated provides a satisfactory correlation for most of the available data and unifies earlier theories into one covering the whole range of vegetable fats. The validity of this theory was, however, illustrated with only two vegetable fats, *Sapium sebiferum* (2) and *Platonia insignis* (3), that contain more than traces of GS_3. Other data reported in the literature for several vegetable fats is not in full agreement with Gunstone's theory. The data were obtained by reasonably reliable oxidation and crystallization methods and are presented in Table I. The data is for fats wherein full glyceride type structures have been determined along with the proportions required according to Gunstone's and Kartha's theories (4,5) and the Tally Number for each fat with reference to each theory.

The Tally Number (TN) is a method for obtaining a reasonable numerical idea of the agreement between experiment and theory in glyceride type structure studies in keeping with present day stan-

TABLE I

Glyceride Type Structures of Some Vegetable Fats Containing Appreciable GS_3

Fat	1(c)[a]	2(c)	3(o)[b]	4(c)	5(o)	6(o)	7(o)	8(o)	9(o)
Sm	73	65	54	53	51	82	74	77.5	92.6
Glyceride type structure, experimental, moles %									
GS_3	21	20	9	8	9	57	42	47	81
GS_2U	77	55	54	54	48	33	40	40	17
GSU_2	2	26	28	32	31	8	15	13	2
GU_3	0	0	9	6	12	2	3	0	0
Glyceride type structure, calculated, Gunstone's theory, moles %									
GS_3	19	0	0	0	0	46	22	33	78
GS_2U	81	96	65	63	58	54	78	67	22
GSU_2	0	5	31	33	37	0	0	0	0
GU_3	0	0	4	4	5	0	0	0	0
Tally number	8	81	28	20	32	42	78	54	10
Glyceride type structure, calculated, Kartha's theory, moles %									
GS_3	19	20	9	8	9	55	40	46	80
GS_2U	81	58	53	51	45	36	43	41	19
GSU_2	0	19	30	33	35	8	15	12	1
GU_3	0	3	8	8	11	1	2	1	0
Tally number	8	13	4	6	8	6	6	4	4

[a] c–GS_3, GS_2U by crystallization.
[b] o–GS_2U by azelaoglyceride estimation: GS_3 by crystallization where S is above C_{18} and by acetic acid-acetone-permanganate oxidation where S is below C_{18}.

Key: 1. *Sapium sebiferum* (Meara and Gupta, *J. Chem. Soc.* p 1337, 1950).
2. *Platonia insignis* (Hilditch and Pathak, *J. Chem. Soc.* Suppl. #1, p 587, 1949).
3. Palm oil (Kartha, *JAOCS 30*, 326, 1953; *31*, 85, 1954).
4. Palm oil (Hilditch and Maddison, *J. Soc. Chem. Ind. 59*, 67, 1940).
5. Palm oil (Luddy, et al., *JAOCS 31*, 266, 1954).
6. *Myristica malabarica* I (Kartha, *J. Sci. Ind. Res. 13A*, 72, 1954).
7. *Myristica malabarica* II (Kartha and Narayanan, quoted in Kartha, *J. Sci. Ind. Res. 21A*, 577, 1962).
8. *Myristica attenuata* (Kartha and Narayanan, *J. Sci. Ind. Res. 21B*, 494, 1962).
9. *Myristica fragrans* (Kartha and Narayanan, *J. Sci. Ind. Res. 21B*, 442, 1962).