

This material was identical to a sample prepared from phenyl isocyanate and *N*-cyclohexylglycine.

*Anal.*—Calcd. for  $C_{15}H_{18}N_2O_2$ : C, 69.74; H, 7.02; N, 10.85. Found: C, 69.46; H, 6.79; N, 10.74.

**1-Phenyl-3-*p*-methoxyphenyl- and 1-*p*-Methoxyphenyl-3-phenylhydantoin.**—A solution of 24.2 Gm. (0.1 mole) of 1-*p*-methoxyphenyl-3-phenylurea, 3.6 Gm. (0.05 mole) of 80% glyoxal, and 20 ml. concentrated hydrochloric acid in 300 ml. of 90% butanol was heated under reflux for 11 hr. The solvent was removed in a rotatory evaporator and the residue collected and crystallized from ethanol to give 12

Gm. (85%) of the mixture 1-*p*-methoxyphenyl-3-phenylhydantoin and 1-phenyl-3-*p*-methoxyphenylhydantoin, m.p. 132–135°;  $\nu_{\max}$ . 1710, 1775  $\text{cm.}^{-1}$  (C=O); NMR,  $\delta$  4.32 ( $\text{CH}_2$ ), 3.79 and 3.80 ( $\text{CH}_3$ ), 6.7–7.6 ( $\text{C}_6\text{H}_4$ ).

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## Separation and Estimation of Methyl and Propyl Esters of *p*-Hydroxybenzoic Acid by Gas Chromatography

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Some of the esters of *p*-hydroxybenzoic acid have been separated and identified by direct gas chromatography. The trimethylsilyl ethers of these compounds lend themselves more readily to gas chromatography since they show little or no tailing and therefore can be measured quantitatively. The method is rapid and sensitive.

**R**ELATIVELY pure single esters of *p*-hydroxybenzoic acid (parabens) can easily be identified by a number of methods (1). However, when more than one ester of *p*-hydroxybenzoic acid is present, especially in very dilute aqueous solution, they are difficult to identify individually. In this case it is necessary to perform a separation prior to the identification (2). Because partition chromatography and adsorption chromatography are only applicable to specific problems of limited complexity and since paper chromatography is time-consuming and insensitive with respect to these esters, gas liquid chromatography was attempted and found to be applicable and furthermore simple and sensitive. This tool will give both the qualitative and quantitative analysis of these compounds (3). The tailing of the esters as shown in Fig. 1 makes the separation and measurement difficult because of the overlapping and indefinite character of the curve. For this reason the esters were converted into the trimethylsilyl ethers which are more volatile, more stable, and better suited to separation by gas chromatography. This is illustrated in Fig. 2.

#### EXPERIMENTAL

The F & M model 609 gas chromatograph equipped with a flame ionization detector was used. The column was 6 ft. long,  $\frac{3}{16}$ -in. O.D. stainless steel, with 10% SE-30 as substrate coated on diatoport S (80–100 mesh) the support.

The reagents were hexamethyldisilazane (JU3), Analabs; trimethylchlorosilane (JU4), Analabs; pyridine (analytical reagent), Mallinckrodt Chemical Works. All the above reagents were used as received, with the exception of pyridine, which was kept over potassium hydroxide pellets.

The procedure was as follows. An aliquot of sample containing an equivalent of 1–20 mg. of

esters was transferred to a separator where the esters were extracted with two portions of 10 ml. each of diethyl ether. The ether phase was collected and washed with distilled water and transferred to a 0.5-oz. vial where the ether phase was evaporated almost to dryness. This was achieved by using both steam and a current of dry air. The last several milliliters of solution were evaporated at room temperature since these compounds are volatile on the steam bath. To this was added 1.0 ml. of anhydrous pyridine, and the dry residue in the vial was mixed with the liquid until it was completely dissolved. Subsequently, 0.2 ml. of hexamethyldisilazane and 0.1 ml. of trimethylchlorosilane were added, and the vial was stoppered with a plastic stopper. The mixture was shaken vigorously for 30 sec. and allowed to stand at room temperature for 15 min. (4, 5). The fine ammonium chloride precipitate formed was not removed since it did not interfere with the subsequent gas chromatography (6). A 1.0- $\mu\text{l.}$  quantity of the trimethylsilyl ethers was introduced into the injection port with a Hamilton microsyringe.

The conditions for operating the F & M 609 were as follows. The injection port was kept at about 235°, the detector block temperature at 200°, a flow rate of nitrogen of 40 ml./min. with an attenuation of 64 and a range of 100. The column temperature was programmed from 90° to 200° at 6.4°/min. The peaks were measured with a compensating polar planimeter. At the present time the author is combining these results with those obtained for the total esters by U. V. spectrophotometry and then quantitatively estimating the per cent of each constituent in the mixture or preparation.

The E(1%, 1 cm.) in water for methyl and propyl *p*-hydroxybenzoate was found to be, respectively, 1012 and 875, with peak at 255.75  $m\mu$  for both, using a Beckman DU spectrophotometer.

Table I shows results found by this method for a

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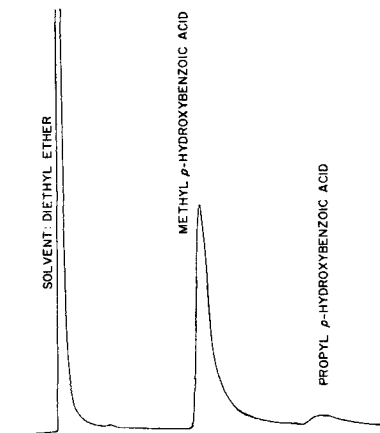


Fig. 1.—Column, 6 ft. stainless steel 0.25 in. 4% XE-60 on Diatoport S (60–80 mesh). Program: 90°–200° at 6.4°/min. Flow: 40 ml./min. (N<sub>2</sub>). Att: 64 × 100. Injection port: 235°C. Det: 200°C. Sample size: 1 μl. Recording speed: 15 in./hr. Used: methyl and propyl paraben (10:1) in diethyl ether. Found: ratio  $A_1/A_2$  = not calculated.

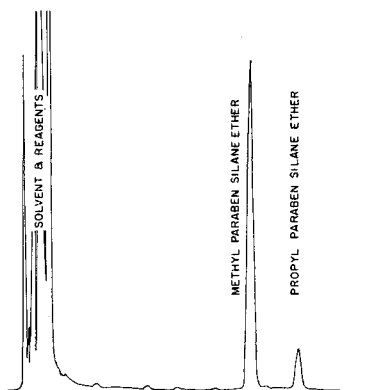


Fig. 2.—Column, 6 ft. stainless steel 3/16 in. 10% SE-30 on Diatoport S (80–100 mesh). Program: 90°–200° at 6.4°/min. Flow: 40 ml./min. (N<sub>2</sub>). Att: 64 × 100. Injection port: 235°C. Det: 200°C. Sample size: 1 μl. Recording speed: 15 in./hr. Used: methyl and propyl paraben silanes (10:1) in pyridine. Found: ratio  $A_1/A_2$  = 10/1.

TABLE I.—QUANTITATIVE DETERMINATION OF METHYL AND PROPYL *p*-HYDROXYBENZOATE (MPHB AND PPHB) BY THE SIMULTANEOUS USE OF BOTH THE GAS CHROMATOGRAPHIC AND ULTRAVIOLET SPECTRA DATA FOR A GROUP OF SAMPLES

Lot No.	Ratio by GC (R)	% Total by U. V. (T)	Theoret. Amt. MPHB, Gm./100 ml.	MPHB, Gm./100 ml. Found (X)	Theoret. Amt. PPHB, Gm./100 ml.	PPHB, Gm./100 ml. Found (Y)
A	10.12	103.5	0.050	0.0518	0.005	0.00510
B	10.00	102.8	0.050	0.0514	0.005	0.00514
C	10.62	100.1	0.050	0.0503	0.005	0.00470
D	10.00	103.2	0.050	0.0516	0.005	0.00516
E	9.65	102.0	0.050	0.0508	0.005	0.00526

group of samples containing these compounds. The formulas used were:

$$\text{Gm. PPHB/100 ml.} = \frac{0.055T}{100(R+1)}$$

$$\text{Gm. MPHB/100 ml.} = \frac{0.055T}{100} - \text{Gm. PPHB/100 ml.}$$

where  $T$  = per cent total esters as determined by U. V. spectrophotometry,  $R$  = ratio as determined by gas chromatography, and 0.055 is the theoretical total of methyl and propyl *p*-hydroxybenzoic acid in Gm./100.0 ml.

#### DISCUSSION

The above procedure has been in constant use in this laboratory for over 1 year. The original column is still being used, showing no deterioration or change in retention times for these two compounds. Polar substrates were tried such as 4% XE-60 on Diatoport S (60–80 mesh), but it was found that this column gave more bleeding than the SE-30 column. Another column that has been used in conjunction with trimethylsilyl ethers is 2.5% QFI on Chromosorb G (AW-DMCS) 60–70 mesh. However, as is the case when using these silicone columns and chromatographing silanes the system in time becomes coated with silicone deposit and must therefore be

cleaned quite often with Freon 12. If this corrective measure is not taken, the sensitivity of the detector becomes impaired.

Recently, the author has found that dimethylformamide is preferable to pyridine since it gives less solvent tailing. Those who prefer to evaporate the solvent should use tetrahydrofuran instead of pyridine, since it can be taken to dryness faster. Furthermore, where quantitative results are critical a glass insert should be applied in the injection port.

#### SUMMARY

Methyl and propyl esters of *p*-hydroxybenzoic acid have been converted to the trimethylsilyl ethers and have been gas chromatographed. Separation has been achieved in less than 1 hr. The method is rapid and has been applied repeatedly to the determination of these compounds in pharmaceutical preparations.

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