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GAS CHROMATOGRAPHIC SEPARATION AND DETECTION OF C_1 TO C_3 MONOCARBOXYLIC ACIDS AS THE *p*-SUBSTITUTED BENZYL ESTERS

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SUMMARY

A rapid, sensitive gas chromatographic method for the separation and detection of microgram and submicrogram quantities of formic, acetic and propionic acids is presented. The procedure involves reaction of microliter volumes of an ethanolic carboxylic acid solution mixture, down to the 0.1 μg/μl concentration level, with an appropriate *p*-substituted benzyl halide in a sealed capillary melting point tube at 110°. The esters formed in the reaction mixture are then isothermally eluted from a general-purpose OV-17 glass column.

INTRODUCTION

Separation and/or detection of the lower monocarboxylic acids in foods and in chemical, biological and ecological media has been achieved using spot tests, paper and thin-layer chromatography[1–5], column partition chromatography[6,7] and by gas chromatography as the free acids or after derivatization under a variety of experimental conditions[8–14].

Color tests are often subject to interference and are therefore of limited utility. Column, paper and thin-layer chromatographic techniques are virtually indispensable to the chemist but the lower volatile fatty acids sometimes demand assiduous sample preparation and control of conditions, and may require relatively long development times for optimum separation. Though excellent results are reported in the literature for most of the gas chromatographic methods for the lower carboxylic acids, many require specialized columns which are not always convenient for those control laboratories which routinely process a wide diversity of chemical substances.

Therefore, in this paper we describe a sensitive method for the rapid separation and detection of microgram and submicrogram quantities of formic, acetic and propionic acids by isothermal gas chromatography on an all-purpose 5% OV-17 on Gas-Chrom Q column as their *p*-methylbenzyl, *p*-bromobenzyl or *p*-nitrobenzyl esters.

EXPERIMENTAL

Materials

The carboxylic acids used were of reagent grade quality.

The following *p*-substituted benzyl halides were used: *p*-nitrobenzyl iodide, K & K (95–99%); *p*-bromobenzyl bromide, K & K (95–99%); *p*-methylbenzyl bromide, Eastman (highest purity); *p*-nitrobenzyl bromide, Eastman (highest purity).

p-Nitrobenzyl, *p*-methylbenzyl and *p*-bromobenzyl esters of formic, acetic and propionic acids were obtained commercially where available (*p*-nitrobenzyl acetate, K & K, 95–99%; *p*-methylbenzyl acetate, K & K, 95–99%) or synthesized according to the procedures described below.

Synthesis of p-substituted benzyl propionate and acetate reference materials. Propionic acid or acetic acid (3 g) dissolved in water (5 ml) was neutralized to a phenolphthalein end-point with 20% aqueous sodium hydroxide solution. A few drops of dilute hydrochloric acid were added so that the final solution was faintly acid to litmus paper. To this was added the appropriate *p*-substituted benzyl bromide (1 g) dissolved in alcohol (25 ml) and the mixture was heated under reflux for 3 h. The mixture was evaporated to half volume and then diluted with water. After extraction into ether, the organic layer was dried over anhydrous sodium sulfate, the solvent removed and the residual oil distilled *in vacuo*.

Synthesis of p-substituted benzyl formate reference materials. The *p*-substituted benzyl alcohol (1 g) was heated under reflux in formic acid (20 ml) containing concentrated HCl (three drops) for 2 h. The mixture was cooled, diluted with ether and shaken with several portions of 3% sodium bicarbonate solution. The organic layer was dried over anhydrous sodium sulfate, the ether removed under a flow of dry nitrogen, leaving an oil which on vacuum distillation yielded the desired product. The halide may also be used, but the yields appeared to be considerably lower.

p-Bromobenzyl formate was synthesized according to the above procedure using *p*-bromobenzyl bromide.

Reference solutions

All reference esters were made up to a concentration of 1 μg/μl in ethanol.

Reagent solutions

The reagent solutions were prepared as follows: Individual carboxylic acid solutions were made up to a concentration of 1 μg/μl in ethanol. An ethanolic mixture of all three carboxylic acids was made up so as to contain 1 μg/μl of each component. Solutions of potassium hydroxide, *p*-nitrobenzyl iodide, *p*-methylbenzyl bromide, and *p*-bromobenzyl bromide each contained 3 μg per μl ethanol.

Micro sample preparation and gas chromatography

The carboxylic acid solution (10 μl) was transferred by means of a Hamilton microsyringe into a capillary melting point tube along with potassium hydroxide in ethanol solution (3 μl) and the appropriate *p*-substituted benzyl halide solution (5 μl) described above (in the case of solution 2, 15 μl were used). The tube was flame sealed and incubated at 110° for 1 h.

After cooling, 2 μl of the reaction mixture were injected into a gas chromato-

graph* equipped with a flame ionization detector unit and fitted with a 5% OV-17 on Gas-Chrom Q (100–120 mesh) glass column (4 ft. × $\frac{1}{8}$ in. I.D.) preconditioned at 280° for 24 h. Column and injection port temperatures were, respectively: for the *p*-methylbenzyl series 100° and 225°, for the *p*-bromobenzyl series 120° and 240°, and for the *p*-nitrobenzyl series 145° and 265°. Nitrogen flow was 80 ml/min, hydrogen flow 50 ml/min, column inlet pressure 8 p.s.i.g., and attenuation × 100.

RESULTS AND DISCUSSION

In order to have an adequate method for the identification of lower molecular weight carboxylic acids by gas chromatography, it was desirable to have at least three different derivatives of each acid. The *p*-substituted benzyl esters offered the most advantages as they are readily prepared and are amenable to gas chromatography.

Reference material preparation

When preparing the reference materials, not all of the ester derivatives were commercially available, hence the appropriate ones were synthesized as described. The feasibility of the synthesis routes was established by gas chromatographic comparison of the pure (95–99%) commercial ester—where available—with the vacuum distillate of the same ester prepared by laboratory synthesis. In addition, IR spectral traces were used to confirm the ester linkage by the presence of the carbonyl stretching frequency band near 1740 cm^{-1}. The correct structure for each of the remaining synthesized esters was then inferred *a posteriori* from these data. The gas chromatograms of the esters synthesized for this investigation did indicate that small quantities of impurities (*e.g.* the corresponding alcohol) were present after a single vacuum distillation but further purification did not appear to be warranted.

Micro sample preparation

In the simulated microanalysis of each carboxylic acid with each *p*-substituted benzyl halide, injection of the reaction mixture was subsequently followed by injection of the ethanolic solution of the reference ester and then finally by injection of a 1:1 mixture of the first two (see Fig. 1, chromatograms a, b and c). In every case, gas chromatography of the reaction mixture afforded a peak of identical retention time to that of the reference ester thus confirming the formation of the expected ester in the capillary tube. In a given series, ester retention times increased with molecular weight of the carboxylic acid such that a mixture of the three acids could be easily resolved after the derivatization step. Fig. 1, chromatogram d, clearly demonstrates a typical separation of a formic, acetic and propionic acid mixture after benzylation, in this instance effected with *p*-bromobenzyl bromide and ethanolic KOH at 110°. Table I gives the retention times, calculated from the point of injection, for each ester and for other relevant compounds in a series. The reaction mixtures gave, in addition to a symmetrical ester peak, a sharp secondary "reaction artifact" peak presumably resulting from isomerization or decomposition of the thermally unstable *p*-substituted benzyl halides but the nature of the product was not investigated. Ethanolic solutions of the halides can, however, be stored at 0° for a week without undergoing appreciable

* Research Specialities Co. "600 series". The FID unit was Model 660.

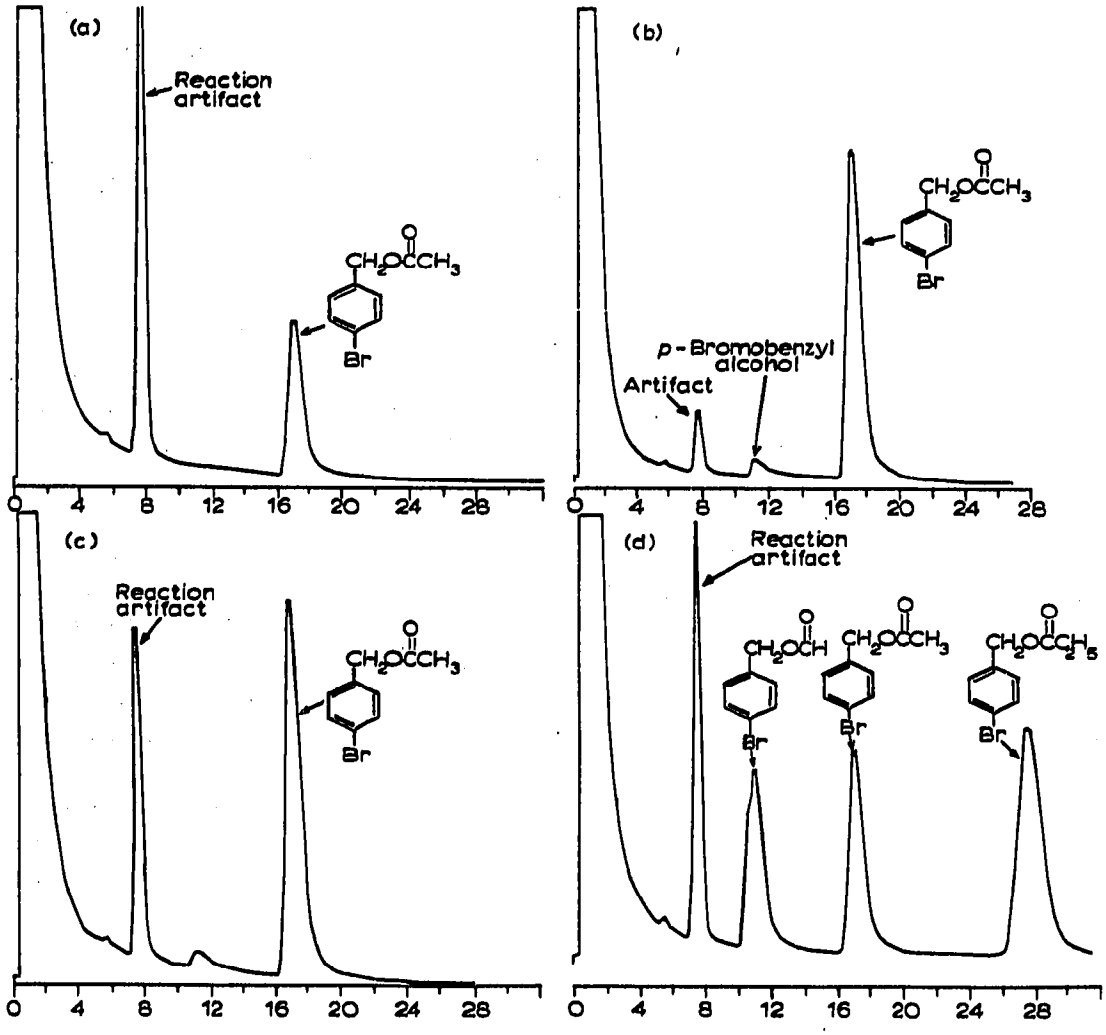

Fig. 1. (a) Reaction mixture (micro preparation using acetic acid solution). (b) Reference standard solution. (c) Reaction mixture of (a) + reference standard of (b) 1:1. (d) Reaction mixture (micro preparation using three-component acid solution).

TABLE I

RETENTION TIMES (min) OF *p*-SUBSTITUTED BENZYL FORMATE, ACETATE AND PROPIONATE ESTERS AND OTHER RELEVANT RELATED COMPOUNDS

	p-Methyl-benzyl (column temp. 100°)	*p-Bromo-benzyl (column temp. 120°)*	*p-Nitro-benzyl (column temp. 145°)*
Formate	8.4	10.9	11.0
Acetate	15.1	17.0	15.3
Propionate	26.3	27.4	22.8
Alcohol	6.6	10.2	12.6
Halide	9.3	12.7	25.5
Artifact	5.7	7.5	7.2

degradation. The presence of some unreacted *p*-nitrobenzyl iodide and its hydrolysis product *p*-nitrobenzyl alcohol was noted in the reaction mixtures of the *p*-nitrobenzyl esters. The iodide was found to be superior to the bromide in this series giving a cleaner reaction mixture and generally enhancing ester formation. In each series, the equilibrium constant for the esterification reaction appeared to be smallest for formic acid, this being suggested by the relative peak heights of the formate, acetate and propionate esters. Optimum results were realised with *p*-bromobenzyl derivatization; peaks were sharp and symmetrical and, aside from the "reaction artifact", the formation of the ester in the capillary tube was attented by no additional observable side reactions except in the case of the formate where, under the stated conditions, a small shoulder peak suggested the presence of some of the corresponding alcohol (see Fig. 1, chromatogram d).

With the appropriate attenuation setting, as little as 10 ng of reference ester were detected. Howevei, in the micro preparation, detection of the carboxylic acid was not usually successful with sample solution concentrations lower than 0.1 $\mu g/\mu l$. The presence of appreciable amounts of water in the reaction mixture promoted virtually complete hydrolysis of the halide to the benzyl alcohol so that aqueous carboxylic acid solutions at the 1 $\mu g/\mu l$ level afforded only a very small ester peak. Nevertheless, detection of the acid was still possible when aqueous solutions of this concentration were diluted tenfold with ethanol and treated as described.

Though a number of chromatographic methods have already been proposed for the detection and separation of C_1 to C_3 monocarboxylic acids in various media such as in foods, in cigarette smoke or as pollutants in river waters, the present method might prove useful for such determinations carried out by control laboratories which, of necessity, employ general-purpose columns in their day to day operation.

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