• *Letter to the Editor*

Preliminary Studies on the Analysis of Mono- and Di-glycerides by GLPC

GAS-LIQUID partition chromatography (GLPC) has been advanced to the point where it may now be used routinely for the determination of fatty acid composition. The only reported work on the analysis of glycerol esters by GLPC is that of McInnes *et al.* (1). Recent work in our laboratory has shown that it may be feasible to analyze both mono- and diglycerides by GLPC.

The basic apparatus used was of conventional design. Block and column temperatures were controlled to ±1.0°C. The helium flow was maintained at 35 ml. per minute and 30 p.s.i. Bare platinum wires drawing 1.25 amperes were used to transmit component response to a 1.0-mv. recorder. Samples were injected through a silicone rubber septum with a 50 μl. capacity syringe. The injection port was cooled with an air coil to preserve the rubber.

The column used in this work was made from a 24-in. length of 3.5-mm.-inside-diameter stainless steel tubing. It was packed with acid washed 40–70 mesh Celite 545 coated with 23% of high vacuum silicone grease by weight. This column was conditioned at 340 to 370°C. for several days before use.

It was found that mono- and diglycerides, as such, could not be eluted from the column. Acetylation assured their complete elution from the column. All samples were acetylated by refluxing with an equal volume of acetyl chloride for 45 min. and removing excess acetyl chloride under vacuum.

The retention times of various acetylated mono- and diglycerides at different temperatures are shown in Figure 1. No differentiation could be made between mono-olein and monostearin. A small peak was clearly evident shortly before the major peak for most of the mono- and diglycerides tested. These peaks are believed to be caused by the B-isomers since they are not present when short-chain triglycerides of equivalent molecular weight are analyzed. Further work needs to be done however before the identity of these peaks is clearly established.

TABLE I

Quantitative Analyses of a Synthetic Glyceride Mixture by GLPC

Component	Calculated composition	Experimentally determined values — Column temperature (°C.)				
		305	305	298	284	284 [a]
	%	%	%	%	%	%
α Monocaprin	0.3	.....	.....	.....	0.6	0.4
β Monolaurin	1.2	.....	.....	0.6	2.7	1.6
α Monolaurin	6.5	5.5	5.0	5.3	8.5	5.1
β Monomyristin	2.6	5.6	3.4	4.7	5.9	3.5
α Monomyristin	10.7	12.4	11.0	12.6	20.6	12.3
β Monopalmitin	2.6	6.7	3.8	5.9	5.1	3.1
α Monopalmitin	8.7	10.3	8.0	9.6	12.7	7.6
β Monoolein	4.1	6.1	4.4	5.6	6.9	4.1
α Monoolein	16.0	15.9	16.6	15.3	27.7	16.6
α Monoarachidin	0.6	.....	1.2	2.0	.....	.....
1,2 Dilaurin	0.3	.....	1.0	.....	3.4	2.0
1,1 Dilaurin	2.6	2.9	2.6	1.9	5.8	3.5
1,1 Lauromyristin	0.7	2.7	2.9	1.9	.....	.....
1,2 Dimyristin	1.2	.....	1.3	.....	.....	.....
1,1 Dimyristin	5.3	3.2	3.7	2.3	.....	.....
1,1 Myristopalmitin	0.4	.....	1.8	.....	.....	.....
1,1 Dipalmitin	1.1	.....	.....	.....	.....	.....
Trilaurin	32.4	28.7	33.3	32.2	.....	.....
1.1 Diolein	2.7	.....	.....	.....	.....	.....

[a] Original values multiplied by 0.6 to compensate for high molecular weight components not eluted at this temperature.

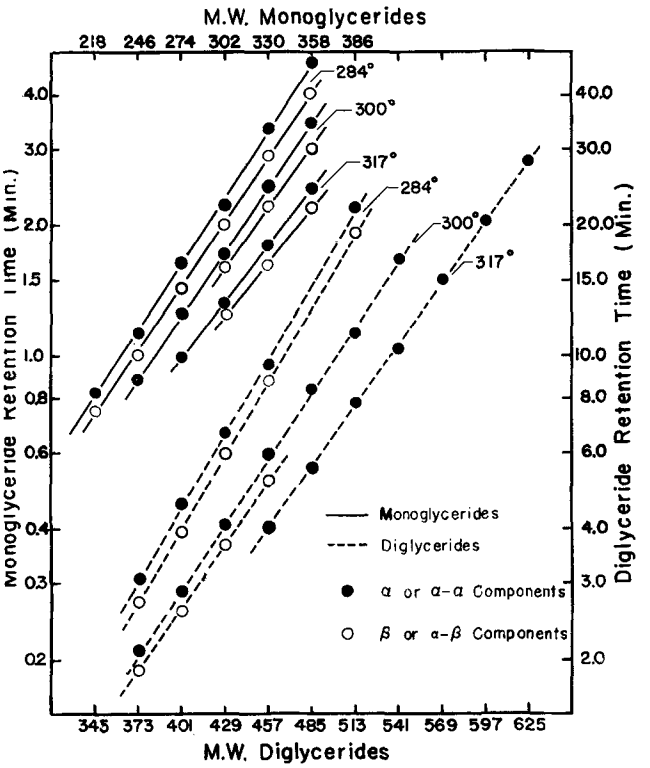

FIG. 1. Plot of log retention time *versus* molecular weight for mono- and diglycerides.

It may be seen that a straight line relationship between molecular weight and the logarithm of the retention time exists for each class of compounds. Raising the column temperature has the advantage of allowing larger components to be eluted but has the disadvantage of reducing the time interval between the monoglyceride peaks.

A mixture of several distilled monoglycerides obtained from Distillation Products Industries and trilaurin was prepared. Since these monoglycerides contained minor amounts of other monoglycerides and diglycerides, the approximate composition of the synthetic mixture was calculated from gas chromatographic analyses of the individual monoglycerides.

An analytical error resulting in a value of 80% purity for a monoglyceride which is actually 90% pure would introduce an error of only 2% into its calculated proportion, when 20% of the substance is present in a mixture. Thus a large analytical error will introduce a relatively small error into the calculated composition of the mixture.

In all GLPC analyses the weight percentage of the individual components were calculated from the following formula:

$$\text{Weight } \% = \frac{\text{rhaM}}{\Sigma(\text{rhaM})} \times 100$$

where r = retention time

h = peak height
a = attenuation factor
M = molecular weight of the corresponding unacetylated component

Using this formula, the calculated composition of the glyceride mixture is compared in Table I with that found in several GLPC determinations at several temperatures. It may be seen that the results are in reasonably good agreement with each other.

It is hoped that these preliminary experiments will serve to increase interest in the quantitative estimation of mono- and diglycerides by gas chromatography.

V. R. HUEBNER
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REFERENCE

1. McInnes, A. G., Tattrie, N. H., and Kates, M., "The Application of Gas-Liquid Partition Chromatography to the Microestimation of Monoglycerides," presented at the 32nd Fall Meeting, American Oil Chemists' Society, Chicago, Ill. October 20–22, 1958.

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Report of the Examination Board, 1958-1959

DURING the year ended May 31, 1959, among their various active members, 40 commercial laboratories were granted referee certificates from the A.O.C.S. Examination Board, as follows:

7—Cottonseed, Oil Cake and Meal, Fatty Oils, and Tallow and Grease
14—Cottonseed, Oil Cake and Meal, and Fatty Oils
7—Cottonseed, Oil Cake and Meal
2—Oil Cake and Meal, Fatty Oils, and Tallow and Grease
1—Oil Cake, Meal and Fatty Oils
6—Oil Cake and Meal
2—Fatty Oils
1—Tallow and Grease

All laboratories certified for Oil Cake and Meal were automatically certified for Protein Concentrates.

During the certificate year C. E. Worthington of Barrow-Agee's Decatur, Ala., laboratory was transferred to Memphis, Tenn., O. M. Bakke of Houston Laboratories, Houston, Tex., and R. M. Dillard, Texas Testing Laboratories, Dallas, Tex., have retired from active chemical participation.

The chairman extends his thanks to all members of the Examination Board and to R. W. Bates and his efficient Smalley Committee for their excellent cooperation.

R. T. DOUGHTIE R. R. KING
E. R. HAHN R. C. STILLMAN
N. W. ZIELS, chairman

ABSTRACTS . . . . R. A. REINERS, Editor

ABSTRACTORS: Lenore Petschaft Africk, R. R. Allen, S. S. Chang, Sini'tiro Kawamura, F. A. Kummerow, and Dorothy M. Rathmann

• Fats and Oils

A SIMPLIFIED PROCEDURE FOR SYNTHESIS OF OLEIC-1-C^{14} ACID. Susanne von Schuching and E. Stutzman (Radioisotope Ser. and General Med. Research, Veterans' Administration Center, Martinsburg, W. Va. and Dept. of Biochem., The George Washington Univ. School of Med., Washington 5, D.C.). *J. Org. Chem.* 24, 345–6 (1959). Bergström's method for the introduction of a C^{14}-atom in the carboxyl position by means of the nitrile synthesis was modified for small scale experiments.

SYNTHESIS OF SOME OCTENOIC ACIDS. J. A. Knight and J. H. Diamond (The School of Chem. and the Engineering Expt. Sta., Georgia Inst. of Technology). *J. Org. Chem.* 24, 400–3 (1959). *Cis*-2-, -3-, -4-, and -6-octenoic acids were prepared by the catalytic semihydrogenation of the octynoic acids. *Trans*-3-, -4-, and -6-octenoic acids were obtained either directly or indirectly starting with a *trans* alkenoic acid obtained by a Knoevengal condensation. Physical properties, including infrared spectra, were determined for all of the acids and most of the intermediates. The infrared spectra of the *trans* compounds showed strong absorption in the region of 10.2–10.35 microns. None of the *cis* compounds showed absorption in this region.

ISOLATION FROM BUTTERFAT OF 14-METHYL PENTADECANOIC (ISOPALMITIC) ACID. R. P. Hansen, F. B. Shorland, and N. J. Cooke (Fats Research Lab., Dept. of Scientific & Ind. Research, Wellington, New Zealand). *Chemistry and Industry* **1959**, 124. C_{16} iso acid 14-methyl pentadecanoic acid was isolated from unhydrogenated butter fat and identified.

EVIDENCE FOR A NEW OXYGENATED FATTY ACID IN THE SEED OIL OF CHRYSANTHEMUM CORONARIUM. C. R. Smith, Jr., K. F. Koch, and I. A. Wolff (Northern Regional Research Lab., Peoria, Ill.). *Chemistry and Industry* **1959**, 259–60. A new epoxy fatty acid occurring in the seed oil of *Chrysanthemum coronarium* (family compositae) was named coronaric acid and its chemical structure proved to be *cis*-9:10-epoxy-*cis*-octadec-12-enoic acid.

INFLUENCE OF THE EXTRACTION OF LIPIDS FROM FLOUR ON GLUTEN DEVELOPMENT AND BREAKDOWN. A. H. Bloksma (Inst. for Cereals, Flour, and Bread T.N.O., Wageningen, The Netherlands). *Chemistry and Industry* **1959**, 253–4. The flour lipids play a role in the gluten development and breakdown. That the original dough properties are not restored completely upon reconstitution may be explained by either of the following three assumptions, namely (i) the contact with the solvent changes other flour constituents, e.g. the protein fraction; (ii) the flour lipids partly lose their essential properties during isolation, or (iii) upon reconstitution they do not reach the areas where they can exert their beneficial influence or reach these areas only after a long mixing time. Experiments also indicated that the mixing tolerance of a flour depends heavily upon the state of the flour lipids.

UNSATURATED FATTY ACIDS OF BUTTERFAT. W. E. Scott, S. F. Herb, P. Magidman, and R. W. Riemenschneider (Eastern Utilization Research and Development Div., Agr. Research Ser., U.S.D.A., Philadelphia 18, Pa.). *J. Agri. Food Chem.* 7, 125–9 (1959). The presence of C_{16} to C_{18} monoethenoid acids in butterfat was confirmed; the C_{12} and C_{14} acids were predominantly the *cis*-form, while the C_{16} and C_{18} acids had both *cis* and *trans* double bonds. The nonconjugated dienoic acids were found to be a mixture of *cis-cis* and either *cis-trans* or *trans-trans* isomers. Conjugated dienoic acids were identified as