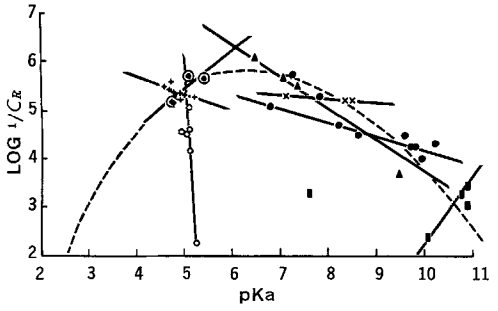

Fig. 1—Key: ⊙, *2-sulfanilamido (substituted) thiadiazoles;* +, meta *and/or* para-*substituted* N^1-*benzoylsulfanilamides;* O, ortho-*substituted* N^1-*benzoylsulfanilamides;* ▲, *2-sulfanilamido (substituted) pyrimidines;* ×, *2-sulfanilamido (substituted) pyridines;* ●, N^1-*(substituted) phenylsulfanilamides;* ■, N^1-*(substituted) methylsulfanilamides.*

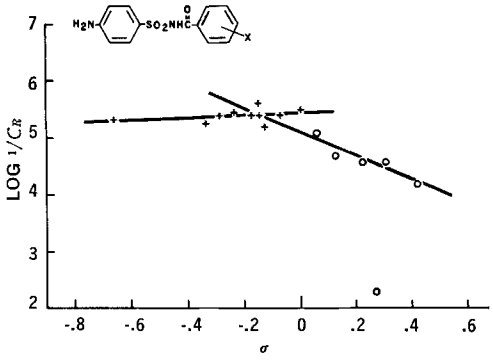

Fig. 2—Key: +, meta *and/or* para-*substituted* N^1-*benzoyl-sulfanilamides;* O, ortho-*substituted* N^1-*benzoyl-sulfanilamides.*

where α defines the "intrinsic" activity which may be associated with a given homologous series, and where log $1/C_R°$ is an arbitrary reference activity, then the break in the line could be interpreted as a possible change in the mechanism of action. This would follow from similar considerations of the Hammett equation in relation to chemical reaction mechanisms (10).

It has been shown (11) that sulfonamides bearing an N^1-aromatic substituent are capable of binding at an enzyme site through each of their aromatic rings. Since all the series considered in this study possess an N^1-aromatic ring except the N^1-(substituted) methylsulfanilamides, the latter is not considered a reliable test series. It is quite conceivable that binding differences, coupled with diminished pKa values, may account for the divergent pattern in activities exhibited by *ortho*-substituted N^1-benzoyl-sulfanilamides.

The linear correlation of *in vitro* bacteriostatic activity with pKa, which is observed in this study for homologous series of sulfonamides, provides little evidence in support of there being a definite pKa which a sulfonamide must possess in order to exhibit maximum activity. Rather, from the data at hand, it appears that many sulfonamides lying outside of the "prescribed" Bell and Roblin maximum, notably those containing electron withdrawing substituents on an N^1-aromatic ring, are potentially capable of high *in vitro* activities, provided they can penetrate the bacterial cell wall.

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Gas Chromatography of Alkyl Ether Derivatives of *p*-Hydroxybenzoate Esters

By MERRILL WILCOX

A procedure for the separation and estimation of mixtures of the four lower normal alkyl *p*-hydroxybenzoates as derivatives is described. The esters were converted to phenyl alkyl ethers by means of diazoalkanes. Diazoethane, diazo-*n*-propane, diazoisobutane, and diazo-*n*-butane, when catalyzed with 0.007 per cent boron trifluoride, alkylated essentially quantitatively in 30 min. at room temperature. Variable yields were noted in the absence of the catalyst for reaction times as long as 18 hr. Diazomethane did not alkylate quantitatively when catalyzed in the same manner. Derivatives prepared from mixtures of the four esters were resolved on the gas chromatograph regardless of the diazoalkane used.

THE ALKYL *p*-hydroxybenzoate esters are important food and pharmaceutical preservatives. Lach and Sawardeker (1) list several reviews of the role of these esters as preservatives. They also review the general methods of analysis which have been published. An additional general method of analysis has recently appeared (2). Higuchi *et al.* (3) developed an assay using column chromatography and U.V. spectrophotometry which could be used to resolve mixtures of the esters. Lach

Received November 17, 1966, from the Agronomy Department, Agricultural Experiment Station, University of Florida, Gainesville, FL 32601
Accepted for publication January 19, 1967.
This investigation was supported in part by grant E294B from the American Cancer Society and grant GB4420 from the National Science Foundation.
Florida Agricultural Experiment Station Journal Series No. 2582.

and Sawardeker (1) and Donato (4) have separated and assayed the esters as their trimethylsilyl ethers by means of gas chromatography. Higher diazoalkanes have been found useful in gas chromatography of phenolic acids (5) because of their superior alkylating activity relative to diazomethane. The alkylating activity of diazomethane has been greatly increased by means of boron trifluoride catalysis (6). Alkyl ethers of phenolic acids have been demonstrated to be somewhat more stable than the corresponding trimethylsilyl ethers (7) in gas chromatography. This report describes the suitability of some higher diazoalkanes under boron trifluoride catalysis for preparation of derivatives of *p*-hydroxybenzoate esters for gas chromatography.

EXPERIMENTAL

Apparatus and Materials—A Perkin-Elmer 202 recording spectrophotometer having linear wavelength presentation was used. The gas chromatograph was a Barber-Colman 10c, equipped with a hydrogen flame ionization detector. The smallest detectable quantity of derivative was approximately 50 nanograms. Sample concentrations were chosen so that injection volumes ranged between 1 and 5 μl. Naphthalene was used as an internal standard. The injection heater and detector were maintained at a temperature 50° above that of the column.

Two columns were used. A stainless steel column 50 M. by 0.25 mm. i.d. coated internally with high vacuum paraffin grease[1] was operated isothermally at 200°, with column flow of 18 ml. argon per minute and inlet pressure of 60 p.s.i. A pre-column splitter allowed 23% of the sample to flow into the column and exhausted the remainder. In later experiments a glass column 2 M. by 4 mm. i.d. without the splitter was substituted. It was packed with 1.5% SE-30 polyester on 90–100 mesh Anakrom ABS and was operated isothermally at 135° with a column flow of 240 ml. argon per minute and an inlet pressure of 28 p.s.i.

Diazomethane was prepared without distillation from *N*-methyl-*N*-nitroso-*N'*-nitroguanidine (5) (Aldrich Chemical Co.). Higher diazoalkanes were prepared without distillation from *N*-alkyl-*N*-nitrosoureas (5). All were used within 30 hr. of their preparation. Alkyl *p*-hydroxybenzoates were provided by Tenneco Chemicals, Inc. Methanolic boron trifluoride (14%, Applied Science Laboratories) was diluted to 0.7% with anhydrous reagent methanol.

Methods—The *p*-hydroxybenzoate esters (usually 10 mg.) were dissolved in 0.5 ml. methanol and treated at room temperature with 3 ml. ethereal diazoalkane, or enough to retain a yellow color. One drop (.03 ml.) of 0.7% methanolic boron trifluoride was added to those samples where catalysis was desired. At the end of the reaction period, the excess diazoalkane was destroyed by bubbling carbon dioxide through the solution. These samples were either injected into the gas chromatograph without further treatment or were evaporated under nitrogen and made to volume in dichloromethane which gave a very narrow solvent peak. Samples used for alkylation assays were treated similarly except that they were made to volume in water rather than dichloromethane.

A phenolate difference spectrum assay was used to measure the remaining phenolic groups after alkylation. A 5-ml. sample aliquot was added to 5 ml. of 0.2 *M* phosphate buffer (pH 10) and read against a reference solution containing an identical aliquot of sample diluted with 5 ml. of 0.2 *M* phosphate buffer (pH 7). The pH values of the solutions were checked by means of a Photovolt model 110 meter after addition of buffer. A graphic method was used to measure the absorbance (*A*). A base line was drawn between the points at 270 and 340 mμ on the spectrum. The height of the curve was then read at 320 mμ relative to the base line, giving the absorbance directly. Since the instrument had linear wavelength presentation, this was equivalent to:

$$A_{\text{corrected}} = A_{320\ m\mu} - \frac{2}{7} A_{270\ m\mu} - \frac{5}{7} A_{340\ m\mu}$$

Calibration curves were prepared in this manner for concentrations which were equivalent to a range of from 0.1 to 10% unalkylated phenol. The data followed Beer's law throughout this range, which included all values of practical interest in this work.

RESULTS AND DISCUSSION

Alkylation—The alkylation activity among the diazoalkanes generally increased with chain length but was not consistently quantitative after uncatalyzed reaction periods as long as 18 hr. Catalysis with 0.007% boron trifluoride greatly increased yields. Alkylation yields following catalyzed reaction periods of 30 min. appear in Table I. The data are averages of single determinations from each of two alkylations in a typical experiment. Reaction times of 2.5 hr. were necessary for consistent alkylation of 95% or better by catalyzed diazomethane.

Chromatography—The relative retention times of the derivatives when chromatographed on the capillary column are listed in Table II. All gave single symmetrical peaks except the *n*-butyl ethers

TABLE I—PER CENT ALKYLATION OF *p*-HYDROXYBENZOATE ESTERS BY DIAZOALKANES CATALYZED WITH .007% BORON TRIFLUORIDE FOR 30 min. AT ROOM TEMPERATURE

	% Alkylation by Diazoalkane				
Ester	Methyl	Ethyl	*n*-Propyl	Iso-butyl	*n*-Butyl
Methyl	<90	97.0	99.8	99.5	99.9
Ethyl	<90	99.2	99.9	99.8	99.6
Propyl	<90	99.6	99.9	99.9	99.9
Butyl	<90	99.5	99.9	99.9	99.9

TABLE II—RELATIVE RETENTION TIMES OF ALKYL ETHERS OF *p*-HYDROXYBENZOATE ESTERS ON PARAFFIN GREASE CAPILLARY COLUMN

	Retention Time of Alkyl Ether Relative to Naphthalene[a] Internal Std.				
Ester	Methyl	Ethyl	*n*-Propyl	Iso-butyl	*n*-Butyl
Methyl	1.3	1.7	2.5	2.9	4.0
Ethyl	1.8	2.2	3.2	3.7	4.9, 5.4[b]
Propyl	2.6	3.4	5.0	5.5	7.2, 7.8[b]
Butyl	4.0	5.2	7.7	8.6	13.3

[1] Marketed as Apiezon L by the James G. Biddle Co., Philadelphia, Pa.

[a] The absolute retention time of naphthalene was 0.90 min. at 200°, 18 ml. argon carrier per min. [b] The second peak appeared to be due to decomposition on the column.

TABLE III—RELATIVE RETENTION TIMES OF ALKYL ETHERS OF *p*-HYDROXYBENZOATE ESTERS ON COLUMN PACKED WITH 1.5% SE-30 POLYESTER ON ANAKROM ABS

Ester	Retention Time of Alkyl Ether Relative to Naphthalene[a] Internal Std.		
	Ethyl	*n*-Propyl	*n*-Butyl
Methyl	2.0	2.5	3.8
Ethyl	2.4	3.2	4.8
Propyl	3.3	4.6	7.3
Butyl	5.0	7.0	11.2

[a] The absolute retention time of naphthalene was 1.0 min. at 135°, 240 ml. argon per min.

of ethyl and propyl *p*-hydroxybenzoate. These two derivatives each gave two overlapping peaks, the first being symmetrical, the second being lower with considerable tailing. The author suspects this effect was due to decomposition on the column. Single peaks without tailing were observed for the *n*-butyl ethers when the column temperature was lowered to 170°. For this reason selected derivatives were chromatographed on a column packed with 1.5% SE-30 polyester on 90-100 mesh Anakrom ABS at a temperature designed to give similar retention times. The data which appear in Table III indicate that this packed column under these conditions is more suitable than the capillary column. The four esters thus may be resolved within each set of ether derivatives under appropriate conditions. Quantitative work was restricted to diazoethane and diazopropane since they produced stable derivatives in good yields. Plots of peak height *versus* concentration were linear for the lower three esters, as were plots of peak area *versus* concentration for the butyl ester when using ethyl derivatives on the capillary column. Plots of peak height *versus* concentration were linear when using propyl derivatives of the four esters on the packed column. This was true whether the esters were alone or mixed on either column.

The results indicate that higher diazoalkanes, when catalyzed with boron trifluoride, are useful for preparation of derivatives for gas chromatography of *p*-hydroxybenzoate esters. Now that *N*-alkyl-*N*-nitroso-*N'*-nitroguanidine precursors of higher diazoalkanes are commercially available,[2] their use should be considered for gas chromatography of these or other phenolic acids.

SUMMARY

Methyl through butyl esters of *p*-hydroxybenzoic acid have been converted to alkyl ethers by means of diazoalkanes catalyzed with .007% boron trifluoride. These ethers were easily resolved on the gas chromatograph. Ethers prepared from diazoethane and diazopropane were found suitable for quantitative work. The method seems generally applicable among phenolic acids where alkylating activity greater than that of diazomethane is needed.

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[2] Cyclo Chemical Corp., Aldrich Chemical Co., and K & K Chemical Co.

Syntheses of Some Hydroindolizines by Reductive Cyclization

By JOSEPH SAM and K. APARAJITHAN

The syntheses of dodecahydrobenzo[*b*] indolizine, octahydrocyclopenta[*b*] indolizine, decahydronaphth[2,1-*b*] indolizine, and 7-methoxydecahydronaphth[2,1-*b*]-indolizine are reported. The preparations of pyridylmethylene and pyridylmethyl derivatives of cyclopentanone, cyclohexanone, 1-tetralone, and 7-methoxy-1-tetralone also are described. Some preliminary pharmacological observations in mice are included.

THE SYNTHESIS of dodecahydrobenzo[*b*] indolizine (11-perhydroazafluorene) (II*b*) involving several steps was reported by Prelog and associates (1). Subsequently the authors reported (2) a two-step synthesis of II*b* by reductive cyclization of I at 200° and 2000 p.s.i. (Scheme I) and also the low pressure (50–60 p.s.i.) catalytic reductive cyclization of 2-pyridylmethylene-1-indanones (III) to the corresponding hydroindolizine (IV) (3, 4). (Scheme II.) The authors have now utilized the latter method for the synthesis of other hydroindolizines (II and XII) from 2-(2-pyridylmethylene)-cycloalkanones (IX and XI). The formation of IX*b* by the reaction of cyclohexanone (V*b*) with 2-pyridinealdehyde (VI) was investigated; however, only 2,6-bis-(2-pyridylmethylene) cyclohexanone (VII) was obtained. The synthesis of IX was accomplished by the reaction of 2-pyridinealdehyde with the morpholine enamine (VIII) (5, 6) of the cycloalkanone (V). The 3-, and 4-pyridylmethylenecycloalkanones also were prepared by this method using the appropriate pyridinealdehydes. The reaction of 2-pyridinealdehyde with 1-tetralones (X) occurred satisfactorily to yield the corresponding pyridylmethylene derivatives (XI). Some of the properties of the products from the reaction of pyridinealdehydes with various cycloalkanones are summarized in Table I.

A number of the unsaturated compounds (IX,

Received December 27, 1966, from the Department of Pharmaceutical Chemistry, University of Mississippi, University, MS 38677

Accepted for publication February 8, 1967.

This work was done during the tenure of a Mississippi Heart Association Research fellowship to K. Aparajithan.

Presented to the Medicinal Chemistry Section, A.PH.A. Academy of Pharmaceutical Sciences, Las Vegas meeting, April 1967.