havior additional homologs and other soils should be examined.

Comparison with Branched Chain Product

Unformulated. Since TPBS is a complicated mixture whose structure is only partially known, a comparison between this commercial product and pure compounds cannot be made on structural grounds. However, the TPBS used is inferior in foam and superior in wetting ability to any of the pure para isomers of comparable molecular weight. Futhermore it is interesting that only TPBS and p-2ϕ-2Me-C_{12} show maxima in their detergency vs. concentration curves. This may be related to the tertiary structure in the pure isomer and to a corresponding predominating structure in the commercial product.

Formulated (Table IV, and V)

Since all of the previous data were obtained for the unbuilt materials, we have attempted to compare the dishwashing foam (9) of formulations with these purified compounds, using a built commercial type formulation from TPBS as an internal standard. The maximum foam for the 1-phenyl compounds was at C_{10} chain length but for the 2-phenyl compounds it was at C_{13}. However, the advantage in dishwashing foam of the tertiary isomer, p-2ϕ-2Me-C_{12}, and central location of the phenyl group, p-5ϕ-C_{12}, are clearly evident.

The profound effect of a detergent additive in the formulation is clearly demonstrated. The actual amount of surfactant in the formula effects the dishwashing performance far less than the amount of additive, a mixture of lauroyl and myristoyl isopropanolamide in this case.

TABLE V

Effect of LMIPA and Sodium (1-Methyldecyl) benzenesulfonate Concentrations on Dishwashing Foam

Test Product: 20-35% p-2ø-C_{11}, 0-5.5% LMIPA, 40% $Na_5P_3O_{10}$ Q.S. Na_2SO_4

Control Product: 20.0% TPBS, 3% LMIPA, 7% Na_2SiO_3, 40% $Na_5P_3O_{10}$, 0.5% CMC, Q.S. Na_2SO_4 (A built commercial type product)

Conditions: Concentration, 0.15%; Temperature, 115F (46C); Hardness, 50 ppm

Test product		Plates washed		▲ Plates
% p-2ø-C_{11}	% LMIPA	Test product	Control product	Test - Control
20	0	12	20	−8
20	3.3	22	22	0
20	4.5	26	21	+5
20	5.5	23	20	+3
23.5	0	15	24	−9
35	0	15	20	−5

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• *Letters to the Editor*

A Simple and Rapid Method for the Determination of Myo-Inositol by Gas-Liquid Chromatography

THE EFFECTIVE APPLICATION of gas chromatography to the analysis of carbohydrates began with the work of Sweeley, Bentley, Makita and Wells in 1963 (1). This work yielded a simple, reliable and sensitive method for the analysis of polyhydroxy compounds with gas-liquid chromatography (GLC) through the formation of O-trimethylsilyl ether (TMS) derivatives. Three publications (2–4) have appeared recently describing methods for the quantitative determination of myo-inositol from various biological sources utilizing essentially the method of Sweeley et al. (1) mentioned above. In each of these publications the limited solubility of myo-inositol in the reaction mixture was pointed out, and, as a result, the time required for derivative formation (TMS) was 3 hr or more. We have found (5) that by the use of dimethylsulfoxide (DMSO), derivatives (TMS) can be ready for injection into the GLC instrument within 10 min after initiation of the reaction. DMSO does not interfere with quantitative trimethylsilylation as shown by parallel assays employing other methods. The derivatives, once formed, are not stable for more than 4–5 hr in DMSO unless stored in the absence of light at OC (6). Under these latter conditions extended periods of stability are obtained.

Aliquots from aqueous solutions of tissue extracts or lipid hydrolysates containing at least 20 μg of free myo-inositol were taken to dryness in 2.5 ml glass-stoppered conical centrifuge tubes by evaporation in vacuo. The tubes were then placed in a vacuum dessicator over NaOH pellets for several hours to remove the last traces of water. The residue was dissolved in 20 μl of dimethylsulfoxide (DMSO) and to this was added 110 μl of a freshly prepared reagent composed of anhydrous pyridine, hexamethyldisilazane and trimethylchlorosilane (8/2/1, v/v). The tubes were gently shaken and allowed to stand at room temperature (23C). After 5–10 min the ammonium chloride by-product was removed by centrifugation at low speed. All tubes were kept stoppered during the entire procedure except during the addition of reagents. Ten microliter aliquots were used routinely for injection into the GLC apparatus, although larger or smaller aliquots can be successfully used. Standard myo-inositol solutions for GLC were prepared in DMSO, and suitable aliquots were treated directly with TMS reagent for GLC.

The analytical data for myo-inositol in Table I were obtained by analysis of acid hydrolysates of the phosphoinositide complex (7) from kidney and tri-

TABLE I

A Comparison of the GLC-Method of Analysis for myo-Inositol with Chemical and Microbiological Methods.

Sample	μg Myo-Inositol		
	GLC	Microbiological	Chemical
Kidney PIC (3)	198	166	197
Brain TPI (5)	480	520	493
		Literature values	
		Hauser (9)	Dawson & Freinkel (10)
Brain [a] (7)	672 ± 125	730 ± 104	—
Testes [a] (7)	256 ± 76	—	280 ± 70
Kidney [a] (8)	546 ± 141	879 ± 223	610 ± 260

[a] Data expressed as μg myo-inositol per gram wet weight of tissue.
PIC — phosphoinositide complex.
TPI — triphosphoinositide.
Numbers in parentheses represent numbers of experiments.
Chromatographic conditions: Instrument, Barber-Colman (radium cell - 0.056 μC); column, ¼ in. × 6 ft. glass column, U-tube; 3% SE · 30 on Chromosorb W; column temperature, 145C; flash heater (inlet), 210C; cell temperature (detector), 190C; inlet pressure, 18 psi (argon); detector sensitivity, 10/2; chart speed, 7.5 in./hr; reaction time, 12 min.

phosphoinositide (8) of beef brain and on tissue extracts. It can be seen that there was satisfactory agreement between the values obtained when using the GLC method as contrasted with the older methods. The agreement between these methods and the reproducibility of our analytical data obtained over a period of time using GLC for myo-inositol analysis indicates the validity of the procedure.

The use of DMSO, thus, increases the utilization of GLC for analysis of myo-inositol by decreasing the time necessary for the formation of TMS derivatives.

This procedure has also been found by Hamilton and his colleagues (6) to be very useful in the analysis of other polyhydroxy compounds.

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D. R. FLINT
TEN-CHING LEE
C. G. HUGGINS
Department of Biochemistry
Tulane University
School of Medicine
New Orleans, Louisiana

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A Sensitive Method for Phosphorus in Lipids[1]

THERE ARE MANY METHODS for the wet ashing of lipid material, and there are many variations of the molybdenum blue colorimetric determination of ortho-phosphate (1). Perchloric acid wet ash methods are the most rapid, and stannous chloride as a reducing agent gives great sensitivity in the colorimetric determination. But one cannot use stannous chloride as the reducing agent in the colorimetric determination in the presence of excess perchloric acid. The method reported here enjoys both speed of digestion with perchloric acid and high sensitivity with stannous chloride. The excess perchloric acid is evaporated before the color is developed.

A solution of lipid material that contained at least 1 μg of phosphorus was put in a 30 ml Kjeldahl flask. A few glass beads (5 mm in diameter) were put in the flask, and the solvent was evaporated on a hot plate. Then, 1 ml of concentrated HNO_3 was added, and the flask was heated until the initial oxidation was completed. This was indicated by the formation of a homogenous solution and the subsidence of vigorous boiling and foaming. The flask was cooled, and 1 ml of 70% $HClO_4$/0.2 g of lipid was added. The flask was heated with a microburner under a perchloric acid fume head, such as described by Diehl and Smith (2), until heavy white fumes were evolved and the digest was clear, indicating that the digestion was complete. The entire digestion procedure takes about 5 min for the average sample.

The excess perchloric acid was evaporated rapidly by placing the flask in a second fume head which was pierced by a piece of glass tubing which extended into the neck of the Kjeldahl flask. Pyrex wool was packed in the space between the fume head and the neck of the flask, so that the head drew a current of air through the tube and into the Kjeldahl flask and quickly evaporated the acid as the flask was heated.

The digested material was transferred to a 25 ml glass stoppered volumetric flask, and the analysis was completed by Fontaine's (3) colorimetric phosphorus determination. For samples containing about 1 μg of phosphorus, greater accuracy was obtained by using a 10 ml volumetric flask and scaling down the amounts of reagents proportionately. One can detect 0.5 μg of phosphorus using this procedure. The use of buffers (1) to increase the sensitivity was investigated. The sensitivity was increased, but the results were not reproducible. A standard curve for phosphorus was prepared by using known quantities of anhydrous KH_2PO_4. The calibration curve needs to be determined only once.

B. C. BLACK
E. G. HAMMOND
Department of Dairy and Food Industry
Iowa State University
Ames, Iowa

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