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## GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC PROPERTIES OF NAPHTHALENEMETHYL ESTERS AND CYCLOHEXANEMETHYL ESTERS OF ORGANIC ACIDS

D. L. CORINA\* and K. ISAAC

*Departement of Biochemistry, University of Southampton, Southampton SO9 3TU (Great Britain)*

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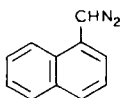
### SUMMARY

The preparation of the esterifying reagents naphthyl diazomethane and cyclohexyldiazomethane is described. These reagents have been used to prepare the naphthalenemethyl and cyclohexanemethyl esters of various types of carboxylic acids. Data are presented for the gas chromatographic retention times of the esters on OV-1 and also for their mass spectral fragmentation patterns.

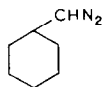
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### INTRODUCTION

Benzyl esters, prepared by esterification with phenyldiazomethane (diazotoluene), have been investigated extensively in our laboratory<sup>1-3</sup> and elsewhere<sup>4,5</sup> by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Continuing our interest in analogues of diazomethane as esterification reagents, and the GC and GC-MS properties of esters prepared from them, we have prepared two further reagents, naphthyl diazomethane (A) and cyclohexyldiazomethane (B).



(A) Naphthyl diazomethane, NDM  
(1-diazomethylnaphthalene)



(B) Cyclohexyldiazomethane, CHDM  
(diazomethylcyclohexane)

In this paper we describe the preparation of these two reagents and outline the GC and MS properties of the resulting naphthalenemethyl (NM) and cyclohexanemethyl (CHM) esters of various types of carboxylic acids.

### EXPERIMENTAL

#### *Materials*

Cyclohexanemethylamine and 1-naphthalenemethylamine were purchased from Aldrich (Gillingham, Dorset, Great Britain); toluene sulphonation and nitro-

sation reagents from BDH (Poole, Great Britain) were recrystallized before use, and carboxylic acids or salts were commercial preparations from various sources and were used without further purification. Tetrahydrofuran-2-carboxylic acid was prepared from tetrahydrofurfural alcohol by Pt/charcoal-air oxidation<sup>6</sup> for 6 h. All solvents were redistilled and stored over 3A molecular sieve. In addition, petroleum spirit, b.p. 30–40°C, was stored over activated alumina.

*Synthesis of diazo precursors N-naphthalenemethyl-N-nitrosotoluene-4-sulphonamide and N-cyclohexanemethyl-N-nitrosotoluene-4-sulphonamide*

These were synthesised via toluenesulphonation (tosylation) and nitrosation by modifications of established procedures for the methyl<sup>7</sup> and benzyl<sup>8</sup> derivatives. The starting amines appeared to be susceptible to atmospheric oxidation, therefore the addition of the amines to the reaction solvent (as soon as possible after weighing) and the initial stages of the tosylation reactions were conducted under an atmosphere of nitrogen. We also found it advantageous to add toluenesulphonyl chloride and alkali in smaller batches more frequently than in previous syntheses<sup>7</sup> and to start the addition of tosyl chloride before the mixture was heated.

*N-Naphthalenemethyltoluene-4-sulphonamide*

1-Naphthalenemethylamine (2.5 ml, 2.7 g) was added to a mixture of 8.0 ml of water and 4.0 ml of dimethylformamide (DMF) with stirring and further DMF was added, as necessary, to render the mixture homogeneous. The addition of tosyl chloride, in 200–400 mg batches, was started immediately and the mixture gradually heated to 70°C with occasional addition of sufficient 50% sodium hydroxide in water–DMF (2:1) to keep the mixture just alkaline. A total of 3.5 g of tosyl chloride was added over a 1.5-h period. On completion of the addition, the mixture was boiled gently for 30 min, ensuring that the mixture remained alkaline. Water (20 ml) was added and the mixture was reheated to melt the precipitated N-naphthalenemethyltoluene-4-sulphonamide. After cooling with rapid stirring the dispersed product was filtered off and air-dried; yield 92% by GC. Purity and progress of the reaction were assessed by GC on a 3% OV-1 column (2.1 m × 2 mm I.D.) programmed 3 min at 160°C, 40°C/min to 340°C when  $R_T$  1-naphthalenemethylamine = 1.6 min and N-naphthalenemethyltoluene-4-sulphonamide = 8.1 min. Material recrystallized from boiling ethanol had m.p. 89–91°C and mass spectrum (direct probe 120°),  $M^+$  at 311 (6.3% base) and  $m/z$  156 (79%), 155 (98%), 154 (100%), 141 (21%), 129 (24%), 128 (19%), 127 (21%) and 91 (29%).

*N-Naphthalenemethyl-N-nitrosotoluene-4-sulphonamide*

This compound was synthesized by prolonged incubation following the procedure in ref. 8; 2.4 g of N-naphthalenemethyltoluene-4-sulphonamide were suspended in 10 ml of glacial acetic acid and cooled to 5°C with stirring. Pre-cooled acetic anhydride was added until solution was complete (60–80 ml). A total of 12 g of solid sodium nitrite was added in small batches over a period of 48 h, the mixture being left stirring overnight at 5°C and for a further 16 h at 5°C after addition of the nitrite was completed. The reaction mixture was then poured into 500 ml of ice-water and warmed to room temperature. On completion of the hydrolysis of the acetic anhydride, a further 200 ml of water were added, the product was filtered off and washed

free from acetic acid with water. The N-naphthalenemethyl-N-nitrosotoluene-4-sulphonamide was dissolved in diethyl ether, washed with dilute sodium hydrogen carbonate solution, water and finally dried over anhydrous sodium sulphate. After evaporation of solvent, the product was recrystallized from diethyl ether-*n*-hexane to yield 2.1 g (80%) pale yellow crystals, m.p. 95–97°C, mass spectrum (direct probe)  $M^+ = 340$  (4.6%) and  $m/z$  310 (9.1%), 308 (7.0%), 232 (2.3%), 185 (6.1%), 156 (32%), 155 (51%), 154 (70%), 141 (100%), 128 (15%), 127 (26%), 115 (10%), 91 (40%) and 30 (23%). Purity checked by thin-layer chromatography (TLC) on silica gel (F-1500) with solvent *n*-hexane–dichloromethane–methanol (70:29:1),  $R_F$  product = 0.55, tosyl intermediate = 0.34. The product stored best in a dark bottle at 5°C.

#### *N-Cyclohexanemethyltoluene-4-sulphonamide*

This compound was prepared in a manner analogous to the NM derivative above with the reagent proportions 6.0 ml, 5.2 g of cyclohexanemethylamine in 15 ml of water (DMF unnecessary) and 10 g of tosyl chloride. The yield was 90%; after recrystallization from hot ethanol, N-cyclohexanemethyltoluene-4-sulphonamide had m.p. 57–59°C and mass spectrum (direct probe 100°C)  $M^+ = 267$  (12%) and  $m/z$  186 (34%), 185 (31%), 184 (99%), 172 (23%), 157 (25%), 156 (29%), 155 (100%), 96 (36%), 92 (31%) and 91 (60%). GC retention on an OV-1 column, programmed as for the NM derivative but 100°C start, cyclohexanemethylamine = 1.8 min, tosyl CHM = 9.2 min.

#### *N-Cyclohexanemethyl-N-nitrosotoluene-4-sulphonamide*

N-Cyclohexanemethyltoluene-4-sulphonamide (12 g) was dissolved in 300 ml of glacial acetic acid and 4 ml of concentrated hydrochloric acid were added. After cooling to below 10°C the material was nitrosated with 7 g of sodium nitrite in 10 ml of water by established procedures<sup>7</sup> over a 1-h period and left a further 45 min. After the addition of 300 ml of water and warming to room temperature, the product was filtered off, washed and dried as described for the NM analogue above. Recrystallization from diethyl ether-*n*-hexane gave bright yellow crystals, yield 82%, m.p. 84–86°C and mass spectrum (direct probe 120°)  $M^+ = 296$  (0.14%) and  $m/z$  266 (1.9%), 210 (1.2%), 184 (11%), 172 (2.7%), 155 (42%), 97 (18%), 96 (20%), 91 (100%) and 30 (18%).

#### *Generation of naphthyl diazomethane and cyclohexyldiazomethane*

Both reagents were generated by the same procedure. A solution of 3 g of potassium hydroxide in 6.0 ml of methanol–water (4:1) was heated to boiling. A total of 300–400 mg of nitroso derivative was added, with shaking, in 50–60 mg batches. The solutions turned deep orange-red (NDM) or yellow (CHDM). Water (3.0 ml) was added, and the mixture cooled to 20°C and extracted with 3 × 2.0 ml of petroleum spirit (b.p. 30–40°C). The combined extracts were washed with water (2 × 4.0 ml) and dried over anhydrous sodium sulphate for NDM or KOH pellets for CHDM, in which form they can be stored at –10°C. Before use, the drying agent was removed, most of the solvent evaporated off under a stream of nitrogen at 10°C and the diazo derivatives dissolved in tetrahydrofuran (THF)–diethyl ether (1:1) to give an approximately 20% solution.

*Preparation of esters*

Free acids were dissolved in diethyl ether, methanol or THF as appropriate to give 1–2 mg per 100  $\mu$ l. A solution of the NDM or CHDM reagent was added in 20- $\mu$ l aliquots until the colour just persisted. Esterification took up to 10 min for the higher molecular weight acids, and slight warming was sometimes necessary for the NDM reaction. The final volume was adjusted to 400–500  $\mu$ l with ethyl acetate and 1–3  $\mu$ l samples were taken for GC or GC–MS analysis. Salts, as solids or concentrated aqueous solutions, were esterified by neutralization with 10% hydrochloric

TABLE I

## RETENTION DATA FOR NAPHTHALENEMETHYL ESTERS OF CARBOXYLIC ACIDS

Column: 2.1 m  $\times$  2 mm I.D. glass, 3% OV-1 on Diatomite CQ, 100–120 mesh. Temperatures as indicated.

| <i>Group</i>  | <i>Parent acid number</i> | <i>Parent acid</i>                | <i>Relative retention</i> |
|---|---------------------------|-----------------------------------|---------------------------|
| Group 1:<br>column 195°C,<br>injector 210°C;<br>reference,<br>methyl palmitate,<br>retention 4.4 min    | 1                         | Acetic                            | 0.41                      |
|   | 2                         | Propionic                         | 0.57                      |
|   | 3                         | Pivalic                           | 0.66                      |
|   | 4                         | Vinylacetic                       | 0.77                      |
|   | 5                         | Glycollic                         | 0.85                      |
|   | 6                         | Monochloroacetic                  | 0.92                      |
|   | 7                         | Crotonic                          | 0.98                      |
|   | 8                         | Dichloroacetic                    | 1.20                      |
|   | 9                         | Monobromoacetic                   | 1.27                      |
|   | 10                        | 4-Hydroxybutyric                  | 1.38                      |
|   | 11                        | Cyclopentylcarboxylic             | 2.00                      |
| Group 2:<br>column 240°C,<br>injector 260°C;<br>reference,<br>methyl behenate,<br>retention 4.9 min     | 12                        | Tetrahydrofuran-2-carboxylic      | 0.34                      |
|   | 13                        | Furan-2-carboxylic                | 0.36                      |
|   | 14                        | Benzoic                           | 0.57                      |
|   | 15                        | Phenylacetic                      | 0.59                      |
|   | 16                        | Cyclohexaneacetic                 | 0.61                      |
|   | 17                        | Nicotinic                         | 0.64                      |
|   | 18                        | 2,6-Dimethylbenzoic               | 0.78                      |
|   | 19                        | Mandelic                          | 0.80                      |
|   | 20                        | 6-Bromohexanoic                   | 0.80                      |
|   | 21                        | Capric                            | 0.88                      |
|   | 22                        | 3-Methoxybenzoic                  | 1.08                      |
|   | 23                        | Cinnamic                          | 1.41                      |
|   | 24                        | Indole-2-carboxylic               | 1.42                      |
|   | 25                        | Indole-2-acetic                   | 1.53                      |
| Group 3:<br>column 295°C,<br>injector 300°C;<br>reference,<br>cholesterol acetate,<br>retention 2.5 min | 26                        | 3,4,5-Trimethoxybenzoic           | 0.90                      |
|   | 27                        | Naphthalene-2-carboxylic          | 0.94                      |
|   | 28                        | Naphthalene-2-acetic              | 0.98                      |
|   | 29                        | 3-Hydroxynaphthalene-2-carboxylic | 0.98                      |
|   | 30                        | Phenylcinnamic                    | 1.35                      |
|   | 31                        | Palmitic                          | 1.39                      |
|   | 32                        | Anthracene-9-carboxylic           | 1.76                      |
|   | 33                        | Stearic                           | 2.16                      |
|   | 34                        | Succinic                          | 2.65                      |
|   | 35                        | Phthalic                          | 5.94                      |
|   | 36                        | Decane-1,10-dicarboxylic          | 12.44                     |

TABLE II

## RETENTION DATA FOR CYCLOHEXANEMETHYL ESTERS OF CARBOXYLIC ACIDS

Column: 2.1 m × 2 mm I.D. glass, 3% OV-1 on Diatomite CQ, 100–120 mesh. Temperatures as indicated.

| <i>Group</i>  | <i>Parent acid number</i>  |                          | <i>Relative retention</i> |      |
|---|--|--------------------------|---------------------------|------|
| Group 1:<br>column 125°C,<br>injector 170°C;<br>reference,<br>methyl caprate,<br>retention 4.4 min      | 1  | Acetic                   | 0.39                      |      |
|   | 2  | Propionic                | 0.73                      |      |
|   | 3  | Pivalic                  | 1.00                      |      |
|   | 4  | Vinylacetic              | 0.76                      |      |
|   | 7  | Crotonic                 | 1.43                      |      |
|   | 8  | Dichloroacetic           | 1.90                      |      |
|   | Group 2:<br>column 175°C,<br>injector 210°C;<br>reference,<br>methyl myristate,<br>retention 3.2 min | 9                        | Bromoacetic               | 0.28 |
|   |  | 11                       | Cyclopentylcarboxylic     | 0.61 |
| 13  |  | Furan-2-carboxylic       | 0.62                      |      |
| 16  |  | Cyclohexaneacetic        | 0.82                      |      |
| 14  |  | Benzoic                  | 1.06                      |      |
| 17  |  | Nicotinic                | 1.15                      |      |
| 15  |  | Phenylacetic             | 1.26                      |      |
| 18  |  | 2,6-Dimethylbenzoic      | 1.72                      |      |
| 19  |  | Mandelic                 | 1.78                      |      |
| 20  |  | 6-Bromohexanoic          | 1.96                      |      |
| 21  |  | Capric                   | 2.43                      |      |
| Group 3:<br>column 235°C,<br>injector 275°C;<br>reference,<br>methyl arachidonate,<br>retention 3.0 min | 22   | 3-Methoxybenzoic         | 2.58                      |      |
|   | 23   | Cinnamic                 | 3.35                      |      |
|   | 24   | Indole-2-carboxylic      | 1.00                      |      |
|   | 27   | Naphthalene-2-carboxylic | 1.09                      |      |
|   | 28   | Naphthalene-2-acetic     | 1.12                      |      |
|   | 25   | Indole-2-acetic          | 1.39                      |      |
|   | 30   | Phenylcinnamic           | 1.61                      |      |
| 32  | Anthracene-9-carboxylic  | 4.18                     |                           |      |

acid in THF, and for aqueous solutions the addition of sufficient THF to render the solution homogeneous. The diazo reagent was then added; esterification was slower under these conditions. The solution was dried with a small amount of anhydrous sodium sulphate before analysis.

*GC and combined GC-MS*

Relative retentions of both NM and CHM esters were measured on a 2.1 m × 2 mm I.D. glass column packed with 3% OV-1 on Diatomite CQ (100–120 mesh) at the temperatures given in Tables I and II, at a nitrogen carrier gas flow-rate of 12 ml/min. GC-MS was performed on a Kratos MS30 instrument using a 2.1 m × 4 mm I.D. glass column packed with OV-1 as above, with a helium carrier gas flow-rate of 40 ml/min. All esters were chromatographed through a temperature programme starting at 180°C (for NM esters) or 130°C (for CHM esters) for 3 min, 25°C/min rise to 340°C for 4 min. Mass spectra were recorded at 24 eV electron impact, 300 μA current and 1000 resolution (naphthalenemethyl dichloroacetate was also scanned at 3000 resolution).

TABLE III

MASSES AND RELATIVE INTENSITIES OF IONS IN THE MASS SPECTRA OF NAPHTHALENEMETHYL ESTERS OF CARBOXYLIC ACIDS

| Ester | $M^+$ |     | Ion a |     | Ion b |     | Ion d |     | Ion e |     | Ion f |     |
|-------|-------|-----|-------|-----|-------|-----|-------|-----|-------|-----|-------|-----|
|       | m/z   | I   | m/z   | I   | m/z   | I   | m/z   | I   | m/z   | I   | m/z   | I   |
| 1 NM  | 200   | 34  | —     | —   | 43    | 61  | 115   | 28  | 129   | 52  | 141   | 100 |
| 2 NM  | 214   | 245 | 29    | 3.0 | 57    | 4.4 | 115   | 22  | 129   | 29  | 141   | 100 |
| 3 NM  | 242   | 7.1 | 57    | 100 | 85    | 5.3 | 115   | 21  | 129   | 7.3 | 141   | 94  |
| 4 NM  | 226   | 44  | 41    | 4.4 | 69    | 4.2 | 115   | 13  | 129   | 7.9 | 141   | 100 |
| 5 NM  | 216   | 24  | 31    | 8.4 | —     | —   | 115   | 40  | 129   | 10  | 141   | 100 |
| 6 NM  | 234   | 27  | 49    | 3.9 | 77    | 3.6 | 115   | 23  | 129   | 22  | 141   | 100 |
| 7 NM  | 226   | 9.2 | 41    | 2.8 | 69    | 100 | 115   | 11  | 129   | 9.2 | 141   | 46  |
| 8 NM  | 268   | 47  | —     | —   | —     | —   | 115   | 12  | 127   | 6.8 | 141   | 100 |
| 9 NM  | 279   | 13  | —     | —   | —     | —   | 115   | 24  | 129   | 34  | 141   | 100 |
| 10 NM | 244   | 19  | —     | —   | 87    | 3.2 | 116   | 1.8 | 129   | 25  | 141   | 100 |
| 11 NM | 254   | 8.9 | 69    | 56  | 97    | 22  | 115   | 13  | 128   | 7.6 | 141   | 100 |
| 12 NM | 256   | 13  | 71    | 65  | —     | —   | 115   | 16  | 128   | 5.0 | 141   | 100 |
| 13 NM | 252   | 45  | —     | —   | 95    | 57  | 115   | 4.2 | 128   | 6.1 | 141   | 100 |
| 14 NM | 262   | 6.1 | 77    | 31  | 105   | 100 | 115   | 18  | 128   | 6.0 | 141   | 69  |
| 15 NM | 276   | 9.4 | 91    | 14  | —     | —   | 115   | 11  | 127   | 3.1 | 141   | 100 |
| 16 NM | 282   | 5.5 | 97    | 12  | 125   | 4.3 | 115   | 15  | 129   | 5.2 | 141   | 100 |
| 17 NM | 263   | 18  | 79    | 32  | 107   | 50  | 115   | 21  | 129   | 12  | 141   | 100 |
| 18 NM | 290   | 36  | 105   | 12  | 133   | 28  | 115   | 11  | 128   | 2.4 | 141   | 100 |
| 19 NM | 292   | 12  | 107   | 46  | —     | —   | 115   | 19  | 128   | 5.5 | 141   | 100 |
| 20 NM | 335   | 10  | —     | —   | 177   | 3.8 | 115   | 12  | 129   | 11  | 141   | 100 |
| 21 NM | 312   | 4.5 | —     | —   | —     | —   | 115   | 4.1 | 128   | 8.0 | 141   | 100 |
| 22 NM | 292   | 5.9 | 107   | 8.5 | 135   | 55  | 115   | 11  | 127   | 4.9 | 141   | 100 |
| 23 NM | 288   | 43  | 103   | 48  | 131   | 65  | 115   | 32  | 127   | 10  | 141   | 100 |
| 24 NM | 301   | 7.6 | 116   | 2.7 | 144   | 1.8 | 115   | 12  | 127   | 6.5 | 141   | 100 |
| 25 NM | 315   | 12  | 130   | 100 | —     | —   | 115   | 8.9 | 128   | 3.8 | 141   | 44  |
| 26 NM | 352   | 18  | 168   | 5.4 | 195   | 65  | 115   | 18  | 127   | 2.3 | 141   | 100 |
| 27 NM | 312   | 19  | *     | —   | *     | —   | 115   | 13  | 127   | 20  | 141   | 100 |
| 28 NM | 326   | 4.6 | *     | —   | *     | —   | 115   | 7.0 | 127   | 1.4 | 141   | 100 |
| 30 NM | 364   | 6.4 | 179   | 50  | 207   | 4.7 | 115   | 16  | 128   | 3.4 | 141   | 100 |
| 31 NM | 396   | 4.3 | —     | —   | —     | —   | 115   | 6.3 | 129   | 5.9 | 141   | 100 |
| 32 NM | 362   | 13  | 178   | 14  | 205   | 6.6 | 115   | 11  | 127   | 2.9 | 141   | 100 |
| 33 NM | 424   | 3.0 | —     | —   | —     | —   | 115   | 6.3 | 129   | 3.8 | 141   | 100 |
| 34 NM | 398   |     |       |     |       |     |       |     |       |     |       |     |
| 35 NM | 446   |     |       |     |       |     |       |     |       |     |       |     |
| 36 NM | 482   |     |       |     |       |     |       |     |       |     |       |     |

\* Ions common to acid and ester groups.

## RESULTS AND DISCUSSION

## GC

For the purposes of retention time measurement, each series of esters was arranged into three temperature groups. Tables I and II list the relative retention data for NM esters and CHM esters, respectively, at the temperatures noted. Long-chain fatty acid methyl esters were suitable reference compounds except for NM Group 3, where cholesterol acetate was used. The reference was co-injected with

| <i>Ion g</i> |          | <i>Other ions</i> |          |            |          |            |          |            |          |            |          |            |          |
|--------------|----------|-------------------|----------|------------|----------|------------|----------|------------|----------|------------|----------|------------|----------|
| <i>m/z</i>   | <i>I</i> | <i>m/z</i>        | <i>I</i> | <i>m/z</i> | <i>I</i> | <i>m/z</i> | <i>I</i> | <i>m/z</i> | <i>I</i> | <i>m/z</i> | <i>I</i> | <i>m/z</i> | <i>I</i> |
| 158          | 69       | 127               | 16       | 128        | 20       | 139        | 28       | 140        | 83       | 142        | 17       | 159        | 7.8      |
| 158          | 62       | 44                | 9.4      | 127        | 10       | 128        | 13       | 139        | 12       | 140        | 47       | 142        | 13       |
| 158          | 10       | 41                | 22       | 44         | 11       | 128        | 5.0      | 139        | 10       | 140        | 7.6      | 142        | 18       |
| 158          | 22       | 41                | 4.4      | 127        | 6.0      | 128        | 6.3      | 139        | 10       | 140        | 17       | 142        | 14       |
| 158          | 4.7      | 63                | 8.5      | 127        | 6.8      | 128        | 12       | 139        | 14       | 140        | 12       | 157        | 1.8      |
| 158          | 28       | 63                | 4.5      | 71         | 6.0      | 127        | 10       | 128        | 11       | 140        | 55       | 157        | 12       |
| 158          | 7.7      | 39                | 8.8      | 41         | 13       | 128        | 6.2      | 139        | 8.8      | 140        | 35       | 181        | 3.6      |
| 156          | 7.3      | 128               | 6.6      | 129        | 5.0      | 139        | 8.7      | 140        | 14       | 158        | 4.7      | 233        | 1.6      |
| 157          | 78       | 127               | 22       | 128        | 23       | 139        | 19       | 140        | 27       | 155        | 11       | 199        | 10       |
| 158          | 44       | 43                | 7.0      | 128        | 15       | 139        | 14       | 140        | 33       | 157        | 29       | 200        | 1.1      |
| 158          | 22       | 41                | 18       | 127        | 5.6      | 139        | 8.4      | 140        | 19       | 156        | 2.8      | 168        | 3.6      |
| 156          | 1.9      | 41                | 13       | 127        | 48       | 129        | 2.8      | 139        | 8.3      | 140        | 19       | 157        | 1.7      |
| 157          | 8.2      | 39                | 11       | 127        | 3.9      | 129        | 5.5      | 139        | 10       | 140        | 19       | 156        | 2.8      |
| 156          | 3.1      | 44                | 5.6      | 51         | 9.2      | 127        | 4.2      | 129        | 5.0      | 139        | 14       | 140        | 23       |
| 157          | 4.0      | 65                | 3.4      | 128        | 2.9      | 129        | 2.3      | 139        | 5.4      | 140        | 4.6      | 142        | 13       |
| 158          | 11       | 41                | 19       | 55         | 43       | 81         | 25       | 83         | 3.7      | 128        | 4.3      | 140        | 7.7      |
| 156          | 3.8      | 52                | 16       | 80         | 8.8      | 127        | 6.5      | 128        | 5.4      | 139        | 23       | 140        | 46       |
| —            | —        | 43                | 5.6      | 77         | 6.8      | 79         | 8.7      | 91         | 1.6      | 118        | 2.1      | 139        | 5.4      |
| 158          | 1.2      | 77                | 18       | 79         | 17       | 105        | 14       | 129        | 4.9      | 139        | 7.9      | 152        | 1.2      |
| 158          | 64       | 69                | 11       | 127        | 5.1      | 128        | 6.6      | 139        | 8.1      | 140        | 26       | 219        | 1.5      |
| 158          | 29       | 43                | 23       | 57         | 9.7      | 71         | 8.3      | 85         | 5.5      | 139        | 5.3      | 140        | 10       |
| 155          | 2.3      | 63                | 5.0      | 77         | 12       | 92         | 5.5      | 139        | 14       | 140        | 12       | 152        | 2.1      |
| 157          | 3.5      | 77                | 48       | 91         | 17       | 140        | 4.4      | 227        | 5.7      | 241        | 20       | 242        | 22       |
| 154          | 4.2      | 89                | 5.9      | 91         | 3.1      | 117        | 3.4      | 130        | 13       | 139        | 4.2      | 155        | 3.5      |
| —            | —        | 40                | 5.6      | 77         | 7.8      | 102        | 3.6      | 103        | 4.7      | 129        | 5.3      | 139        | 3.4      |
| 155          | 1.5      | 40                | 6.5      | 53         | 6.1      | 66         | 4.6      | 77         | 5.0      | 139        | 9.2      | 140        | 7.7      |
| 155          | 70       | 126               | 3.9      | 128        | 7.3      | 139        | 7.3      | 140        | 9.3      | 267        | 3.9      | 294        | 2.1      |
| 155          | 0.65     | 40                | 1.5      | 113        | 0.55     | 126        | 0.55     | 128        | 1.3      | 139        | 2.8      | 158        | 0.40     |
| 157          | 0.88     | 152               | 3.8      | 178        | 31       | 223        | 2.9      | 229        | 5.6      | 241        | 1.6      | 318        | 7.4      |
| 158          | 61       | 43                | 21       | 57         | 15       | 71         | 7.9      | 85         | 4.8      | 128        | 3.3      | 140        | 16       |
| —            | —        | 40                | 17       | 73         | 14       | 139        | 4.1      | 176        | 8.6      | 177        | 7.9      | 221        | 5.2      |
| 158          | 53       | 43                | 25       | 57         | 21       | 71         | 7.7      | 128        | 3.3      | 140        | 12       | 157        | 2.9      |

the sample. The molecular weights of the esters are given in Tables III and IV. Based on the order of elution of the NM esters, each compound was given a "parent acid number" which is used to denote the parent acid component in Tables III and IV, and in discussion of the MS data. For example, 14 NM signifies benzoic acid naphthalenemethyl ester. Although fewer acids were investigated in these NM, and CHM series than in our previous work<sup>1-3</sup> on benzyl esters, we have retained a broad range of structural types of acid groups.

Naphthalenemethyl esters generally produced sharp symmetrical peaks, in-

TABLE IV

MASSES AND RELATIVE INTENSITIES OF IONS IN THE MASS SPECTRA OF CYCLOHEXANEMETHYL ESTERS OF CARBOXYLIC ACIDS

| Ester  | $M^+$ |      | Ion a |     | Ion b |      | Ion c |     | Ion h |     | Ion i |     |
|--------|-------|------|-------|-----|-------|------|-------|-----|-------|-----|-------|-----|
|        | m/z   | I    | m/z   | I   | m/z   | I    | m/z   | I   | m/z   | I   | m/z   | I   |
| 1 CHM  | 156   | —    | —     | —   | 43    | 100  | —     | —   | 81    | 40  | 96    | 20  |
| 2 CHM  | 170   | —    | 29    | 21  | 57    | 100  | 74    | 2.1 | 81    | 75  | 96    | 43  |
| 3 CHM  | 198   | —    | 57    | 93  | 85    | 12   | 103   | 5.7 | 81    | 39  | 96    | 100 |
| 4 CHM  | 182   | —    | 41    | 28  | 69    | 19   | 86    | 24  | 81    | 27  | 97    | 48  |
| 7 CHM  | 182   | —    | 41    | 62  | 69    | 100  | 87    | 14  | 81    | 56  | 96    | 71  |
| 8 CHM  | 224   | —    | 83*   | 6.4 | 111*  | 0.38 | —     | —   | 81    | 62  | 97    | 40  |
| 9 CHM  | 234   | —    | —     | —   | 121   | 8.0  | —     | —   | 81    | 89  | 96    | 97  |
| 11 CHM | 210   | 0.17 | 69    | 52  | 97    | 52   | 114   | 13  | 83    | 4.5 | 96    | 100 |
| 13 CHM | 212   | 1.4  | 68    | 27  | 95    | 81   | 113   | 27  | 81    | 69  | 96    | 100 |
| 14 CHM | 218   | —    | 77    | 38  | 105   | 96   | 122   | 13  | 81    | 50  | 96    | 100 |
| 15 CHM | 232   | —    | 91    | 61  | —     | —    | 136   | 3.0 | 81    | 21  | 95    | 69  |
| 16 CHM | 238   | 1.4  | **    | —   | 125   | 15   | 142   | 4.5 | 81    | 26  | 96    | 100 |
| 17 CHM | 219   | 4.6  | 78    | 41  | 106   | 53   | 124   | 100 | 81    | 53  | 96    | 34  |
| 18 CHM | 246   | 5.8  | 105   | 32  | 133   | 100  | 149   | 97  | 81    | 26  | 96    | 53  |
| 19 CHM | 248   | 2.0  | 107   | 100 | 134   | 0.42 | —     | —   | 81    | 33  | 96    | 24  |
| 20 CHM | 291   | —    | —     | —   | 177   | 13   | 194   | 2.8 | 81    | 44  | 96    | 100 |
| 21 CHM | 268   | —    | —     | —   | 155   | 12   | 172   | 6.1 | 81    | 40  | 96    | 100 |
| 22 CHM | 248   | 8.4  | 107   | 10  | 135   | 42   | 152   | 100 | 81    | 12  | 96    | 5.7 |
| 23 CHM | 244   | 3.4  | 103   | 51  | 131   | 87   | 148   | 25  | 81    | 63  | 96    | 100 |
| 24 CHM | 257   | 26   | 115   | 23  | 143   | 100  | 161   | 67  | 81    | 11  | 97    | 6.5 |
| 25 CHM | 271   | 33   | 132   | 100 | 159   | 2.5  | 177   | 7.4 | 81    | 2.8 | 97    | 4.5 |
| 27 CHM | 268   | 7.2  | 127   | 27  | 155   | 33   | 172   | 100 | 81    | 7.9 | 96    | 4.4 |
| 28 CHM | 282   | 26   | 141   | 100 | 168   | 7.4  | 186   | 45  | 81    | 12  | 97    | 26  |
| 30 CHM | 320   | 28   | 178   | 100 | 206   | 9.5  | 223   | 86  | 83    | 3.6 | 97    | 13  |
| 32 CHM | 318   | 52   | 177   | 38  | 205   | 33   | 222   | 100 | 81    | 8.3 | 96    | 3.9 |

\* Identified from 3000 resolution spectrum (see text).

\*\* Ions common to acid and ester groups.

cluding the Group 3 esters which required high temperatures for elution. Dicarboxylic acids, even of simple structure, chromatographed very slowly. Of several tested, only 34 NM, 35 NM and 36 NM could be eluted from the column, the latter two esters having very long retention times. This result renders the NM derivative probably unsuitable for the GC of dicarboxylic acids.

Cyclohexanemethyl esters showed retention characteristics similar to those of benzyl esters<sup>1,2</sup> and hence only a limited number of these were prepared (Table II) with a consideration more to the types for which mass spectral information was required. In CHM Groups 2 and 3, there were some reversals in the order of elution in comparison with the corresponding NM esters.

Peaks ascribed to remaining diazo reagent and by-products of the esterification reaction eluted before any of the esters. Small peaks due to impurities and residual tosyl intermediate were sometimes observed at higher temperatures, especially in the CHDM preparations, but they did not interfere with the GC or GC-MS analyses. It was not possible to obtain a CHDM preparation of high purity, but we did not attempt to purify either diazo reagent by vacuum distillation. Our



*Other ions*

| <i>m/z</i> | <i>I</i> | <i>m/z</i> | <i>I</i> | <i>m/z</i> | <i>I</i> | <i>m/z</i> | <i>I</i> | <i>m/z</i> | <i>I</i> | <i>m/z</i> | <i>I</i> | <i>m/z</i> | <i>I</i> |
|------------|----------|------------|----------|------------|----------|------------|----------|------------|----------|------------|----------|------------|----------|
| 41         | 32       | 54         | 23       | 55         | 54       | 67         | 34       | 68         | 21       | 83         | 9.2      | 95         | 2.8      |
| 41         | 58       | 54         | 23       | 55         | 80       | 67         | 43       | 68         | 27       | 71         | 16       | 97         | 9.5      |
| 41         | 47       | 55         | 83       | 57         | 93       | 74         | 76       | 87         | 29       | 97         | 34       | 143        | 4.2      |
| 39         | 12       | 41         | 37       | 55         | 100      | 67         | 19       | 68         | 13       | 83         | 59       | 96         | 43       |
| 39         | 17       | 41         | 35       | 55         | 56       | 67         | 33       | 68         | 21       | 95         | 7.9      | 137        | 1.1      |
| 41         | 32       | 43         | 19       | 55         | 100      | 67         | 37       | 68         | 20       | 83         | 39       | 96         | 31       |
| 41         | 41       | 54         | 31       | 55         | 100      | 67         | 62       | 68         | 40       | 83         | 40       | 95         | 13       |
| 41         | 29       | 54         | 11       | 55         | 47       | 67         | 31       | 68         | 18       | 73         | 8.9      | 115        | 8.3      |
| 39         | 18       | 41         | 18       | 54         | 4.5      | 55         | 18       | 67         | 38       | 97         | 10       | 126        | 1.8      |
| 41         | 9.8      | 51         | 8.6      | 55         | 19       | 67         | 29       | 68         | 15       | 135        | 1.7      | 173        | 0.12     |
| 41         | 21       | 55         | 100      | 65         | 12       | 67         | 13       | 68         | 6.5      | 96         | 26       | 135        | 5.6      |
| 55         | 45       | 67         | 14       | 83         | 9.3      | 97         | 69       | 141        | 2.8      | 155        | 9.7      | 156        | 5.9      |
| 41         | 45       | 55         | 65       | 67         | 38       | 79         | 22       | 95         | 26       | 127        | 11       | 137        | 26       |
| 77         | 23       | 79         | 17       | 91         | 8.4      | 97         | 42       | 103        | 15       | 132        | 76       | 150        | 23       |
| 55         | 97       | 67         | 27       | 77         | 51       | 79         | 49       | 105        | 31       | 118        | 6.8      | 201        | 2.5      |
| 55         | 54       | 67         | 32       | 69         | 35       | 97         | 34       | 115        | 4.8      | 156        | 2.4      | 195        | 3.3      |
| 41         | 15       | 55         | 41       | 67         | 20       | 71         | 11       | 97         | 49       | 129        | 3.6      | 173        | 3.5      |
| 41         | 4.8      | 55         | 9.3      | 67         | 6.0      | 77         | 8.7      | 92         | 7.6      | 95         | 2.5      | 153        | 13       |
| 55         | 54       | 67         | 40       | 68         | 27       | 77         | 37       | 102        | 21       | 149        | 27       | 150        | 31       |
| 55         | 51       | 89         | 37       | 116        | 11       | 144        | 30       | 155        | 10       | 184        | 7.0      | 198        | 6.3      |
| 57         | 54       | 79         | 18       | 99         | 7.8      | 104        | 12       | 105        | 13       | 157        | 2.4      | 200        | 2.0      |
| 41         | 8.3      | 55         | 13       | 67         | 6.1      | 77         | 3.9      | 91         | 4.0      | 184        | 1.9      | 198        | 1.7      |
| 41         | 24       | 55         | 92       | 67         | 12       | 91         | 8.3      | 115        | 17       | 139        | 17       | 198        | 2.9      |
| 41         | 26       | 55         | 67       | 107        | 23       | 118        | 23       | 1562       | 10       | 177        | 81       | 205        | 6.7      |
| 41         | 24       | 55         | 33       | 81         | 7.9      | 151        | 9.6      | 176        | 25       | 178        | 22       | 281        | 6.5      |


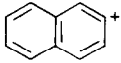
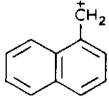
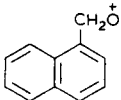


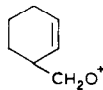
impression was that CHDM was not sufficiently stable to be purified in this manner. Dimethylformamide, an excellent solvent for esterification using phenyldiazomethane<sup>2</sup>, appeared to catalyse the rapid decomposition of CHDM. Stock solutions of CHDM in petroleum spirit could be kept for only 3–4 days at  $-10^{\circ}\text{C}$  and in general the stability and behaviour of CHDM resembled that of diazomethane. In contrast, NDM showed excellent stability, stock solutions being stable for 2–3 weeks at  $-10^{\circ}\text{C}$ . Presumably both phenyldiazomethane and NDM are stabilised by the proximity of an aromatic group.

*Mass spectrometry*

The principal ions in the mass spectra of the NM and CHM esters are listed in Tables III and IV, respectively. Table V gives "formal" structures of the ions of most interest. Many of the fragments appeared as clusters of ions as a result of various hydrogen rearrangements and migrations, so that the ion notation in Table V refers to the group of ions. The most abundant ion in that group is given under the appropriate ion heading in Tables III and IV. Other ions are listed by abundance

TABLE V

PRINCIPAL IONS IN THE MASS SPECTRA OF NAPHTHALENEMETHYL AND CYCLOHEXANEMETHYL ESTERS OF CARBOXYLIC ACIDS

| <i>Ion</i> | <i>Formal structure</i>   | <i>Formula</i>   |
|------------|---|--|
| a          | R <sup>+</sup>  | from   |
| b          | RCO(H) <sup>+</sup>   | acid   |
| c          | RCOOH(H) <sup>+</sup>   | moiety   |
| d          |    | C <sub>9</sub> H <sub>7</sub> <sup>+</sup> <i>m/z</i> 115 group                                |
| e          |    | C <sub>10</sub> H <sub>7</sub> <sup>+</sup> <i>m/z</i> 127 group                               |
| f          |    | C <sub>11</sub> H <sub>9</sub> <sup>+</sup> <i>m/z</i> 141                                     |
| g          |    | C <sub>11</sub> H <sub>9</sub> O <sup>+</sup> <i>m/z</i> 157 group                             |
| h          |    | C <sub>6</sub> H <sub>9</sub> <sup>+</sup> <i>m/z</i> 81 group                                 |
| i          |   | C <sub>7</sub> H <sub>12</sub> <sup>+</sup> <i>m/z</i> 96 group                                |
| j          |  | C <sub>7</sub> H <sub>11</sub> O <sup>+</sup> <i>m/z</i> 111 group<br>(not listed in Table IV) |

or structural significance. Ion abundances are normalized to percent base peak. For halide-containing fragments only the peak due to the more abundant isotope is listed. Fragments which can be derived from both parts of the molecule are indicated by asterisks for 27 NM and 28 NM in Table III and by double asterisks for 16 CHM in Table IV.

The stabilizing nature of the NM group resulted in the major ions predictably arising from naphthalene and naphthalenemethyl with ion groups centred at 115 (ion d), *m/z* 127 (ion e), *m/z* 141 (ion f, usually base peak) and *m/z* 157 (ion g) with varying degrees of hydrogen migration to these ions. Molecular ions, usually of good abundance, were observed for all NM esters. Increasing aromaticity and, to some extent, increasing molecular weight, tended to increase the abundance of fragments from the acid group (ions a and b) although in the NM series the "free" acid ion c

was observed only in four esters (19 NM and 22 NM at  $m/z$  152, 30 NM at  $m/z$  223 and 32 NM at  $m/z$  221) in contrast to CHM esters (see below).

Certain types<sup>3</sup> of benzyl esters undergo a minor rearrangement<sup>9</sup> involving the migration of the benzyl group to an unsaturated centre (usually phenyl) in the acid group, accompanied by a two-step elimination of H<sub>2</sub>O and CO, or one-step elimination of (H)COOH from the molecular ion. As part of our interest in defining some of the structural requirements for this rearrangement, we wished to see whether any NM esters would undergo a similar aryl migration by applying the simple criterion<sup>3</sup> of loss of 18 and 28 a.m.u., or 44–46 a.m.u., from M<sup>+</sup>. Such losses, however, were only observed in four esters, 7 NM ( $m/z$  181), 23 NM ( $m/z$  242), 27 NM ( $m/z$  294 and 267) and 30 NM ( $m/z$  318), where the last three presumably formed naphthylfluorene-type ions analogous to  $m/z$  166 in benzyl benzoate<sup>9</sup>. From this limited occurrence, we concluded that the types of NM esters studied were much less amenable to this type of migration than are benzyl esters. Possibly a study of further examples of substituted naphthoic acid NM esters may show that the rearrangement is more favoured when both acid and esterifying groups are more structurally equivalent. Of course, loss of these molecules from M<sup>+</sup> does not necessarily mean that the proposed rearrangement has occurred. With the exception of this possible rearrangement, and other rearrangements observed in the cinnamic group (23 NM, 30 NM), the NM spectra were fairly straightforward. Simple cleavage was favoured over group migrations, although hydrogen migration of one to three atoms occurred to all ions containing the naphthyl group (ions d to g) in contrast to benzyl esters<sup>3</sup> where hydrogen migration was observed only to the benzyloxy equivalent of ion g. Loss of halide or hydrogen halide was observed in 6 NM, 8 NM, 9 NM and 20 NM.

A selection representative of various acid types was analysed in the CHM series. General considerations of esters with alicyclic esterifying groups predict less useful mass spectra, particularly for esters of non-aromatic acids. Fragments from the CHM group dominated the spectra of low-molecular-weight acids, and showed varying degrees of hydrogen loss from the CHM group to give the principal ions centred at  $m/z$  81 (ion h, formally cyclohexene) and  $m/z$  96 (ion i, possibly methylcyclohexene) and hydrocarbon ions therefrom ( $m/z$  41, 55, 67). The oxygen-carrying species, cyclohexenemethoxy ion j ( $m/z$  111) was observed as a minor peak in a few esters only (3 CHM, 7 CHM, 8 CHM, 11 CHM, 13 CHM, 20 CHM and 21 CHM) with intensities ranging from 0.8 to 3.5%. These have not been listed in Table IV.

Molecular ions were absent from low-molecular-weight acids but appeared with increasing molecular weight and charge-stabilizing nature of the acid; there was also a corresponding appearance of ions a and b from the acid group. Ions of the free or protonated acid (ion c) were also observed in the CHM series. The entry for ions a and b in 8 CHM based on a mass spectrum taken at 3000 resolution, when  $m/z$  82.95 for CHCl<sub>2</sub> was distinguished from  $m/z$  83.085 for C<sub>6</sub>H<sub>11</sub> and  $m/z$  110.947 for CHCl<sub>2</sub>O from  $m/z$  111.080 for C<sub>7</sub>H<sub>11</sub>O. The migration of hydrogen from the CHM group to the acid ions a, b and c was observed in many CHM esters.

Our investigations of higher esters are directed chiefly towards derivatives which may be applicable to direct GC-MS analysis of heavy isotopes, especially carboxyl <sup>18</sup>O atoms in low-molecular-weight acids from biological systems<sup>10,11</sup>. Ease of purification, esterification, extraction and satisfactory molecular ion intensities are all important in this respect (M<sup>+</sup> often is the only ion containing both oxygens of

the carboxyl group). NM esters show promise in this direction, with  $M^+$  present in all monocarboxylic acids tested, including long-chain fatty acids. Benzyl esters of long-chain fatty acids do not give  $M^+$ , although their methyl esters normally do. The present initial study of both NM and CHM esters was concerned with the collection of GC and MS data and a brief comparison with benzyl esters. We have therefore not yet tested either derivative on a biologically derived system, but would predict that the bulky NM group may have the advantage of moving an ester of interest away from other non-esterifiable components or impurities in a GC run. Similarly, good solubility of the NM esters in a solvent such as hexane may prove useful in purification. The esterification procedure is fairly straightforward and also can be performed in the presence of small amounts of water. The analogous anthracenemethyl esters have been described<sup>12</sup> and used as fluorescent derivatives for fatty acid detection in HPLC, but esterification times are very long. Limitations noted in the GC behaviour of NM esters would appear to be the upper limit of about molecular weight 450 for successful GC on OV-1 packed columns, and the very long retention times of dicarboxylic acid NM esters.

Naphtyldiazomethane has been synthesised previously<sup>13</sup>, by a different route, from naphthaldehyde via the unstable hydrazone<sup>14</sup> and used in the HPLC of long-chain fatty acids<sup>15</sup>.

CHM esters would appear to have limited analytical applications. Although it was desirable to document the MS behaviour of CHM esters to maintain the completeness of our study, the spectra ultimately proved to be of little diagnostic value. The formation of potentially useful ions from the acid moiety in CHM esters was found to be neither consistent nor predictable.

## REFERENCES

- 1 D. L. Corina, *J. Chromatogr.*, 87 (1973) 254.
- 2 D. L. Corina, K. E. Ballard and K. Isaac, *J. Chromatogr.*, 210 (1981) 146.
- 3 D. L. Corina, J. N. Wright and K. E. Ballard, *Org. Mass Spectrom.*, 18 (1983) 60.
- 4 U. Hintze, H. Röper and G. Gercken, *J. Chromatogr.*, 87 (1973) 481.
- 5 J. Oehlschläger, U. Hintze and G. Gercken, *J. Chromatogr.*, 110 (1975) 53.
- 6 K. Heyns and H. Paulsen, *Chem. Ber.*, 86 (1953) 833.
- 7 T. J. Boer and H. J. Backer, *Rec. Trav. Chim. Pays-Bas*, 73 (1954) 229.
- 8 G. C. Overberger and J. B. Anselme, *J. Org. Chem.*, 28 (1963) 592.
- 9 J. M. Beynon, R. M. Caprioli, R. H. Shapiro, K. B. Tomer and C. W. J. Chang, *Org. Mass Spectrom.*, 6 (1972) 863.
- 10 D. L. Corina and D. C. Wilton, *Biochem. J.*, 157 (1976) 573.
- 11 M. Akthar, M. R. Calder, D. L. Corina and J. N. Wright, *Biochem. J.*, 201 (1982) 569.
- 12 S. A. Barker, *Anal. Biochem.*, 107 (1980) 116.
- 13 T. Nakaya, T. Tomomoto and M. Imoto, *Bull. Soc. Chem. Jap.*, 40 (1967) 691
- 14 S. W. Staley, C. K. Dustman and G. E. Linkowski, *J. Amer. Chem. Soc.*, 103 (1981) 1069.
- 15 D. P. Matthees and W. C. Purdy, *Anal. Chim. Acta*, 109 (1979) 61