# Selective Determination of Pentafluorobenzyl Ester Derivatives of Carboxylic Acids by GC Using Microwave Plasma and Mass Selective Detection

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A highly selective, sensitive method for determination of carboxylic acids in water has been developed that is based on fluorine-selective gas chromatography (GC) detection of pentafluorobenzyl ester derivatives. The atmospheric-pressure helium microwave-induced plasma detector is well suited for fluorine-specific detection of these derivatives, which were well resolved by capillary GC and displayed a characteristic fragmentation behavior when examined by GC-mass selective detection. For demonstration purposes, a number of carboxylic acids, ranging from  $C_1$  to  $C_{20}$ , were examined and found to be derivatized with yields greater than 90%, and a sample of creek water was examined for its carboxylic acid content. Index Headings: Microwave-induced plasma detector; Fluorine; Carboxylic acids; Pentafluorobenzyl esters.

## INTRODUCTION

A need for selective, quantitative analysis of lactic acid in aqueous solutions and an interest in the atmosphericpressure helium microwave-induced plasma detector (MIPD) for highly selective capillary gas chromatography (GC) quantitative analysis of complex mixtures led to a study of the application of this instrumentation for quantitative analysis of carboxylic acids in aqueous solutions, which we now report.

In recent years, studies of capillary GC-MIPD capabilities have shown it to offer valuable element-specific detection with impressive sensitivity.<sup>1-6</sup> Most relevant for this work, as little as 5 pg-fluorine/s can be detected selectively and with no significant interference.<sup>6,7</sup> Continuing studies are making further contributions regarding the instrumentation.<sup>8-12</sup> Furthermore, the recent introduction of a commercially available GC-MIPD instrument should facilitate application of this technology.<sup>13</sup>

The biological and environmental importance of carboxylic acids has led to extensive methods development for their measurement, the importance of which is reflected by the estimated 25% of GC publications devoted to these compounds.<sup>14</sup> Carboxylic acids are important metabolites in many biological processes and also are common environmental water contaminants derived from humic substances during water treatment processes.<sup>15</sup>

Because of their high polarity, the more volatile, shorter-chain fatty acids are difficult to analyze by capillary GC, while the longer-chain, less volatile compounds require high temperatures to elute. The most widely used procedure to overcome these problems is to prepare volatile, thermally stable derivatives of the fatty acids.

Of the numerous types of derivatives that can be pre-

pared,<sup>16,17</sup> pentafluorobenzyl esters are easy to prepare, stable, volatile, and amenable to the use of a selective detector: The electron capture detector (ECD) and the MIPD both yield high responses to these derivatives, owing to the electrophoric pentafluorobenzyl moiety. The MIPD offers the advantage of fluorine selectivity, which the ECD lacks. Furthermore, pentafluorobenzyl bromide effectively derivatizes acids in the presence of water, a distinct advantage for aqueous samples.<sup>18</sup> The use of pentafluorobenzyl derivatives of carboxylic acids [namely, the derivative of (Z)-3-decenoic acid in furniture carpet beetles] has been reported in the analysis of insect pheromones.<sup>19</sup>

This communication reports the application of GC-MIPD in the fluorine mode for the selective detection of pentafluorobenzyl derivatives of carboxylic acids.

#### **EXPERIMENTAL**

The gas chromatograph (a Varian 3700 fitted with a split/splitless injection port and a flame ionization detector) and the MIPD detection system used for GC-MIPD have been described elsewhere.<sup>13</sup> A 15-m  $\times$  0.32mm-i.d. fused-silica column, coated with a  $0.25 - \mu m$  film of DB-5 phase (J & W Scientific, Inc., Folsom, CA), was used. The outlet of the GC column was split to a flame ionization detector (FID) and the MIPD, as described previously.<sup>13</sup> The transfer line from the effluent splitter to the MIPD was a length of aluminum-clad fused-silica capillary (o.d. 0.32 mm), coated with a  $0.10-\mu m$  film of high-temperature HT5 phase (Alltech Associates, Deerfield, IL) and was maintained at 320°C. The microwave input power was 90 W, and the reflected power was minimized (approximately 0 W). The temperature of the MIPD torch assembly was 320°C. The fluorine emission from the plasma was monitored at a wavelength of 685.6 nm, while the flow rate of the plasma support gas (helium) was 60 mL/min.

A Hewlett-Packard 5890A gas chromatograph coupled to a Hewlett-Packard 5790B Mass Selective Detector (MSD) was used for qualitative analysis of samples. The GC was fitted with a Grob-type split/splitless injection port and a 30-m  $\times$  0.25-mm-o.d. fused-silica capillary column coated with a 0.25- $\mu$ m film of DB-1 phase (J & W Scientific, Inc., Folsom, CA). The column was coupled to the MSD via a capillary-direct interface. The following conditions were used: injection temperature, 220°C; column temperature program, 40°-290°C at 8°C/min; interface temperature, 280°C; injection volume, 1  $\mu$ L; split ratio,  $\sim$  30:1. Mass spectra were recorded in the mass

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FIG. 1. Comparison of FID and MIPD traces of pentafluorobenzyl esters of a carboxylic acid mixture. (A) MIPD (fluorine) signal, monitored at 685.6 nm (atten.  $\times$  32); (B) FID signal (atten.  $\times$  8). (1) Formate ester; (2) acetate ester; (3) propionate ester; (4) lactate ester; (5) valerate ester; (6) hexanoate ester; (7) benzoate ester. Column temperature: 40° to 290°C at 10°C/min.

range m/z 30-350, at a scan rate of approximately 1.5 Hz.

Pentafluorobenzophenone (used as an internal standard) and pentafluorobenzyl bromide were obtained from Aldrich Chemical Co. All solvents used were of chromatography grade.

The linear dynamic range (LDR) of the MIPD was determined by making injections of a dilution series of pentafluorobenzophenone in dichloromethane (partition ratio, k = 10).<sup>20</sup> The concentrations of the standard samples ranged from  $1.0 \times 10^{-9}$  to  $1.0 \times 10^{-5}$  g-F/µL. The split ratio was approximately 25:1.

The derivatization procedure used to prepare the pentafluorobenzyl esters was similar to that described by Kawahara.<sup>21,22</sup> The acid or acids ( $\sim 100 \text{ mg of each}$ ) in acetone ( $\sim 20$  mL) were treated with one equivalent of pentafluorobenzyl bromide dissolved in methanol (1 g/mL) and one equivalent of powdered potassium carbonate. The reaction mixture was then refluxed for 3 h. Thereafter, the solution was concentrated under reduced pressure, the residue was extracted  $(4 \times 50 \text{ mL diethyl})$ ether), and the resulting extract was washed  $(4 \times 10 \text{ mL})$ distilled water) and then dried (anhydrous sodium sulfate). The resulting mixture was concentrated under reduced pressure, and the residue was taken up in dichloromethane. It may be advantageous to use a small excess of derivatizing agent, which would both promote complete derivatization and take into account the possibility that the amount of organic acid to be derivatized is not precisely known.

A number of acid samples were prepared and deriva-

tized as follows: (1) The efficiency of the reaction was determined with the use of hexanoic, benzoic, and lactic acids. (2) The general utility of this method was tested by using a mixture of formic, acetic, propionic, valeric, lactic, caproic, and benzoic acids (in approximately equal amounts, except for formic and benzoic acid, which were at twice the level of the other acids). (3) The practical utility was tested on an environmentally relevant sample, an extract of creek water (150 mL) obtained by acidification to pH 1 and extraction with dichloromethane.<sup>23</sup>

#### **RESULTS AND DISCUSSION**

The linear dynamic range of the MIPD for pentafluorobenzophenone was determined to be  $8 \times 10^4$ . This value is slightly larger than values reported in the literature for monofluorinated compounds.<sup>1,3,5,13</sup> The upper range for the LDR is limited by carbon overloading and degrading of the plasma.<sup>1</sup> Evidently, the carbon overloading for these pentafluorobenzyl esters occurs at slightly greater sample sizes, perhaps owing to the presence of the five fluorine atoms.

The MIPD affords particularly good sensitivity for these derivatives because the detector response is correlated with the number of fluorine atoms per molecule of analyte.<sup>13</sup> Thus, the LDR for these pentafluorobenzyl esters is nearly 10<sup>5</sup>, a large range that is primarily due to the very good sensitivity.

These pentafluorobenzyl esters are prepared in good yields. For three representative acids, the esterification was carried out on a scale that permitted measurement of yields by weight. The yields were reproducible and ranged from 92% to 98%: 105 mg (0.91 mmol) of hexanoic acid provided 247 mg (0.83 mmol, 92%) of ester, 93 mg (0.76 mmol) of benzoic acid provided 221 mg (0.73 mmol, 96%) of ester; and 38 mg (0.42 mmol) of lactic acid provided 112 mg (0.41 mmol, 98%) of ester.

A sample of pyruvic acid was also derivatized, but the derivatization under reflux required exclusion of air, achieved by using an atmosphere of nitrogen, to obtain a satisfactory yield (67%) of the desired ester [55 mg (0.63 mmol) of the acid provided 112 mg (0.42 mmol) of ester], apparently owing to decomposition of pyruvic acid when heated in the presence of air.

A recent publication<sup>24</sup> reports the derivatization of carboxylic acids on a micro-scale using a Keele microreactor/extractor. Although this procedure avoids the concentration steps, in our experience problems can arise owing to relatively large amounts of unreacted pentafluorobenzyl bromide, which overloads the GC column and the MIPD discharge tube.

The effective GC separation of all the pentafluorobenzyl esters derived from the trial mixture of acids is shown in Fig. 1. Trace 1A shows the fluorine signal from the MIPD, while 1B shows the FID signal. The particularly good signal-to-noise ratio of the MIPD output, which approaches the FID signal-to-noise ratio, is clear demonstration of the advantage of having five fluorine atoms in the derivative and is far better than the signal-tonoise ratio typical of monofluorinated compounds.<sup>13</sup>

The pentafluorobenzyl derivatives of carboxylic acids have a characteristic behavior upon electron impact (EI) typified by generally simple mass spectra that usually



FIG. 2. Electron impact (EI) (70 eV) mass spectrum of pentafluorobenzyl hexanoate.

show the molecular ion (1-20%). Figure 2 shows the EI mass spectrum of pentafluorobenzyl hexanoate as an illustrative example. Of the 19 acids examined, only pyruvic and succinic acids failed to show a molecular ion for the pentafluorobenzyl ester.

All of the alkanoic acid derivatives showed a base (100%) peak at m/z 181 (C<sub>7</sub>H<sub>2</sub>F<sub>5</sub><sup>+</sup>, presumably the pentafluorotropylium ion). Moreover, all the derivatives showed an ion at m/z 197 (1–5%), due to the pentafluorobenzyloxy fragment (C<sub>6</sub>F<sub>5</sub>CH<sub>2</sub>O<sup>+</sup>). The mass spectra also showed a peak at m/z 161 (7–18%), probably due to the loss of HF from the pentafluorobenzyl fragment. This ion is also present in the mass spectrum of pentafluorobenzyl bromide. The typical pattern for the relative abundances of the molecular ion and those at m/z 197, 181, and 161 is clearly evident from the data for 19 carboxylic acids summarized in Table I.

Because application of this method commonly could require determination of carboxylic acids in the presence of other compounds bearing different but reactive functional groups, it is of interest to point out that the ions occurring at m/z 195, 196, and 197 may permit the qualitative distinction between pentafluorobenzyl derivatives of carboxylic acids, phenols, and some amines (although tertiary amines do not react with pentafluorobenzyl bromide under the conditions used for this work). In our experience, the mass spectra of these carboxylic acid derivatives always contain an ion at m/z 197 (C<sub>6</sub>F<sub>5</sub>CH<sub>2</sub>O<sup>+</sup>), an ion that is absent in the spectra of the derivatives of phenols and primary amines. A fragment ion at m/z 196  $(C_6F_5CH_2NH^+)$  is present in the mass spectra of primary amine derivatives, but this ion usually is absent in the derivatives of carboxylic acids and phenols. The ion at m/z 195 usually is present (at low abundances) in the

TABLE I. Relative abundances of characteristic mass fragments of pentafluorobenzyl esters of carboxylic acids.»

|                                  | Molecular ion | Molecular ion Relative abundances (%) of characteristic ions |                |         |         |         |           |
|----------------------------------|---------------|--|----------------|---------|---------|---------|-----------|
| Pentafluorobenzyl ester of       | m/z (%)       | m/z 181  | <i>m/z</i> 161 | m/z 197 | m/z 196 | m/z 195 | m/z (%)   |
| methanoic acid                   | 226 (29)      | 100  | 18             | 3       | 0       | 4       | 31 (11)   |
| ethanoic acid                    | 240 (26)      | 100  | 14             | 16      | 0       | 6       | 43 (61)   |
| propanoic acid                   | 254 (11)      | 100  | 8              | 3       | 0       | 3       | 57 (34)   |
| 2-methylpropanoic acid           | 268 (14)      | 100  | 10             | 3       | 0       | 4       | 43 (46)   |
| 2-hydroxypropanoic acid (lactic) | 270 (2)       | 75   | 13             | 2       | 0       | 4       | 45 (100)  |
| butanoic acid                    | 268 (10)      | 100  | 10             | 5       | 0       | 0       | 43 (28)   |
| 2-methylbutanoic acid            | 282 (8)       | 100  | 9              | 3       | 0       | 3       | 57 (59)   |
| pentanoic acid                   | 282 (5)       | 100  | 7              | 4       | 0       | 3.      | 43 (16)   |
| hexanoic acid                    | 296 (2)       | 100  | 8              | 3       | 0       | 3       | 69 (25)   |
| benzoic acid                     | 302 (41)      | 78   | 13             | 3       | 0       | 4       | 105 (100) |
| butanedioic acid (succinic)      | 478 (0)       | 100  | 7              | 5       | 1       | 2       | 101 (36)  |
| 2-oxopropanoic acid (pyruvic)    | 268 (0)       | 90   | 14             | 3       | 0       | 3       | 43 (100)  |
| octanoic acid                    | 324 (3)       | 100  | 4              | 2       | 0       | 1       | 57 (15)   |
| decanoic acid                    | 352 (2)       | 100  | 3              | 2       | 0       | 1       | 69 (15)   |
| dodecanoic acid                  | 380 (1)       | 100  | 3              | 1       | 0       | 1       | 43 (21)   |
| tetradecanoic acid               | 408 (2)       | 100  | 2              | 1       | 0       | 1       | 43 (14)   |
| hexadecanoic acid                | 436 (1)       | 100  | <b>2</b>       | 1       | 0       | 3       | 43 (21)   |
| octadecanoic acid                | 464 (2)       | 100  | 2              | 1       | 1       | 3       | 43 (18)   |
| eicosanoic acid                  | 492 (1)       | 100  | 2              | 2       | 1       | 3       | 43 (22)   |

" The data listed above are averages from three different samples for each pentafluorobenzyl ester.



FIG. 3. Comparison of FID and MIPD traces of a sample of creek water treated with pentafluorobenzyl bromide reagent. (A) MIPD (fluorine) signal, monitored at 685.6 nm (atten.  $\times$  32); (B) FID signal (atten.  $\times$  8). (1) Hexanoate ester; (2) benzoate ester; (3) nonanoate ester; (4) decanoate ester.

mass spectra of carboxylic acid derivatives, but absent in the spectra of the phenol and amine derivatives.

Figure 3 compares the FID and MIPD (fluorine signal) traces of the mixture produced by treating a creek water extract with pentafluorobenzyl bromide. The MIPD trace is dramatically simpler than the trace from the FID. Four of the components in this mixture that showed clear signals in the MIPD trace could be identified from their mass spectra. The levels of these acids present in the sample were estimated to be in the ng/mL range. From the relative responses of the MIPD and FID traces, it was apparent that the other fluorine peaks in the MIPD chromatogram also were derived from pentafluorinated compounds. Although structures were not assigned, they most likely were derivatives of unsaturated acids, phenols, or mercaptans (which also are known to react with pentafluorobenzyl bromide).<sup>16,18</sup>

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