observed since VII > VIII > IX. However, the main interest in the synthesis of this series of compounds was to determine if the *trans*-hydroxymethyl group of the cyclohexyl nucleus could bridge to a binding point which could not be reached by the corresponding *cis*-derivative. An examination of Table I reveals that VII is approximately as effective an inhibitor as XIII or XIV, compounds whose hydroxymethyl groups are *cis*. Since it has been established previously that the hydroxymethyl groups of XIII and XIV contribute little to binding to the enzyme (5), the *trans* hydroxymethyl group of VII does not make a significant contribution to the binding of this compound to the enzyme.

REFERENCES

(1) Buchanan, J. M., "Biosynthesis of Purine Nucleotides" in "The Nucleic Acids," Vol. III, Chargaff, E., and Davidson, J. N., eds., Academic Press Inc., New York, N. Y., 1960, p. 304.
(2) Handschumacher, R. E., and Welch, A. D., "Agents which Influence Nucleic Acid Metabolism," *ibid.*, p. 453.
(3) Schaeffer, H. J., Marathe, S., and Alks, V., THIS JOURNAL, **53**, 1368(1964).
(4) Schaeffer, H. J., and Bhargava, P. S., *Biochemistry*, in press.
(5) Schaeffer, H. J., Godse, D. D., and Liu, G., THIS JOURNAL, **53**, 1510(1964).
(6) Temple, C., Jr., Kussner, C. L., and Montgomery, J. A., *J. Med. Pharm. Chem.*, **5**, 866(1962).
(7) Kaplan, N. O. in "Methods in Enzymology," Vol. II, Colowick, S. P., and Kaplan, N. O., eds., Academic Press Inc., New York, N. Y., 1955, p. 473.
(8) Baker, B. R., and Sachdev, H. S., THIS JOURNAL, **52**, 933(1963).

Rapid Method for the Determination of Mixtures of *p*-Hydroxybenzoate Esters by Gas Chromatography

By J. L. LACH and J. S. SAWARDEKER

A procedure for the separation and quantitative determination of mixtures of the *p*-hydroxybenzoate esters is described employing vapor phase chromatography.

THE ALKYL *p*-hydroxybenzoate esters more commonly known as the parabens constitute one of the most important group of pharmaceutical preservatives. The role of parabens as preservatives has been reviewed in the literature from time to time by Aalto *et al.* (1), Barkeley (2), Neidig and Burrell (3), Sokol (4), and Gottfried (5). However, the literature cites only a few general methods for the detection and the estimation of the parabens (6–8). Furthermore, none of these methods are useful for the determination of exact quantities of individual parabens in the presence of other parabens when used in combinations. Even the U.S.P. method fails to permit such an analysis. The analysis of the parabens is complicated by the fact that the total paraben concentrations employed as preservatives seldom exceed 0.2% of the formulation. The most promising assay for these parabens has been developed by Higuchi and co-workers (9). Their procedure is based on the preliminary extraction of these parabens from the formulation, separation of the component esters by partition chromatography, and their subsequent determination by ultraviolet spectrophotometry.

The increasingly important role of the parabens as preservatives emphasized the need for a rapid method of their determination. This report deals with the qualitative and quantitative determination of the parabens by gas chromatography. Although these parabens were gas chromatographed directly, the separation of methyl paraben from the ethyl paraben offered considerable difficulty even though the propyl and the butyl parabens were well resolved. We have, therefore, converted the hydroxyl groups on the *p*-hydroxybenzoates to the corresponding ethers and gas chromatographed the trimethylsilyl derivatives. The method has the advantage of speed and accuracy and is applicable over a wide range of concentrations.

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EXPERIMENTAL

Apparatus and Materials.—A F & M model 500 linear programmed high-temperature chromatograph with model 1609 flame ionization attachment, equipped with Minneapolis Honeywell Y143 recorder and model 201 Disc Integrator was used.

A 2-ft. copper tube packed with Diatoport S (diatomaceous earth specially treated and silanized, offered by F & M Scientific Co.) and coated with 2% SE-30 and a 4-ft. copper tube packed with the same support and coated with 10% butanediol-succinate was used.

Hexamethyldisilazane and trimethylchlorosilane were obtained from Applied Science Laboratories,

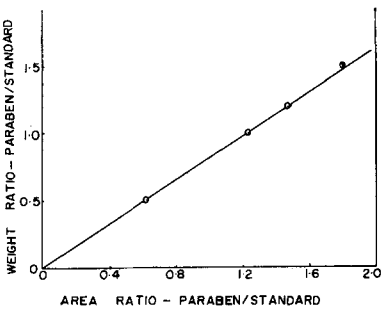

Fig. 1.—Calibration curve for the paraben derivatives with the internal standard.

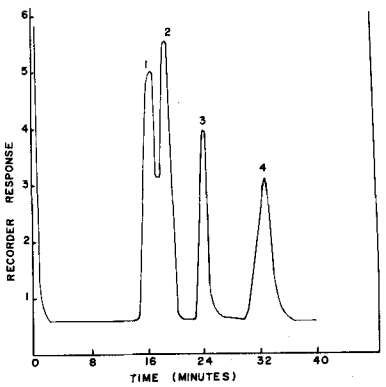

Fig. 2.—Response obtained for paraben mixture using 4-ft., 10% butanediolsuccinate column. Sensitivity: range, 100; attn. 64×; column temp., 190°; injection port temp., 300°; detector temp., 250°; helium flow, 150 ml./min.; sample size 1 μl. Key: 1, methyl *p*-hydroxybenzoate; 2, ethyl *p*-hydroxybenzoate; 3, propyl *p*-hydroxybenzoate; 4, butyl *p*-hydroxybenzoate.

TABLE I.—RELATIVE RETENTION TIMES FOR PARABENS

Component	Retention Time,[a] min.
Methyl paraben	16.4
Ethyl paraben	18.4
Propyl paraben	24
Butyl paraben	32.4

[a] Relative to solvent peak.

State College, Pa. Pyridine, analytical reagent grade, was dried over potassium hydroxide pellets prior to use.

The recrystallized melting points were as follows: methyl *p*-hydroxybenzoate, 131°; ethyl *p*-hydroxybenzoate, 116–118°; propyl *p*-hydroxybenzoate, 96–97°; butyl *p*-hydroxybenzoate, 68–69°; and phenanthrene, 100°.

Method.—The trimethylsilyl derivatives were prepared according to the method of Bentley *et al.* (10). In a small glass vial was placed 10 mg. each of the four parabens and to this was added 10 mg. phenanthrene. The parabens and phenanthrene were dissolved in 1 ml. anhydrous pyridine, and 0.1 ml. each of hexamethyldisilazane and trimethylchlorosilane were then added. The vials were stoppered with polyethylene stoppers, shaken vigorously for 1 min., and allowed to stand at room temperature for 1 hr. One-fifth to 1 μl. of this was injected directly onto the gas chromatograph.

Extraction of the Samples from the Dosage Forms.—Chloroform was used to extract the parabens from the aqueous preparations. Three extractions were sufficient to extract the parabens because of their high solubilities in chloroform compared to water. Chloroform then was evaporated and the residue dissolved in pyridine and converted to the trimethylsilyl derivative as described earlier.

Internal Standard.—Among a number of compounds investigated, phenanthrene was chosen because it met the requirements for an effective standard (11, 12) which are: (*a*) readily separable from parabens, (*b*) retention time very close to the retention times of the parabens, (*c*) detector response linearly related to the response of the parabens, (*d*) inertness and ready availability, (*e*) absence from pharmaceutical systems, and (*f*) ease of solubility in pyridine.

Calibration Curve.—A constant amount of the internal standard was added to a specified volume

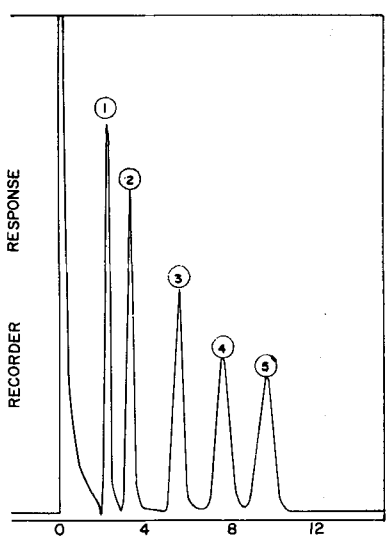

Fig. 3.—Gas chromatogram of paraben mixture chromatographed as the trimethylsilyl derivatives along with the internal standard using a 2-ft. SE-30 column. Sensitivity: range, 100; attn., 64×; Column temp., 110°; injection port temp., 300°; detector temp., 250°; helium flow, 70 ml./min.; sample size, 0.5 μl. Key: 1, methyl *p*-hydroxybenzoate; 2, ethyl *p*-hydroxybenzoate; 3, propyl *p*-hydroxybenzoate; 4, internal standard; 5, butyl *p*-hydroxybenzoate.

TABLE II.—RELATIVE RETENTION TIMES OF TRIMETHYLSILYL DERIVATIVES OF PARABENS

Component	Retention Time,[a] min.
Methyl paraben	1.8
Ethyl paraben	2.8
Propyl paraben	5.0
Butyl paraben	8.6

[a] Relative to solvent peak.

TABLE III.—RESULTS OF ANALYSIS OF KNOWN SAMPLES

Mixture No.	Components	Amt. Present, %	Amt. Recovered, %
1	Methyl	0.750	0.739
	Ethyl	0.750	0.740
	Propyl	0.750	0.742
	Butyl	0.750	0.742
2	Methyl	0.250	0.242
	Ethyl	0.250	0.246
	Propy	0.250	0.251
	Butyl	0.250	0.249
3	Methyl	0.050	0.045
	Propyl	0.150	0.143
	Butyl	0.150	0.144

TABLE IV.—RESULTS OF ANALYSIS OF COMMERCIAL FORMULATIONS

Dosage Form	Components	Amt. Labeled, %	Amt. Recovered, %
Decadron[a]	Methyl	0.150	0.144
	Propyl	0.020	0.0181
Xylocaine hydrochloride[b]	Methyl	0.010	0.0092

[a] Trademarked by Merck Sharp & Dohme, Division of Merck & Co., Inc., West Point, Pa. [b] Trademarked by Astra Pharmaceutical Products Inc., Worcester, Mass.

of several synthetic mixtures containing known concentrations of solutes under investigation. The weight ratio of each solute to the internal standard when plotted against the area ratio of the solute to the internal standard resulted in the desired calibration curve. (See Fig. 1.) The analysis of the unknown sample then was carried out by adding the same amount of the internal standard to the unknown mixture and by direct determination of the solute concentration in the unknown from comparison with the calibration curve.

RESULTS AND DISCUSSION

Hydroxybenzoate esters being phenolic compounds offered considerable difficulty in their direct quantitative analysis, because of the slight adsorption effects exhibited. Among a number of stationary phases, apiezon L, SE-30, Dow Corning silicone fluid 200, Dow Corning silicone fluid 550, Carbowax 20M, Carbowax 4000, ethylene glycol succinate, and butanediol succinate, only the last phase gave best symmetrical peaks, even though none of these phases could resolve methyl paraben from ethyl paraben.

The chromatogram obtained using a 4-ft. column packed with 10% butanediolsuccinate is illustrated in Fig. 2. The retention times for the parabens are given in Table I. As is evident, propyl and butyl parabens are well resolved on this column. A complete resolution of methyl paraben from ethyl paraben would necessitate the use of a long column. However, such a long column causes an increase in the retention times of the parabens resulting in slight distortion of the sharp peaks and thus rendering it undesirable for accurate quantitative analysis.

Therefore, the authors attempted to derivatize the parabens to their simplest derivatives, the trimethylsilyl ethers which can be prepared easily as described earlier. These derivatives when chromatographed on a 2-ft., 2% SE-30 column resolved all the four parabens with symmetrical peaks readily suitable for quantitative analysis. The chromatogram with the operating conditions is shown in Fig. 3. The retention times for these ethers are given in Table II.

The recoveries from three synthetic mixtures are shown in Table III.

To demonstrate the feasibility of this method for the quantitative analysis of parabens in commercial formulations, the procedure was applied to the analysis of two such formulations and the data obtained are presented in Table IV. Although two formulations were subjected to this method of analysis, the data obtained suggest that this procedure can be applied satifactorily to other pharmaceutical formulations. It should be pointed out that when the parabens are used as preservatives in combinations other than methyl and ethyl, their derivatization to the ether derivatives is not necessary since they can be gas chromatographed directly on the butanediolsuccinate column to obtain quantitative results. However, when the methyl and ethyl parabens are used in combinations, the derivatization to the ethers provides a suitable means for their analysis.

SUMMARY

Data have been presented for the separation and quantitative determination of mixtures of the *p*-hydroxybenzoate esters using gas chromatography. The method has been shown to be applicable to the analysis of these esters in several commercial formulations.

REFERENCES

(1) Aalto, T. R., Firman, M. C., and Rigler, N. E. THIS JOURNAL, **42**, 449(1953).
(2) Barkeley, E. L., *Am. Perfumer*, **73**, 33(1959).
(3) Neidig, C. P., and Burrell, H., *Drug Cosmetic Ind.*, **54**, 408(1944).
(4) Sokol, H., DRUG STANDARDS, **20**, 89(1952).
(5) Gottfried, N. S., *Am. J. Hosp. Pharm.*, **19**, 310(1962).
(6) Boyce, E. G., *J. Assoc. Offic. Agr. Chemists*, **28**, 301 (1945).
(7) Jones, C. N., *ibid.*, **30**, 486(1947).
(8) Ettinger, M. B., Ruchoft, C. C., and Lishka, R. J., *Anal. Chem.*, **23**, 1783(1952).
(9) Higuchi, T., *et al.*, THIS JOURNAL, **41**, 293(1952).
(10) Bentley, R., Sweeley, C. C., and Makita, M., *Biochem. Biophys. Res. Commun.*, **11**, 14(1963).
(11) Ray, N. H., *J. Appl. Chem. London*, **4**, 121(1954).
(12) Harvey, D., and Chalkeley, D. E., *Fuel*, **34**, 191 (1955).