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## Note

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### Further calibration data for the gas chromatography of benzyl esters

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Benzyl esters have been found useful for gas chromatography (GC)<sup>1-3</sup> and GC-mass spectrometry (MS)<sup>4-7</sup> of carboxylic acids, especially short-chain and other low-molecular-weight species. Various data on GC retention times<sup>1,2</sup> of benzyl esters and MS studies<sup>2,6,7</sup> have been published; this note presents retention time data for further benzyl esters prepared during a mass spectral study. A range of chemical types has been included, with some of biological interest.

#### EXPERIMENTAL

##### *Preparation of samples and esterification*

Acids or salts were obtained from various commercial sources and used without further purification. Benzyl chloroformate was purchased as the ester. Tetrahydrofuran 2-carboxylic acid was prepared by platinum oxidation<sup>8</sup> of tetrahydrofurfuryl alcohol. Benzyl esters were prepared by esterification using diazotoluene (phenyldiazomethane) prepared from N-benzyl-N-nitroso-toluene-4-sulphonamide<sup>9</sup> as previously described<sup>10</sup>; excess solvent was removed under vacuum and the diazotoluene mixed with dry dimethylformamide to give an approximately 25% (v/v) solution. Acid salts were converted to the free acid using an ion-exchange resin<sup>10</sup> and dried or lyophilised. Esters were prepared by the addition of a slight excess of diazotoluene solution to the acid (1 to 2 mg) and on completion of the reaction the solution was diluted with ethyl acetate to give a final concentration of approximately 4 mg/ml.

##### *Gas chromatography*

Retention times were measured on a column packed with 3% OV-1 on Diatomite CQ, 100-120 mesh; column dimensions and other parameters were as previously described<sup>1</sup> except that column temperatures were as given in Table I. Identities of the peaks were confirmed by GC-MS<sup>4</sup> at GC column temperatures 30° higher than those in Table I.

#### RESULTS AND DISCUSSION

The results are summarised in Table I, which is divided into three temperature groups: (a) 140°, reference benzyl 2-bromopropionate, (b) 200°, reference benzyl

TABLE I

## RETENTION DATA FOR BENZYL ESTERS OF CARBOXYLIC ACIDS

Column, 1.52 m × 4 mm I.D. glass, 3% OV-1 on Diatomite CQ, 100–120 mesh. Temperatures (°C) as indicated.

<i>Parent acid</i>	<i>Mol.wt. of benzyl ester</i>	<i>Relative retention*</i>
<i>Group 1 —140° column, 200° injector</i>		
Chloroformic	170	0.16
Pivalic (2,2-dimethylpropanoic)	192	0.51
Bromoacetic	229	0.65
2-Bromopropanoic	243	1.00 <sup>a</sup>
Cyclohexylacetic	232	1.17
Tetrahydrofuran 2-carboxylic	206	1.18
Cyclopentylcarboxylic	204	1.21
Furan 2-carboxylic	202	1.46
3-Methoxybenzoic	242	1.84
<i>trans</i> -Cinnamic	238	1.97
<i>Group 2 —200° column, 250° injector</i>		
5-Bromofuran 2-carboxylic	281	0.33
2,6-Dimethylbenzoic	240	0.40
6-Bromohexanoic	285	0.43
Indole 2-carboxylic	251	0.92
1- and 2-naphthoic	262	1.00 <sup>b</sup>
3,4,5-Trimethoxybenzoic	286	1.16
2-Naphthaleneacetic	276	1.27
2,3-Dihydroxymaleic	296	1.30
3-Hydroxy-2-naphthoic	278	1.53
Anthracene 9-carboxylic	312	1.85
Benzilic (2-Hydroxy-2,2-diphenylpropanoic)	318	1.95
Phenylcinnamic	314	2.03
<i>Group 3 —250° column, 300° injector</i>		
11-Bromoundecanoic	359	0.46
Palmitic	346	0.59
Oleic	372	0.93
Linoleic	370	0.93
Linolenic	368	0.94
Stearic	374	1.00 <sup>c</sup>
Naphthalene 2,3-dicarboxylic	396	1.36
Decane 1,10-dicarboxylic	382	1.53

\* Retention times (min) of reference esters: <sup>a</sup>11.9, <sup>b</sup>13.1, <sup>c</sup>12.3.

naphthoate and (c) 250°, reference benzyl stearate. (Retention characteristics of benzyl stearate have been published<sup>1,6</sup> but the ester is included here as a suitable reference and for comparison with the C<sub>18</sub> unsaturated acid esters).

With regard to the previous use<sup>1,4,6,7</sup> of diazotoluene in light petroleum (b.p. 30–40°C) or diethyl ether, some difficulty was experienced with the solubility of certain acids in the present series. Use of dimethylformamide as a solvent for both diazotoluene and sample was found to overcome this difficulty and showed that dimethylformamide was an excellent solvent for the esterification system.

As observed previously<sup>1</sup>, most esters eluted in approximately molecular weight order, but with the notable exception of the bromine-containing acids. The series benzyl bromoacetate, benzyl 2-bromopropionate, benzyl 5-bromofuroate, benzyl 6-bromohexanoate and benzyl 11-bromoundecanoate all gave much shorter retention times, relative to comparable molecular weight acids, of between one third and one half of the predicted value.

In one aspect of this study, we investigated the esterification of acids exhibiting varying degrees of steric hindrance about the carboxyl group as judged by standard esterification techniques. In common with other diazo-esterifying reagents, and although the reagent possesses the relatively bulky benzyl group, esterification with diazotoluene proceeded rapidly (within 5 min) and smoothly with acids such as 2,6-dimethylbenzoic, 3-hydroxy-2-naphthoic, anthracene 9-carboxylic, phenylcinnamic, benzoic, naphthalene 2,3-dicarboxylic and pivalic. Dicarboxylic acids also reacted smoothly; certain shorter-chain dicarboxylic acid benzyl esters have been chromatographed previously<sup>1,7</sup>. The positional isomers 1- and 2-naphthoic acid benzyl esters did not resolve.

Fatty acid benzyl esters of the C<sub>18</sub> group were tested on OV-1 in comparison with EGSS-X phase<sup>6</sup>. In common with observations of methyl esters on non-polar phases<sup>11</sup>, C<sub>18:0</sub> resolved, albeit poorly, from others in the group, but C<sub>18:1</sub>, C<sub>18:2</sub> and C<sub>18:3</sub> essentially co-eluted. This reflects the known property of OV-1 in often giving incomplete resolution of levels of unsaturation; the benzyl esters did not enhance the resolution. The separation of the benzyl esters of the C<sub>18</sub> group on 10% SILAR-10C was similar to that of the methyl esters on this phase. Various aspects, of the esterification reaction and applications, in the use of diazotoluene have already been discussed<sup>1,2,6,7</sup>.

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