Derivatization and Mass Spectrometric Investigations of Substituted Benzeneboronic Acids. The Use of Linked Scanning During Gas Chromatography Mass Spectrometry

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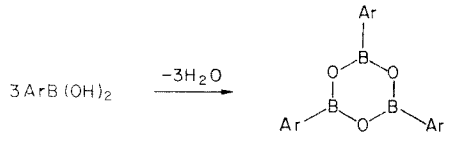
***Substituted* benzeneboronic acids are important intermediates in the synthesis of support matrices for affinity chromatography but their analysis by mass spectrometry is hindered by thermal reactions in the ion source. A simple derivatization with 1,2- or 1,3-diols removes this difficulty and imparts sufficient volatility for application of gas chromatography/mass spectrometry. The mass spectra of the resulting boronate esters are discussed with reference to high resolution measurements, isotope labelling studies and observation of metastable ions. *ortho* Substituents are shown to interact strongly during fragmentation. Linked scanning at constant *B/E* was used to characterize fragmentation pathways and the compatibility of linked scanning and GC/MS is reported.**

INTRODUCTION

Substituted boronic acids are growing in importance as reactive ligands for affinity chromatography.[1] They are useful in medical, academic and industrial spheres for separating membrane proteins, nucleotides, glycoproteins and polyhydroxy compounds.[1,2] During the synthesis of boronic acid ligands in this department,[1] a rapid and simple means of monitoring reactions and elucidating the structures of products was required as an adjunct to nuclear magnetic resonance spectroscopy. Mass spectrometric analysis of the aromatic boronic acids, $ArB(OH)_2$, themselves was not suitable owing to partial dehydration and cyclization (Scheme 1) affording boroxines (trioxatriborans) in the inlet.[3,4] Deliberate derivatization to boroxines and analysis by gas chromatography/mass spectrometry (GC/MS)[4] was not adequate. For instance, for many substituted benzeneboronic acids after conversion to the heterocycle, more than one GC peak is observed and retention times are inordinately long. As derivatives for structural analysis, trimethylsilyl and acyclic alkyl esters of boronic acids are too unstable towards hydrolysis to have received much attention. They also afford molecular ions of low abundance.[5]



Scheme 1

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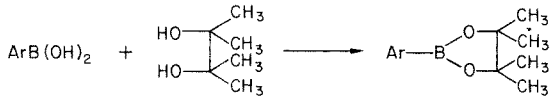
Simple boronic acids are well known as derivatizing agents for 1,2- and 1,3-diols and related compounds.[6–8] Using the same principle, but in reverse, simple diols were assessed as derivatizing agents for analysis of the more complex boronic acids. A very brief report[9] described the characterization of electronegative aromatic boronic acids after reaction with pinacol (2,3-dihydroxy-2,3-dimethylbutane) as shown in Scheme 2. Synthesized as derivatizing reagents for use with electron capture GC detectors, the acids were derivatized on a preparative scale. After recrystallization, the resulting boronate esters were analysed by ultraviolet (UV) and infrared (IR) spectroscopy, thin-layer chromatography (TLC), GC and direct probe mass spectrometry.[9] Herein is reported derivatization on an analytical scale with other diols, not requiring a work-up procedure.



Scheme 2

Mass spectral fragmentation mechanisms of boronate esters have been subject to considerable study.[10–18] The main area of interest has been the origin of hydrocarbon ions, thought to be due to complex rearrangement processes such as that shown (Scheme 3). For the purposes of structural elucidation, such rearrangements may not be desirable and because they are notably absent from the spectra of pinacol boronates,[15] these derivatives have been favoured by other workers.[9] For the more complex boronic acids reported here, propane-1,3-diol was chosen as the principal derivatizing agent. Analogous rearrangements are much less prevalent for the resulting dioxaborans

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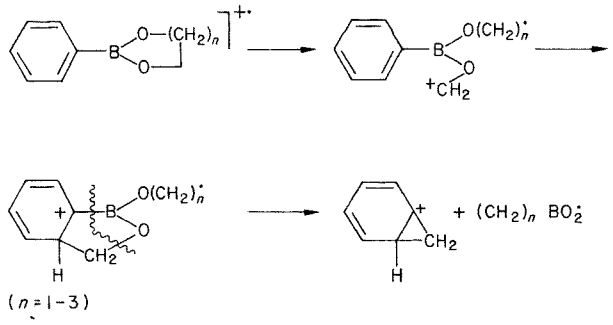
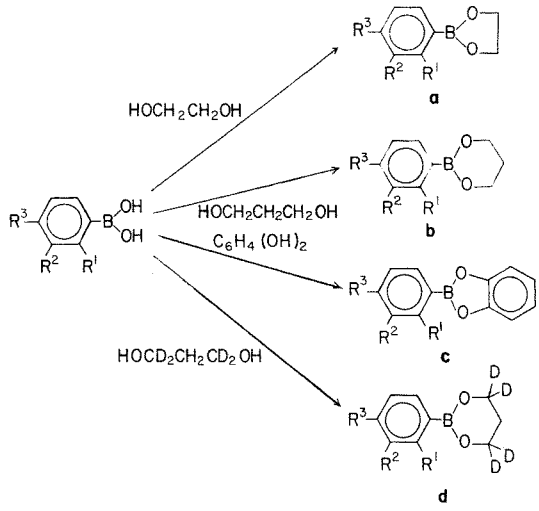


Scheme 3



1 $R^1 = R^2 = R^3 = H$
2 $R^1 = R^2 = H, R^3 = CH_3$
3 $R^1 = H, R^2 = NO_2, R^3 = CH_3$
4 $R^1 = H, R^2 = NH_2, R^3 = COOH$
5 $R^1 = H, R^2 = NH_2, R^3 = COOCH_3$
6 $R^1 = CH_2N(CH_3)_2, R^2 = R^3 = H$
7 $R^1 = R^2 = H, R^3 = NO_2$
8 $R^1 = H, R^2 = NO_2, R^3 = H$
9 $R^1 = NO_2, R^2 = R^3 = H$
10 $R^1 = H, R^2 = NO_2, R^3 = COOH$
11 $R^1 = NO_2, R^2 = H, R^3 = COOH$
12 $R^1 = NO_2, R^2 = H, R^3 = COOCH_3$
13 $R^1 = H, R^2 = NO_2, R^3 = COOCH_3$

Scheme 4

because of competing fragmentations of the labile aromatic substituents. As discussed below, the predominant rearrangements occur as a result of *ortho* effects. The loss of halogen from molecular ions of the pinacol derivatives of 2-halobenzeneboronic acids has also been attributed to the influence of the neighbouring boron function.[9]

Metastable ion techniques, in particular mass analysed ion kinetic energy spectroscopy (MIKES) and linked scanning at constant *B/E*, are useful for differentiating isomeric components and for direct analysis of mixtures.[19–21] For the former purpose, methods based on metastable ions are said to yield data more diagnostic of structure than does routine mass spectrometry. Typical applications occur in the steroid,[22] aromatic[23,24] and carotenoid[21] fields. For direct analysis of mixtures, metastable ion techniques (often given the unhelpful term, MS/MS[25]) suffer from a number of disadvantages.[20] For example, isomeric components of mixtures cannot be resolved by mass selection for separate examination. Mixtures of isomers are best analysed by combined chromatography and mass spectrometry. Thus, the novel combination of GC/MS and linked scanning at constant *B/E*, as described below, is useful because isomers can be separated prior to analysis by a method that is sensitive to fine differences in structure. Linked scanning during GC/MS is used here as a convenient means of establishing precursor/product ion relationships and as a highly structure-sensitive probe. Some initial results have been communicated.[26]

RESULTS AND DISCUSSION

The boronic acids discussed in this paper are shown (**1–13**), with ethane-1,2-diol, propane-1,3-diol, 1,2-dihydroxybenzene and [1,1,3,3-$^2H_4$]propane-1,3-diol being used as derivatizing agents (Scheme 4). Aliquots of the reaction solution were examined without work-up for cyclic boronates by direct insertion probe (for high resolution studies) and GC/MS. Excess of diol was separated from boronate products by applying a temperature gradient to the probe or by the gas chromatograph.

Derivatives of benzene- and 4-methylbenzeneboronic acids (1a–1c, 2a–c)

Some details of the mass spectra of these boronate esters are given in Table 1. Trends are readily discernible from the table. Hydrocarbon ions, resulting from the previously reported[10–13,15–18] rearrangement process shown in Scheme 3, decrease in abundance on changing from the dioxaborolans, **a**, to dioxaborans, **b**. To reduce the complexity of the resulting spectra, derivatization with propane-1,3-diol to boronates **b** was selected for structural elucidation of the more complex compounds discussed below. However, it should be noted that the mass spectrum of compound

Table 1.[a] Some data from the routine mass spectra of boronates 1a–1c and 2a–2c

Compound	Abundance of molecular ion (% rel. ab., %TIC)	Abundance of rearrangement ion[b] (%TIC)	Base peak
1a	74.9, 17.5	23.4	$[C_7H_7]^+$
1b	76.4, 22.4	3.9	$[C_6H_5BO]^{+\cdot}$
1c	100, 30.3	—	$[M]^{+\cdot}$
2a	100, 13.8	7.2	$[M]^{+\cdot}$
2b	100, 19.0	1.9	$[M]^{+\cdot}$
2c	100, 22.8	—	$[M]^{+\cdot}$

[a] Percentage relative abundance, % rel.ab.; percentage of total ion current, % TIC.
[b] Rearrangement process shown in Scheme 3 ($[C_7H_7]^+$ for derivatives of compound **1**; $[C_8H_9]^+$ for derivatives of compound **2**).

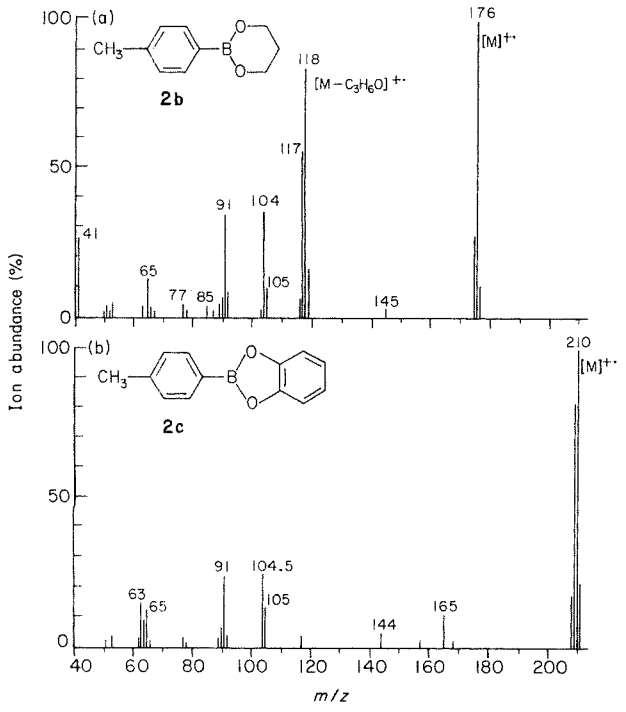


Figure 1. Routine mass spectra of compounds **2b** (a) and **2c** (b) recorded during GC/MS. All peaks over 2% rel. ab. are included.

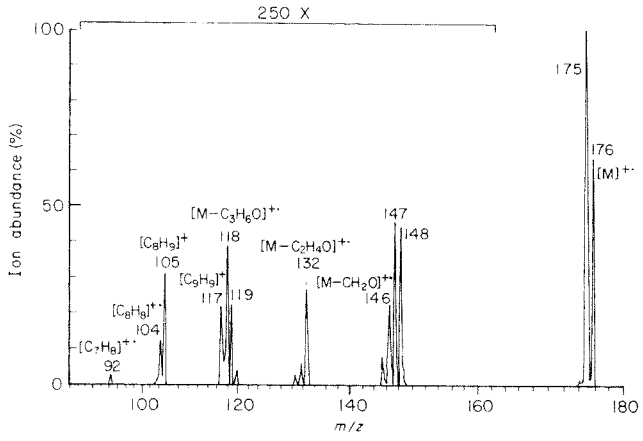


Figure 2. *B/E* linked scan spectrum of the molecular ion of cyclic boronate **2b** recorded during GC/MS.

2b contained rearrangement ions not only of composition C_8H_9 (*m/z* 105) but also of C_8H_8 (*m/z* 104) and C_9H_9 (*m/z* 117) as seen in Fig. 1(a). These hydrocarbon ions are due to primary cleavages of the molecular ion as shown by linked scanning with a constant ratio of magnetic field to electric sector voltage (*B/E*)[27] to detect all products of unimolecular decompositions of $[M]^{+\cdot}$ occurring in the first field-free region (1st FFR) of the conventional geometry, double focusing mass spectrometer. The *B/E* mass spectrum is shown in Fig. 2 for boronate **2b**, the same result being obtained by direct probe or chromatographic inlet. Ejection of $C_3H_5BO_2$, $C_2H_5BO_2$, $C_2H_4BO_2^{\cdot}$ and $CH_4BO_2^{\cdot}$ leads to $[C_7H_8]^{+\cdot}$, $[C_8H_8]^{+\cdot}$, $[C_8H_9]^{+}$ and $[C_9H_9]^{+}$, respectively. Other losses include those of $(CH_2)_nO$.

The percentage of total ion current (TIC) carried by the molecular ions of each type of boronate derivative increases in the order **a**<**b**<**c** (Table 1). For boronic acids affording derivatives **b** with molecular ions of only low abundance, derivatization with 1,2-dihydroxybenzene readily leads to confirmation of molecular weight. The mass spectrum of the aromatic derivative **2c** is given in Fig. 1(b). Notable in this, and other derivatives **c**, are particularly abundant doubly charged ions. For compound **2c**, $[M]^{2+}$ ions containing the major ^{11}B isotope (*m/z* 105) have 13.2%, and $[M]^{2+}$ with the minor ^{10}B isotope and $[M-H]^{2+}$ ions together (*m/z* 104.5) have 24.6% relative abundance.

Mass spectra of derivatives **1a**,[10–13] **2b**,[11] **1c**[28] and **2a**[18] have been discussed elsewhere.

Cyclic esters of 4-methyl-3-nitrobenzeneboronic acid (3b and 3c)

Normal and linked scan spectra of compound **3b** have been published[26] with reference to the ambiguities attendant on assignment of fragmentation pathways by metastable ion studies.

As expected for the 2-nitrotoluene functionality[29,30] an *ortho* effect dominates the mass spectra (Scheme 5) and nearly all fragmentation proceeds through the highly abundant $[M-HO^{\cdot}]^{+}$ ion at *m/z* 204. Its *B/E* linked scan spectrum, obtained during GC/MS of compound **3b**, is reproduced here (Fig. 3) for comparative purposes. In the linked scan spectrum of compound **2b** (Fig. 2), the precursor (molecular) ion is seen to eject 28 (CO or C_2H_4), 29 ($HCO^{\cdot}$ or $C_2H_5^{\cdot}$) and 58 (C_3H_6O) u, yielding ions at *m/z* 148, 147 and 118 respectively. The ejected neutral species necessarily orginate from the dioxaboran ring and all three peaks are small. For the $[M-HO^{\cdot}]^{+}$ ion of compound **3b** (Fig. 3), the loss of 58 u gives a similarly small peak (*m/z* 146) and indicates, not surprisingly, the same origin for the C_3H_6O ejected. However, the peaks due

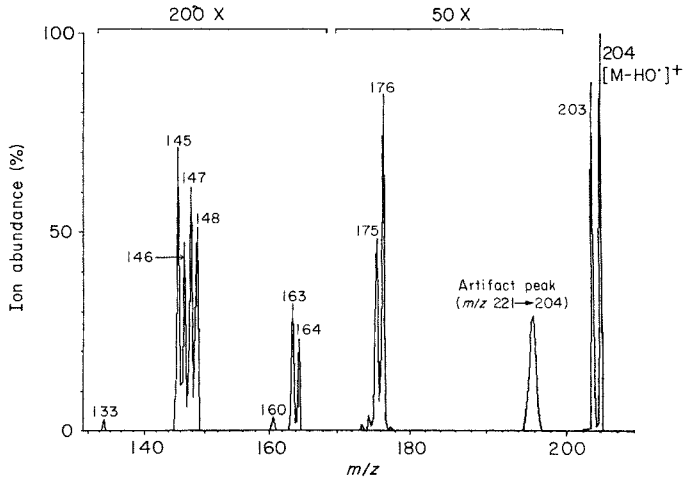
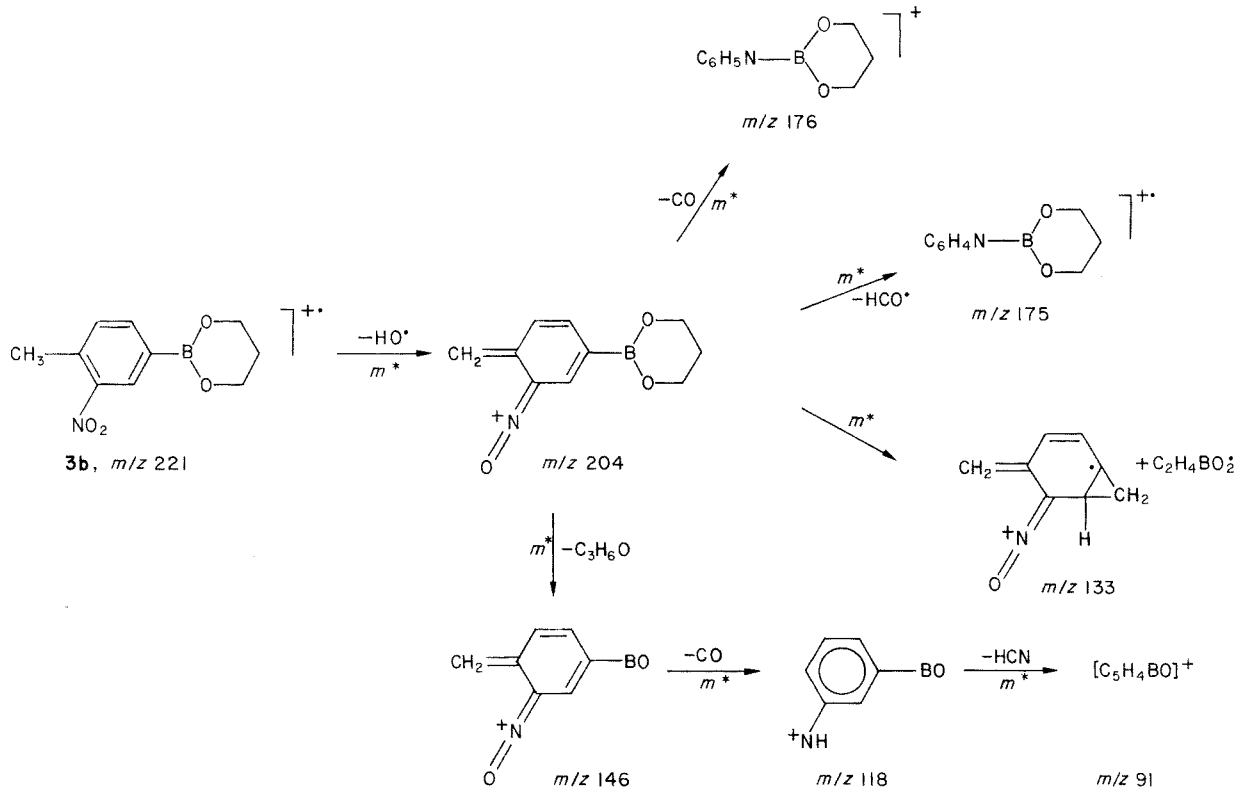


Figure 3. *B/E* linked scan spectrum of the $[M-HO^{\cdot}]^{+}$ ion of compound **3b** recorded during GC/MS.



Scheme 5

to losses of 28 and 29 u (*m/z* 176 and 175) are much larger than the corresponding ones for compound **2b** and this suggests a different origin for the CO and CHO· (or remotely CH_3N) eliminated from the $[M-HO]^+$ ion of boronate **3b**, viz. the aromatic substituents as drawn in Scheme 5. The ions depicted in Scheme 5 account for the major peaks in the routine mass spectrum with two exceptions: abundant ions $[M-NO_2\cdot]^+$ and $[M-NO_2\cdot-C_3H_6O]^+$ were observed and characterized by accurate mass measurement.[26]

The molecular ion of boronate **3b** has 18% of the abundance of $[M-HO\cdot]^+$, the latter being the base peak of the spectrum.[26] For the aromatic derivative **3c**, the molecular ion constitutes the base peak (Fig. 4)

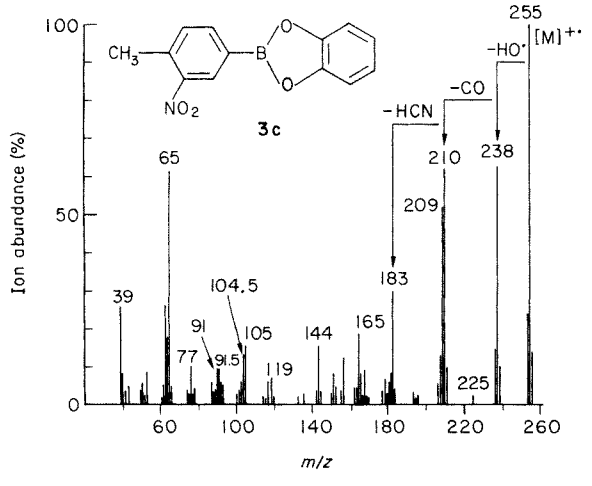
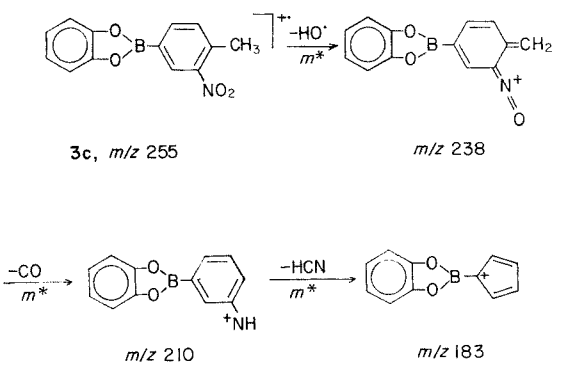
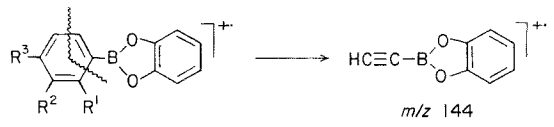


Figure 4. Routine mass spectrum of boronate **3c** recorded during GC/MS. All peaks greater than 2% rel.ab. are shown.



Scheme 6

and the well-characterized sequential losses of HO·, CO and HCN of the 2-nitrotoluene grouping (*ortho* effect; see Scheme 6)[29,30] are clear in the spectrum. Primary cleavages of NO· (*m/z* 225) and $NO_2\cdot$ (*m/z* 209) are also observed. As with all derivatives **c**, an ion at *m/z* 144 is observed and may be attributed to cleavage of the aromatic ring attached to boron (Scheme 7). The double charged ions, $[M-HO\cdot]^{2+}$ (*m/z* 119), $[M-HO\cdot-CO]^{2+}$ (*m/z* 105), $[M-NO_2\cdot]^{2+}$ (*m/z* 104.5) and $[M-HO\cdot-CO-HCN]^{2+}$ (*m/z* 91.5), are all abundant (7–15% rel.ab.). Thus,



Scheme 7

the spectrum is highly diagnostic of structural features and readily interpreted.

Propane-1,3-diol 3-amino-4-carboxybenzeneboronate (4b)

This compound affords a very simple mass spectrum (Fig. 5(a)), most of the ion current being carried by the ions of Scheme 8. The well-known proximity effect of *ortho* amino acids,[31] whereby H_2O and HCN are eliminated consecutively, is clear for compound **4b**. As with all dioxaborans, C_3H_6O is ejected from the heterocyclic ring giving rise to a second series of peaks separated by 18 and 27 u as shown in Scheme 8.

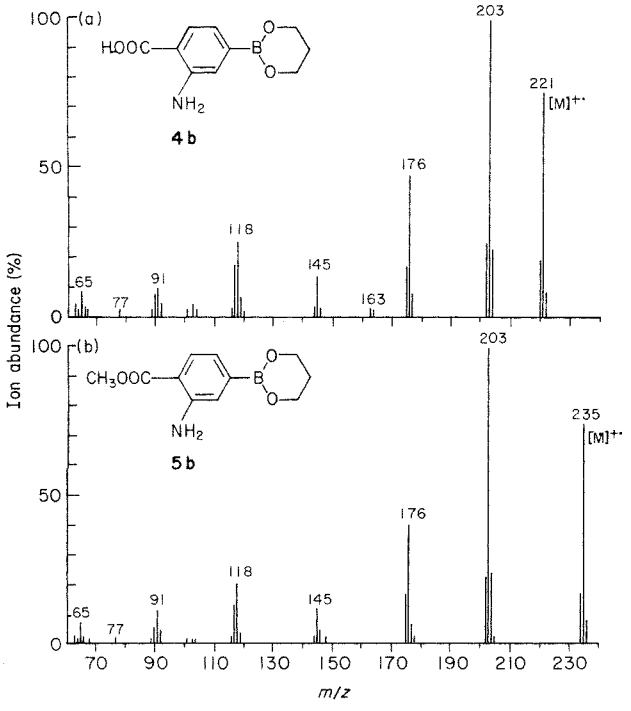
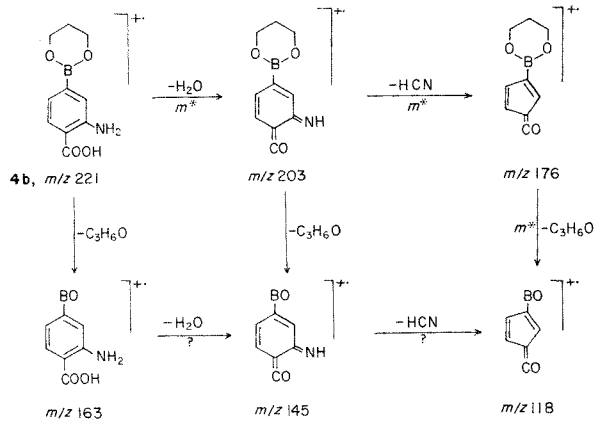


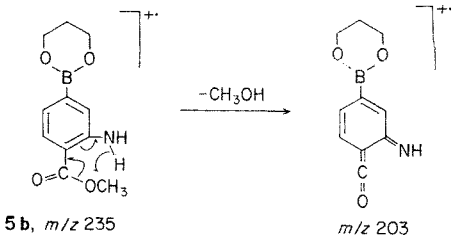
Figure 5. Routine mass spectra of (a) compound **4b** recorded by direct insertion and (b) derivative **5b** recorded during GC/MS. All peaks greater than 2 % rel.ab. are included in the figure.



Scheme 8

Propane-1,3-diol 3-amino-4-carboxymethylbenzeneboronate (5b)

Whilst the acid **4b** affords a readily interpreted mass spectrum, it is not suitable for GC/MS. The methyl ester of the parent boronic acid can be analysed by GC/MS of its cyclic boronate derivative **5b** and this also affords a simple spectrum (Fig. 5(b)). The *ortho* effect is again in evidence inasmuch as methanol is ejected from the molecular ion of compound **5b**. This reaction gives rise to a peak at *m/z* 203 and is represented in Scheme 9. The mass spectra of the acid **4b**

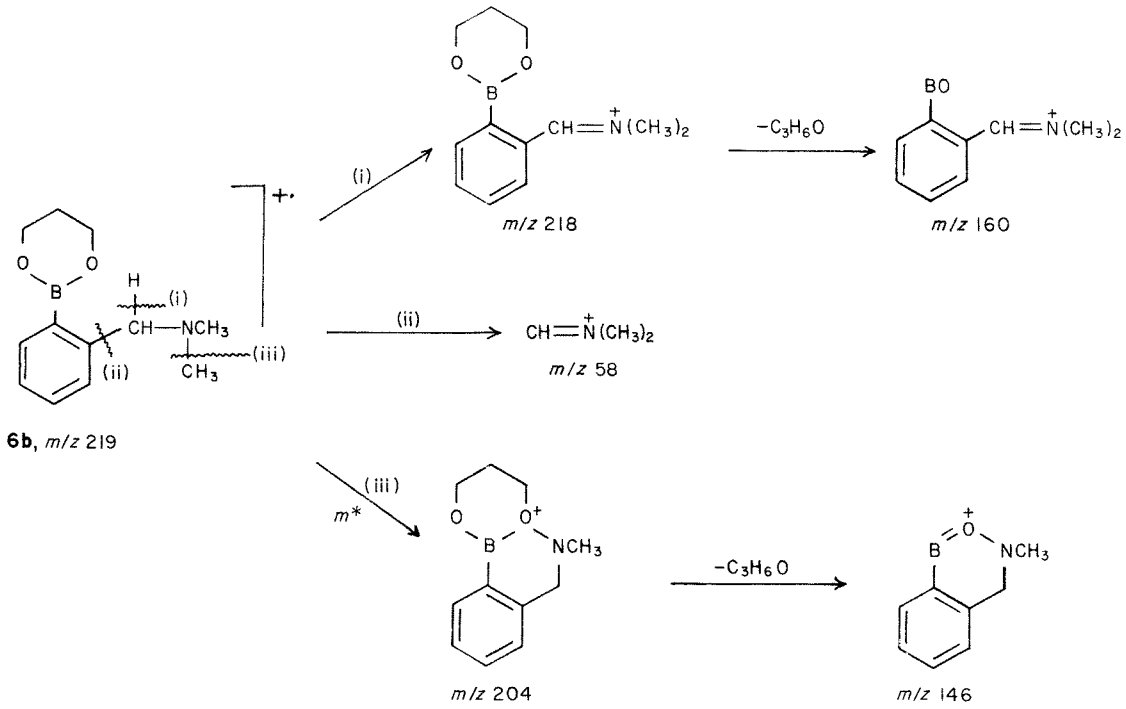


Scheme 9

and ester **5b** are virtually coincident from *m/z* 203 downwards (Fig. 5(a and b)), suggesting the same subsequent decomposition of a common ion. Thus, it is reasonable to draw the ion at *m/z* 203 with the same structure for each compound.

Propane-1,3-diol 2-methylaminomethylbenzeneboronate (6b and 6d)

Consistent with the behavior of *N*-dimethylbenzylamine, the molecular ion of compound **6b** undergoes expected α-cleavages to yield $[M-H]^+$ ions and $CH_2=\overset{+}{N}(CH_3)_2$ as shown in Scheme 10 and Fig. 6(a). Of more interest is the unusually facile cleavage of the C—N bond to eject a methyl radical and afford an ion at *m/z* 204. *N*-Dimethylbenzylamine does not yield significant $[M-CH_3^{\cdot}]^+$ ions, so it may be supposed that in compound **6b** the neighbouring boronate group facilitates the cleavage, possibly in stabilizing the resulting ion as indicated in Scheme 10. The only significant metastable ions for this compound correspond to losses of 15 and 91 u from the molecular ion. The latter process is similarly noteworthy and again attributable to an *ortho* effect. A possible genesis and structure for this ion are shown in Scheme 11. The initial $H^{\cdot}$-transfer is common to the McLafferty rearrangement, which itself results in ejection of $CH_2{=}NCH_3$ and to an ion at *m/z* 176. However, it is proposed that an alternative outcome is possible leading to ejection of $C_7H_7^{\cdot}$ as a result of interaction between *ortho* substituents (Scheme 11). The nature of the resulting ion at *m/z* 128 was supported by isotope labelling. In the mass spectrum of the tetradeuteriated derivative **6d**, the peak was shifted to *m/z* 132 (Fig. 6(b)). Ions containing the dioxoboran ring commonly eject C_3H_6O (58 u) and for compound **6b**, this fragmentation is in evidence (*m/z* 128 → *m/z* 70). For the labelled compound **6d** the equivalent process leads to ejection of $C_3H_2D_4O$ (62 u) from *m/z* 132, also to give



Scheme 10

m/z 70 (see Fig. 6(a and b)). Ejection of C_3H_6O and $C_3H_2D_4O$ occur also from $[M-H]^+$ ions of derivatives **6b** and **6d**, respectively, affording an ion at m/z 160 which like m/z 70, does not shift upon deuterium labelling. These data argue strongly for the novel *ortho* effect as illustrated in Scheme 11.

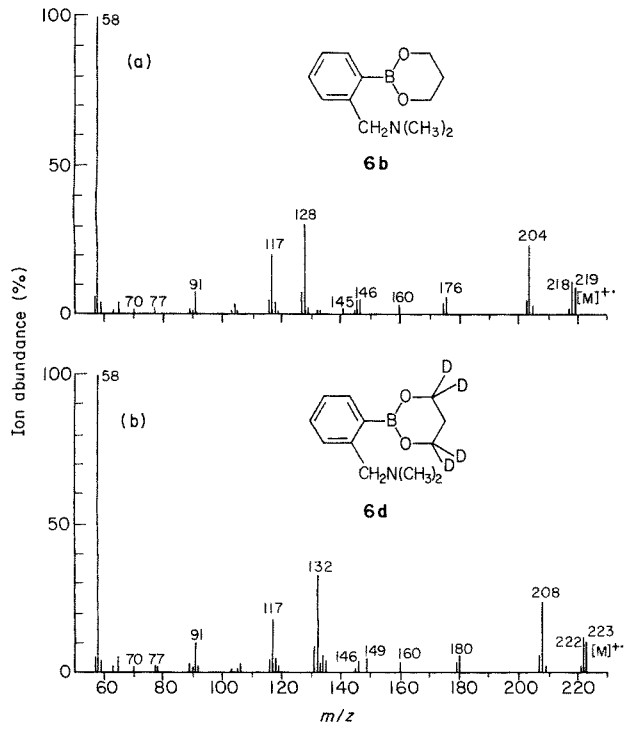
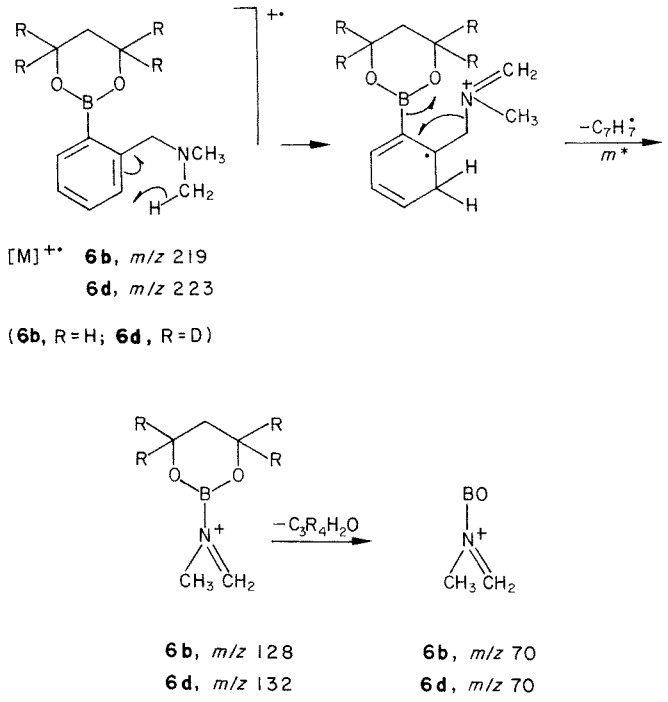


Figure 6. Routine mass spectra of compound **6b** (a) and its deuterium labelled analogue **6d** (b) recorded during GC/MS. All peaks over 1.5 % rel.ab. are shown.



Scheme 11

Propane-1,3-diol nitrobenzeneboronates (7–9b, 9d)

Nitration of benzeneboronic acid leads to a mixture of mainly 2- and 4-nitrobenzeneboronic acids. After cyclic boronate formation, this mixture can be analysed by GC/MS. The mass spectrum of the *para* isomer **7b** displays the normal behaviour[32a] of the nitro group in that peaks are observed corresponding to $[M]^{+\cdot}$ (m/z 207), $[M-O]^{+\cdot}$ (m/z 191), $[M-NO^{\cdot}]^+$

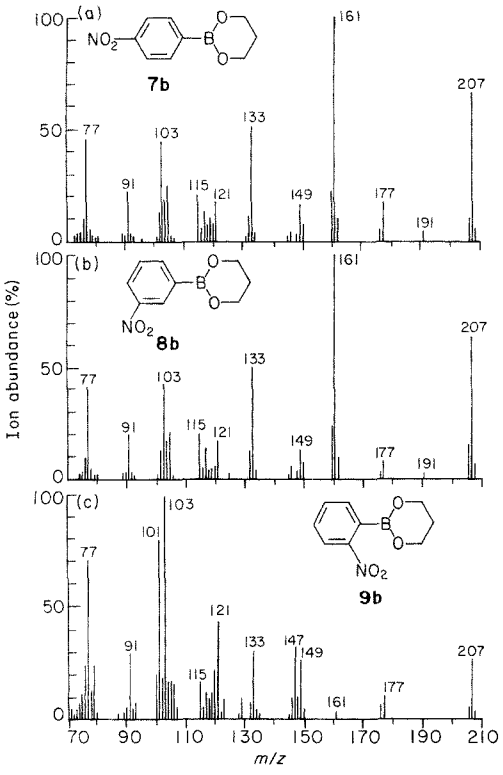
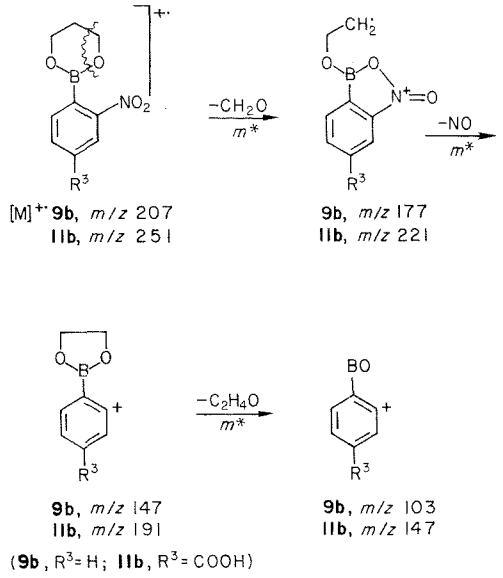


Figure 7. Partial routine mass spectra of (a) *p*-, (b) *m*- and (c) o-nitrobenzeneboronates of propane-1,3-diol. Peaks of more than 2% rel.ab. are shown.

(m/z 177), $[M-NO_2^{\cdot}]^+$ (m/z 161) and $[M-NO^{\cdot}-CO]^+$ (m/z 149) as seen in Fig. 7. These ions are accompanied by standard cleavages of the boronate group as shown by examination of the deuteriated analogue **7d**. It was noted that both derivatives **7b** and **7d** afforded $[M-58]^+$ peaks, thus showing that the ion involved was $[M-NO^{\cdot}-CO]^+$ and not $[M-C_3H_6O]^{+\cdot}/[M-C_3H_2D_4O]^{+\cdot}$. The peak due to $[M-C_3H_2D_4O]^{+\cdot}$ in the mass spectrum of **7d** was relatively small. The *meta* isomer **8b** gave a routine mass spectrum virtually identical to that of the *para* isomer **7b** with a base peak due to $[M-NO_2^{\cdot}]^+$ ions. On the other hand, the mass spectrum of propane-1,3-diol 2-nitrobenzeneboronate **9b** showed very few ions indicative of the standard behaviour of the nitro group (Fig. 7). For example, the $[M-NO_2^{\cdot}]^+$ ion at m/z 161 was only 3.5% rel. ab. The different behaviour exhibited when $R^1 = NO_2$ is a result of *ortho* effects competing successfully with direct cleavages of the nitro group. Two novel *ortho* effects between the boronate ester and nitro group are apparent from Fig. 7(c) and are rationalized in Schemes 12 and 13. The $[M-30]^+$ peak at m/z 177 was due to ejection of CH_2O, not $NO^{\cdot}$ as in the case of isomers **7b** and **8b**, from the molecular ion. (In compound **9d**, CD_2O was ejected and $[M-NO]^+$ ions were not observed.) Since derivatives **b** otherwise show negligible loss of CH_2O, its observation here is ascribed to participation by the neighbouring NO_2 (Scheme 12). Similar interactions have been proposed[33,34] to account for unusual carbon–boron bond cleavage in the cyclic boronates of

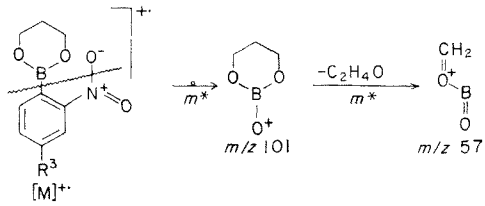


Scheme 12

sugars. It is thought that acetate and phosphate groups anchimerically assist bond cleavage at boron, affording ions in which boron is bonded intramolecularly to appropriately orientated acetate or phosphate functions through their oxygen atoms. For compound **9b**, subsequent loss of $NO^{\cdot}$ affords a peak at m/z 147 which is not present in the mass spectra of the *meta* and *para* isomers **7b** and **8b** (Fig. 7). Further fragmentation by loss of C_2H_4O, supported by observation of the appropriate metastable ion, leads to the base peak of the spectrum at m/z 103.

The second, unique *ortho* effect of compound **9b** is illustrated in Scheme 13 ($R^3 = H$) and is supported by metastable ion data and isotope labelling. The large peak at m/z 101 (m/z 105 for the $[^2H_4]$ compound, **9d**) is attributed to transfer of an oxygen atom from the nitro group to the cyclic boronate ester grouping and cleavage with charge retention on the aliphatic product. Loss of C_2H_4O from the rearrangement ion at m/z 101 affords a peak at m/z 57 (Scheme 13). This fragmentation pathway (Scheme 13) is not an accessible one for isomers **7b** and **8b** because the boronate and nitro groups are spatially separated (Fig. 7).

Isotope labelling showed that the $[M-58]^{+\cdot}$ peak of compound **9b** was comprised mainly of $[M-CH_3BO_2]^{+\cdot}$ with only few $[M-C_3H_6O]^{+\cdot}$ ions. Again, this is not common in derivatives **c** and may be a further example of neighbouring group participation.



Scheme 13

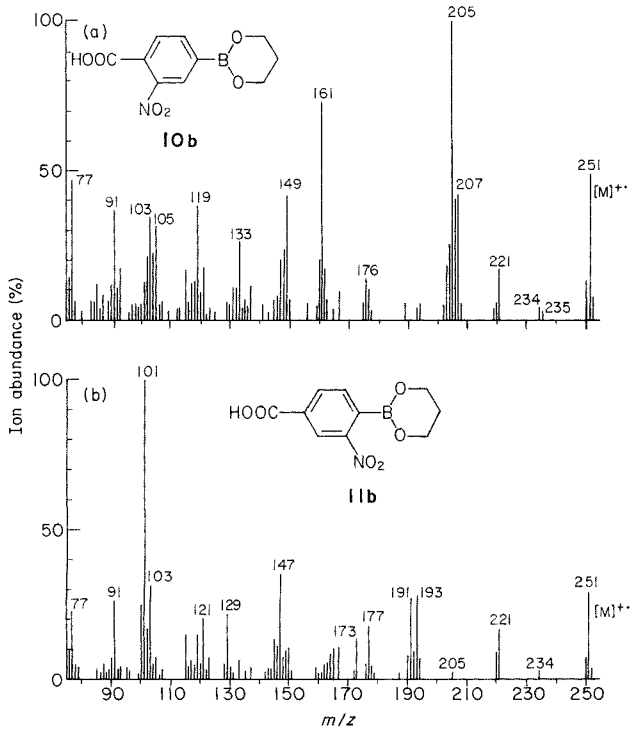


Figure 8. Direct insertion mass spectra of cyclic boronates **10b** (a) and **11b** (b). All peaks over *m/z* 75 and greater than 2 % rel.ab. are included.

Propane-1,3-diol carboxynitrobenzeneboronates (10b and 11b)

Oxidation of boronic acid (**3**) leads to 4-carboxy-3-nitrobenzeneboronic acid (**10**). The mass spectrum of derivative **10b** was compared with that of the isomeric species **11b** (Fig. 8). As with the simple nitrobenzeneboronates, the spectra show considerable differences, attributable to neighbouring group effects.

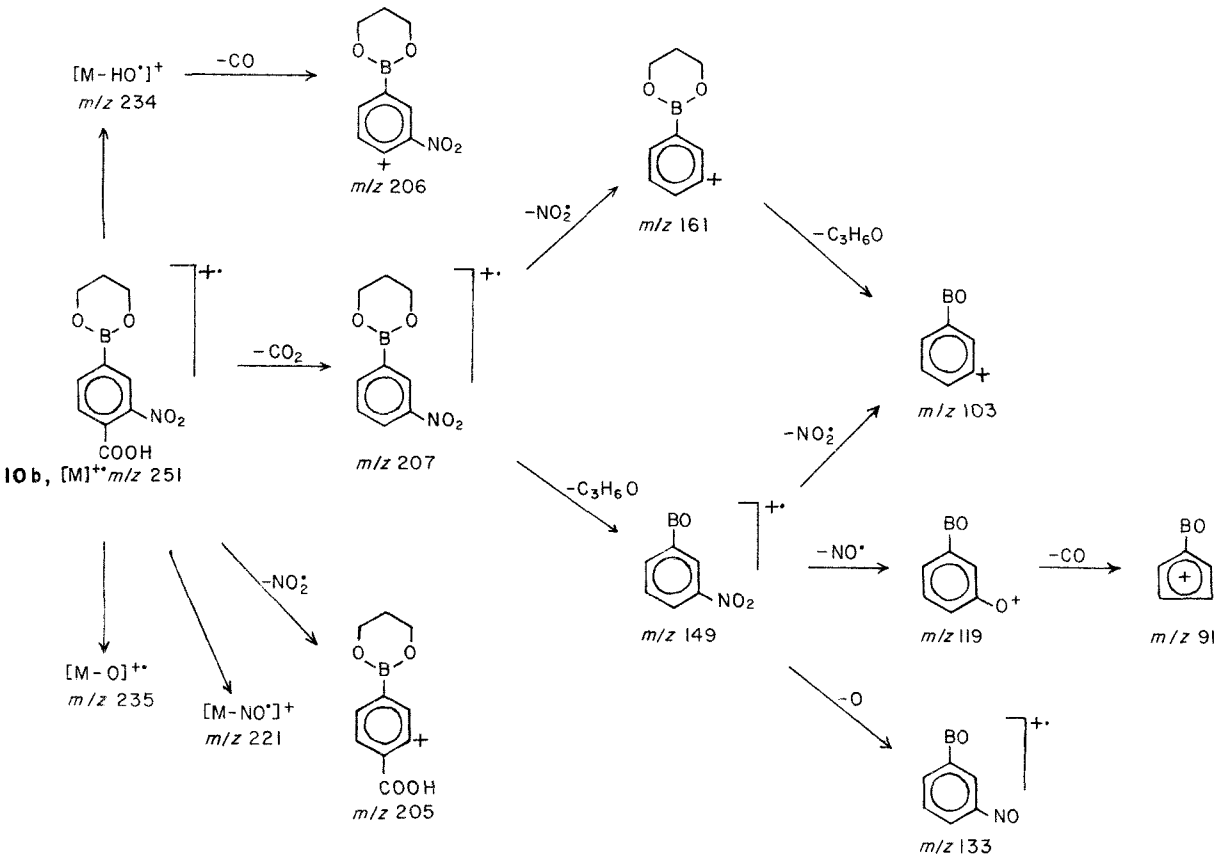
The two new *ortho* effects reported above for the 2-nitrobenzeneboronate derivative are observed also for the analogous 4-carboxy-2-nitrobenzeneboronate as shown in Schemes 12 and 13 ($R^3 = COOH$). For the acid **11b** the rearrangement ion at *m/z* 101 gives rise to the base peak of the spectrum (Fig. 8(b)) and its elemental composition was confirmed by accurate mass measurement (*m/z* 101.0413; required for $[C_3H_6BO_3]^+$, 101.0410). Similarly, by high resolution study the $[M-30]^{+\cdot}$ and $[M-60]^+$ ions were confirmed to be due to $[M-CH_2O]^{+\cdot}$ and $[M-CH_2O-NO^{\cdot}]^+$ ions. The fragmentation pathway shown in Scheme 12, which receives some support[26] from metastable ion data, is proposed to account for these observations. Accurate mass measurement also proved that the $[M-58]^{+\cdot}$ ions at *m/z* 193 were comprised mainly of $[M-CH_3BO_2]^{+\cdot}$ ions, like those of the analogue **9b**. The rearrangement that affords $[M-CH_3BO_2]^{+\cdot}$ ions may well be facilitated by a third *ortho* effect of the boronate and nitro groups.

As seen in Fig. 8(a), the isomeric 4-carboxy-3-nitrobenzeneboronate derivative **10b**, in which the nitro group and boronate ester function are well separated, does not afford important peaks at *m/z* 101 or *m/z* 191 ($[M-CH_2O-NO^{\cdot}]^+$). Furthermore, its $[M-30]^+$ ion at *m/z* 221 corresponds only to $[M-NO^{\cdot}]^+$. As with the simple nitrobenzeneboronates (**7–9**), the 'normal' behaviour of the nitro group is observed only when that group is spatially removed from the boronate grouping. In compound **10b** this 'normal' behaviour is manifest as $[M-O]^{+\cdot}$, $[M-NO^{\cdot}]^+$ and $[M-NO_2^{\cdot}]^+$ ions, the last affording the base peak as it does for the 3- and 4-nitrobenzeneboronates (**7b** and **8b**). (The $[M-NO_2^{\cdot}]^+$ ion of isomer **11b** comprises less than 3% rel. ab.). Whilst isolated aromatic carboxyl groups eliminate $HO^{\cdot}$ and $(HO^{\cdot}+CO)$ after electron impact, as evidenced by the mass spectrum of benzoic acid,[32b] this fragmentation is to some extent suppressed if neighbouring NO_2 or COOH groups occur when CO_2 is lost. For example, 2-nitrobenzoic acid shows a prevalent $[M-44]^{+\cdot}$ peak owing to ejection of CO_2. In accord with this, only the isomer **10b** with *ortho* carboxy and nitro groups eliminates carbon dioxide from its molecular ion to yield an ion at *m/z* 207 (Fig. 8). The major peaks in the mass spectrum of compound **10b** are rationalized in Scheme 14.

Mixture analysis and linked scanning during GC/MS

The analysis of mixtures of boronic acids is illustrated here with reference to a mixture containing isomeric derivatives **12b** and **13b**. Several other mixtures, such as that from nitration of benzeneboronic acid and containing unreacted starting material and 2- and 4-nitrobenzeneboronic acids, were analysed successfully in the same way by GC/MS with packed and/or capillary columns.

Since free carboxylic acids are not suitable for gas chromatography, the methyl esters of the carboxynitrobenzeneboronic acids **10** and **11** were prepared. The resulting esters were derivatized with propane-1,3-diol and the reaction mixture analysed directly. The compounds were separated readily by GC and analysed by repetitive linked scanning at constant *B/E* for unimolecular decompositions of the molecular ions (*m/z* 265 for both compounds). The resulting total ion current trace is shown in Fig. 9 and two major peaks are clear. The linked scan spectra of each peak are given (Fig. 10). Precursor ion resolution is poor due to the wide 'energy window' of the electric sector of the instrument used (±0.5%). Thus, the precursor ion beam contains some molecular ions with ^{10}B (*m/z* 264) and ^{13}C (*m/z* 266) isotopes. After correction for these isotope contributions, the two spectra can be presented as in Table 2. The 'normal' behaviour for an aromatic nitro-ester is elimination from the molecular ion of $NO_2^{\cdot}$ and $CH_3O^{\cdot}$. These two processes are observed for the second-eluting substance which is hence assigned to the 3,4-disubstituted species **13b**. The 2,4-disubstituted isomer **12b** shows fragmentations typical for an *o*-nitrobenzeneboronate ester. Ejection of 30 u from the molecular ion probably corresponds to $[M]^{+\cdot} \rightarrow [M-CH_2O]^{+\cdot}$ (Scheme 12, $R^3 = COOCH_3$). Additionally, formation of $[M-CH_3BO_2]^{+\cdot}$ at *m/z* 207 is similarly characteristic for



Scheme 14

the neighbouring boronate and nitro groups (cf. compounds **9b** and **11b** above). The most striking *ortho* effect in such molecules is the formation of $[C_3H_6BO_3]^+$ (*m/z* 101) by transfer of an oxygen from the nitro group to the boron ring (Scheme 12). In fact, this species constitutes the base peak of the routine spectrum of compound **12b**. However, this rearrangement ion was not formed significantly in the 1st FFR, but the process $[M]^{+\cdot} \rightarrow [M-101]^+$ was observed. That is, the rearrangement occurred with the charge retained instead on the aromatic moiety. Ejection of methoxy radical is much less favoured for this isomer, presumably because the *ortho* effects compete effectively. Thus, the qualitatively different linked scan spectra are highly diagnostic of the isomeric structures.

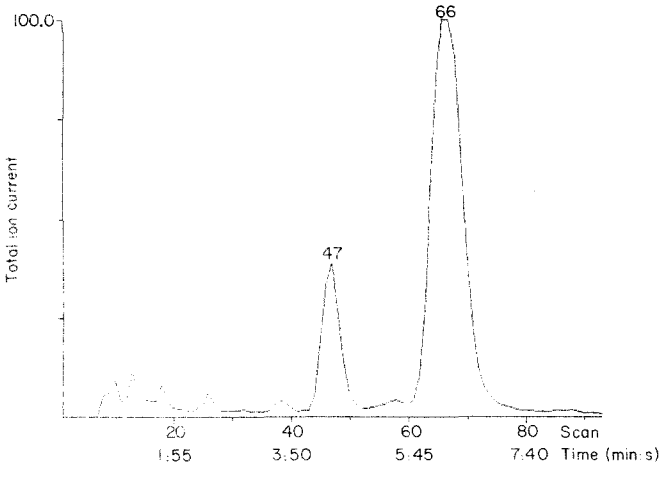


Figure 9. Total ion current chromatogram obtained by repetitive linked scanning at constant *B/E* for all product ions of *m/z* 265, the molecular ion of the esters **12b** and **13b**.

The example discussed illustrates the feasibility and utility of recording full linked scan spectra during GC/MS. The technique employs the power of GC to separate, and of metastable ion studies to probe the structures of, isomeric compounds. The scan rate employed for this experiment was 4 s decade^{-1} and no attempt was made to increase the rate since, with a packed column, it provided six or more linked scan spectra over each GC peak. Any distortion of ion abundance due to changing sample concentration during the scan is thereby kept within acceptable limits. The problem of changes in mass spectra due to varying total ion current during GC is not unique to linked scanning; it has not significantly inhibited conventional GC/MS. If necessary, the ion abundances can be corrected computationally to steady pressure conditions by reference to the recorded variation in total ion current. The difference between linked and routine scanning situations is that the major limitation on scan speed for the former is probably not instrumental but due to ion statistics. The number of decompositions occurring in the 1st FFR, and hence the number of detectable ions, can be increased by use of collisional activation.[19,20] Thus, the use of a collision gas is expected to allow an improvement in scan speed.

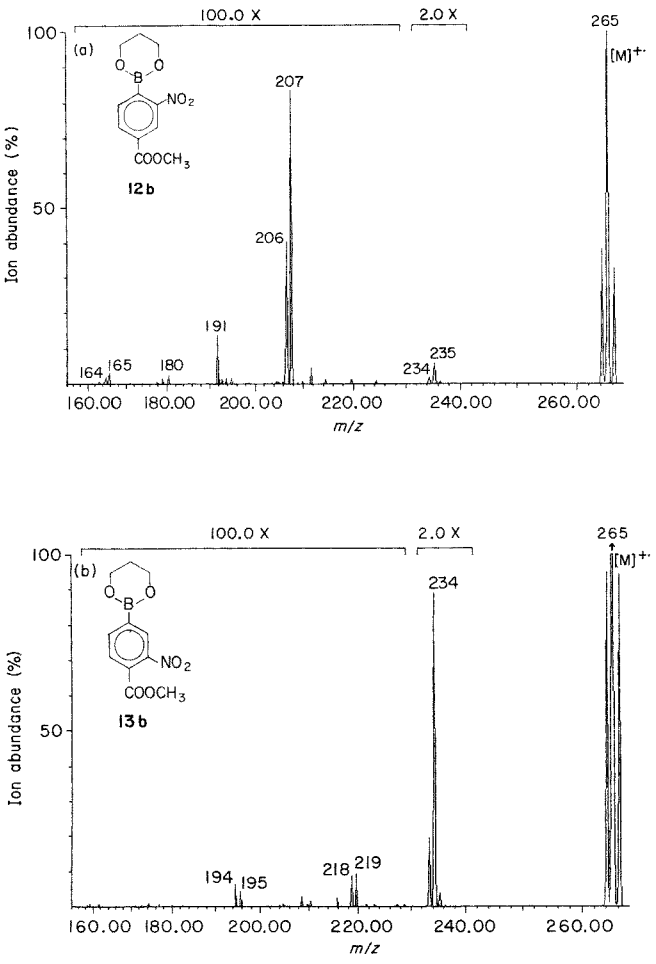


Figure 10. *B/E* linked scan spectra obtained from the GC/MS analysis shown in Fig. 9; (a) spectrum of the first eluting component (scan number 47) due to compound **12b**; (b) spectrum of the second eluting component (scan number 66) due to compound **13b**.

Table 2. Normalized *B/E* linked scan spectra obtained during GC/MS of a mixture of cyclic boronates 12b and 13b (corrected for boron isotopes where appropriate)

Compound	Product m/z values	Mass lost	Probable species ejected	% rel.ab.[a]
12b	235	30	CH_2O	3.10
	234	31	$CH_3O^{\cdot}$	0.10
	207	58	CH_3BO_2	0.80
	206	59	$CH_4BO_2^{\cdot}$	0.41
	191	74	$C_2H_7BO_2$	0.14
	180	85	$C_3H_6BO_2^{\cdot}$	0.02
	179	86	$C_3H_7BO_2$	0.01
	165	100	$C_3H_5BO_3$	0.03
	164	101	$C_2H_6BO_3^{\cdot}$	0.02
13b	234	31	$CH_3O^{\cdot}$	15.3
	219	46	$NO_2^{\cdot}$	0.03
	218	47	HNO_2	0.02
	195	70	$C_2H_3BO_2$?	0.01
	194	71	$C_2H_4BO_2$?	0.02

[a] Relative to precursor ion at *m/z* 265 (100%). All peaks of rel. ab. 0.01% or more are tabulated.

CONCLUSIONS

(1) Boronic acids may be analysed by direct probe or by GC/MS following a simple derivatization with propane-1,3-diol or 1,2-dihydroxybenzene. The resulting mass spectra are readily interpreted.

(2) The cyclic boronate functionality interacts strongly with any *ortho* substituents. Other workers[9] have reported that cleavage of a halo substituent assumed importance only when it occupied the position *ortho* to the cyclic boronate function. The carbon–boron bond of cyclic boronates is cleaved only infrequently, presumably because of the instability of gaseous cyclic borenium ions.[9,28] Yet it is thought that when an electron-rich functionality is able to interact intramolecularly with the electron-deficient boron atom, carbon–boron bond cleavage can occur.[9] Such interaction has been proposed to account for facile carbon–boron bond cleavages in the cyclic boronates of sugar acetates,[33] sugar phosphates[34] and sphingosine *N*-dimethylaminomethylene derivatives,[35,36] the electron-rich groups being acetate, phosphate and *N*-dimethylaminomethylene respectively. The argument receives strong support from the observation here of ions resulting from the actual migration of an oxygen atom derived from a nitro group (Scheme 13) and of the $CH_2{=}NCH_3$ unit derived from the dimethylamino group (Scheme 11) to the electrophilic boron as the carbon–boron bond is broken. The interaction between the nitro group and the boron proposed in the first step of Scheme 12 is of the same type.[33–36]

(3) Selected metastable ion monitoring during GC is a well-known and selective technique for quantification of organic substances such as steroids[37] and cannabinoids.[38] For these analyses, the analyser is static. This paper is the first publication to describe computerized acquisition of full linked scan spectra during GC/MS with a magnetic sector instrument, a technique which permits separation and mass spectral differentiation of isomeric substances in a mixture. The technique is under active investigation in these laboratories also for analysis of mixtures of isomeric steroids.

EXPERIMENTAL

Materials

The boronic acids were synthesized towards production of selective ligands for affinity chromatography. Their synthesis will be the subject of future publications from this department. Ethane-1,2-diol, propane-1,3-diol and 1,2-dihydroxybenzene were laboratory reagent grade, commercial samples. [1,1,3,3-$^2H_4$]Propane-1,3-diol was prepared by reaction of malonic acid with lithium aluminium deuteride in dry ether. For derivatization of the boronic acids, an equimolar amount or excess of a solution of the diol (0.5 mmol ml^{-1} in ethyl acetate or ethyl acetate/pyridine (1:1)) was added to the boronic acid, present at the nanogram–microgram level. After standing at room temperature for a few minutes (reaction was complete

within 30 min), an aliquot of the reaction mixture was subjected directly to mass spectrometric analysis.

Methods

The mass spectrometer employed was a VG Micromass 7070F (conventional geometry, double focusing instrument) coupled to a Pye Unicam 204 gas chromatograph and a Finnigan Incos 2300 data system. Accelerating voltage, 4 kV; emission current, 200 μA; electron beam energy, 70 eV; ion source temperature, *c.* 200 °C. Direct probe analyses were carried out at a resolution of 800 or 5500. The temperature of the sample was increased gradually by passing the probe slowly into the hot ion source whilst monitoring the ion current due to the excess of diol. This formed a simple and effective means of separating any excess of diol from the less volatile boronate esters.

Studies by GC/MS employed repetitive scanning and (i) 3% OV-17 column at temperatures between 145 and 240 °C, (ii) 3% OV-1 column at temperatures between 130 and 220 °C, (iii) OV-101 wall-coated open tubular column (20 m) at 150–200 °C and (iv) SE-30 support-coated open tubular column (40 m) at 230 °C. The carrier gas was helium at a flow rate of 40 ml min^{-1} for the packed columns and a pressure of 5 or 10 lb in^{-2} for the 20 m and 40 m capillary columns, respectively. The GC/MS interface was a jet separator for the packed columns and a direct coupling via glass-lined tubing for the capillary columns.

Metastable ion detection was performed during GC/MS with either of the packed columns by linked scanning such that the magnetic flux (B) and voltage (E) of the electric sector remained constant throughout the scan.[27] This type of scanning was achieved through standard hardware from VG Analytical Limited, based on a Hall effect sensor for measuring the magnetic field. The data system was used to control repetitive scanning at constant B/E with total scan cycle times of 5–6 s.

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