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The use of boronate derivatives in the characterization of catecholamines and related β-hydroxy-amines by gas liquid chromatography-mass spectrometry

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The gas chromatographic separation of various β-hydroxy-amines in the form of derived 1,3,2-oxazaborolidines is complemented by a mass-spectrometric study of cyclic boronates of natural catecholamines. These derivatives and their synthetic analogues yield characteristic mass spectra. Closely similar compounds, such as the n-butylboronates of the diastereoisomers (−)-ephedrine and (+)-ψ-ephedrine, give almost identical spectra but can be readily distinguished by their GLC retention times. The same can be said for the positional isomer pairs synephrine and phenylephrine, and for octopamine and 4-deoxynoradrenaline. Mass-spectrometric fragmentation modes are postulated from a comparison of the shifts in the masses of fragment ions corresponding to various substituents in the group of biological amines studied. Additional correlations are derived from studies of methyl-, cyclohexyl- and phenyl-boronates.

Various methods have been published for the separation of β-hydroxy-amines by gas chromatography (GLC), chiefly in connection with catecholamines and related compounds.

Biological amines of moderate polarity have been separated without prior modification, good resolution being obtained by coating the support with potassium hydroxide (Brochmann-Hansen & Svendsen, 1962; Parker, Fontan & Kirk, 1962; Beckett, Moffat & others, 1967; Beckett, Tucker & Moffat, 1967) or by using high percentages of stationary phase (Zwol, 1966). Other methods usually depend upon the use of derivatives having greatly reduced polarity in order to improve stability and resolution in GLC. Among the modifications described for this purpose are Schiff's base formation (Brochmann-Hansen & Svendsen, 1962; Beckett, Tucker & Moffat, 1967; Beckett & Wilkinson, 1965); silylation of hydroxyl groups coupled with conversion of the amine to a Schiff's base or oxazolidine (Clarke, Wilk & others, 1967; Capella & Horning, 1966; Kawai & Tamura, 1967); silylation of hydroxyl groups and primary amino-groups (Sen & McGeer, 1963; Horning, Moss & Horning, 1967); silylation of hydroxyl groups followed by acetylation on the nitrogen atom (Horning, Moss & others 1968); and acetylation of hydroxyl and amino-groups (Brooks & Horning, 1964). The formation of trifluoroacetates (Kawai & Tamura, 1968), or the silylation of hydroxyl groups followed by formation of *N*-heptafluorobutyryl derivatives (Horning & others, 1968), afford products with good electron-capture properties, thus allowing the detection of catecholamines in very small quantities. Many of the above derivatives are of value in mass spectrometry, especially for the more polar hydroxy-amines. The mass spectra of various parent phenethylamines have been studied

(Teeter, 1966; Reisch, Pagnucco & others, 1968), but have the disadvantage that the molecular ion is of very low abundance in most cases.

Recently we have been examining various cyclic boronates to evaluate their use as derivatives for gas chromatography and mass spectrometry. Among the advantages boronates possess for analytical purposes are (i) the ease of their formation—the reaction taking place in most instances at room temperature without the use of a catalyst; (ii) their selectivity, enabling the proximity of functional groups to be verified; (iii) their potential value for separating diastereoisomers by GLC, as observed with (−)-ephedrine and (+)-ψ-ephedrine (Anthony, Brooks & others, 1969); and (iv) their characteristic mass spectra, usually including molecular ions in appreciable abundance (Brooks, Middleditch & Anthony, 1969). Earlier workers have described the preparative reactions of boronic acids (or their anhydrides) with a variety of bifunctional compounds, including 1,2-diols (Kuivila, Keough & Soboczenski, 1954; Sugihara & Bowman, 1958; Finch & Lockhart, 1962; Bowie & Musgrave, 1963; Ferrier, Prasad & others, 1964; Foster, Haines & others, 1965; Brooks & Watson, 1969) and β-hydroxy-amines (Pailer & Fenzl, 1961; Pribyl, Louis & Bernstein, 1961), to give cyclic derivatives. Thus catechol readily forms boronates (Fig. 1a; R = Cl, Bu^n, Bu^t, Ph, etc.):

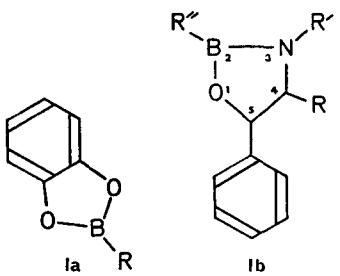


FIG. 1. a. General formula of cyclic boronates derived from catechol. b. General formula of cyclic boronates derived from β-hydroxyphenethylamines.

similarly the phenylboronate (Fig. 1b; R = R′ = Me; R″ = Ph) of (−)-ephedrine has been prepared. It was therefore of interest to explore the use of cyclic boronates in the protection of catecholamines for gas chromatography and in their characterization by mass spectrometry. Boronates of various β-hydroxy-amines, and bisboronates of β-hydroxy-catecholamines have been prepared. Their GLC properties have been found to be satisfactory (Anthony & others, 1969), provided that strongly polar groups are absent. The reactions did not proceed quantitatively under the mild conditions described: the best yields were observed if large substituents were present in the oxazaborolidine ring. Compounds having unsubstituted phenolic groups (e.g. octopamine) gave poorer yields.

EXPERIMENTAL

Combined gas chromatography-mass spectrometry was carried out with an LKB 9000 instrument: the ionizing voltage was 70 eV, source temperature 290° and accelerating voltage 3·5 kV. GLC retention data were obtained on a Carlo Erba "Fractovap GB" gas chromatograph with matching silanized glass U-tube columns (6 ft). The stationary phase was 1% OV-17 on Gas Chrom Q (100–120 mesh). n-Alkanes were used as standards.

The n-butylboronates were prepared by treatment of the β-hydroxy-amine (1 mg), in the form of its free base, hydrochloride, sulphate or tartrate, with n-butylboronic acid (1–1·5 molar equivalents) in pyridine (1 ml) which had been dried and distilled over sodium hydroxide. The free base could be conveniently prepared from the hydrochloride by exposing the pyridine solution of the hydroxy-amine salt to ammonia vapour and separating the precipitated ammonium chloride before derivative formation. For hydroxy-amines, such as isoprenaline sulphate, which were not sufficiently soluble in pyridine, a suitable reaction solvent was dimethylformamide which had been dried by azeotropic distillation with benzene and further distilled over anhydrous sodium sulphate.

In most cases, aliquots of the reaction mixture were injected directly on to the GLC column. In the reactions involving octopamine and 4-deoxynoradrenaline, cyclic derivatives appeared to be formed in low yield, and vacuum sublimation at 250°/0·01 mm Hg was used to separate the derivative (in its free-base form) from non-volatile material.

Mass spectrometry

The cyclic nature of boronate derivatives of bifunctional compounds in some cases directs the mode of mass-spectrometric fragmentation: this is observed for the 2-substituted 1,3,2-oxazaborolidines derived from β-hydroxy-amines. Although the relative intensity of certain fragments is influenced by the substituent on the boron atom, the general mode of breakdown is the same for the methyl-, n-butyl-, cyclohexyl- and phenyl-boronates studied. The present discussion is concerned mainly with n-butylboronates, which have convenient gas-chromatographic properties (e.g. capacity for resolution of diastereoisomers, combined with moderate retention times). It should be noted that certain ions observed in the mass spectra of n-butylboronates evolve from fragmentation of the n-butyl substituent.

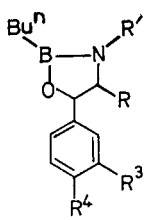
General formula	Parent compound	Substituents			
		R	R′	R^3	R^4
	β-Hydroxyphenethylamine	H	H	H	H
	Norpseudoephedrine	Me	H	H	H
	Phenylpropanolamine	Me	H	H	H
	Pseudoephedrine	Me	Me	H	H
	Ephedrine	Me	Me	H	H
	Octopamine	H	H	H	OH
	4-Deoxynoradrenaline	H	H	OH	H
	Synephrine	H	Me	H	OH
	Phenylephrine	H	Me	OH	H
	Normetanephrine	H	H	OMe	OH
	Metanephrine	H	Me	OMe	OH
	Noradrenaline	H	H	$Bu^n \cdot BO_2$	
	Adrenaline	H	Me	$Bu^n \cdot BO_2$	
	Isoprenaline	H	Pr^i	$Bu^n \cdot BO_2$	
	3,4-Dihydroxynorephedrine	Me	H	$Bu^n \cdot BO_2$	

FIG. 2. Structural formulae of n-butylboronates of β-hydroxy-amines.

Fig. 2 shows the general formula of the 2-n-butyl-1,3,2-oxazaborolidines, together with the substituents in the compounds studied.

Postulated representations of the main fragments from the mass spectra of the n-butylboronates of β-hydroxy-β-arylethylamines are shown in Fig. 3, and the principal ions observed in the compounds studied are listed in Table 1.

Table 1. *Mass spectral breakdown of n-butylboronates of β-hydroxy-β-arylethylamines*

Parent compound	Molecular ion	M/e and type of major fragments*										M/e and type of other diagnostic fragments				
β-Hydroxyphenethylamine	203 (21%)	161 X	91 VI	90VIa	202 III VII	120 VIII	118 IV	89 VIb	146 II	117 V	104 —	174 I (6%)				
Norpseudoephedrine	217 (33%)	202 III	91 VI	90 VIa	160 II	118 IV	216 VII	117 V	175 X	89 VIb	188 I	134 VIII (4%)				
Phenylpropanolamine	217 (26%)	202 III	91 VI	90 VIa	118 IV	160 II	117 V	89 VIb	216 VII	175 X	132 —	188 I (6%)	134 VIII (5%)			
Pseudoephedrine	231 (9%)	216 II	91 VI	132 IV	117 V	90 VIa	89 VIb	230 VII	118 —	105 —	174 II	202 I (2%)	189 X (2%)	148 VIII (1%)		
Ephedrine	231 (10%)	216 III	91 VI	132 IV	117 V	90 VIa	230 VII	174 II	89 VIb	159 —	118 —	189 X (2%)	148 VIII (2%)	202 I (2%)		
Octopamine	219 (100%)	107 VI	218 III VII	134 IV	177 X	133 V	105 VIb	162 II	136 VIII	106 VIa	135 IX	190 I (19%)				
4-Deoxynoradrenaline	219 (100%)	107 VI	218 III VII	134 IV	177 X	133 V	105 VIb	162 II	136 VIII	106 VIa	135 IX	190 I (18%)				
Synephrine	233 (74%)	191 X	232 III VII	107 VI	150 VIII	148 IV	106 VIa	133 V	120 —	105 VIb	176 II	149 IX (13%)	204 I (7%)			
Phenylephrine	233 (84%)	191 X	232 III VII	107 VI	149 IX	148 IV	120 —	133 V	150 VIII	134 —	105 VIb	176 II (21%)	204 I (11%)			
Normetanephrine	249 (100%)	137 VI	248 III VII	219 —	218 —	163 V	232 —	135 VIb	166 VIII	136 VIa	150 —	192 II (16%)	164 IV (15%)	165 IX (15%)	220 I (14%)	207 X (9%)
Metanephrine	263 (100%)	262 III VII	137 VI	180 VIII	179 IX	221 X	163 V	164 —	246 —	146 —	136 VIa	178 IV (19%)	206 II (8%)	234 I (5%)		
Noradrenaline	301 (84%)	300 III VII	189 VI	217 IX	216 IV	218 VIII	215 V	188 VIa	244 II	259 X	272 I					
Adrenaline	315 (71%)	273 X	314 III VII	231 IX	232 VIII	189 VI	230 IV	188 VIa	258 II	202 —	215 V	187 VIb (16%)	286 I (9%)			
3,4-Dihydroxynorephedrine	315 (46%)	300 III	189 VI	314 VII	188 VIa	216 IV	215 V	230 —	273 X	258 II	231 —	286 I (5%)	232 VIII (4%)			

* In order of abundance.

As can be seen in Table 1, the molecular weight was easily determined in each of the compounds studied, as a fairly prominent parent ion was obtained in each case. The substituents on C-4 of the oxazaborolidine ring (hydrogen atom or methyl group) can readily be identified by their loss, principally to give ions of type III (Fig. 3). The ratio of ^{10}B to ^{11}B in this fragment indicates whether one or two molecules of n-butylboronic acid have been incorporated into the molecule. The nature of the substituents on N-3 (hydrogen atom or a methyl group) can be inferred from the transition IV → V (Fig. 3) where the group is eliminated as a radical (the exceptional case of the *N*-isopropyl derivative, isoprenaline butylboronate, is discussed later).

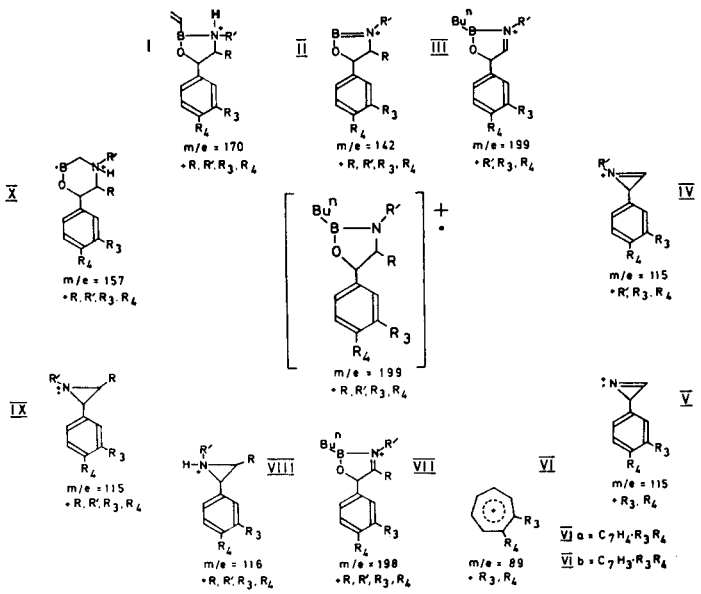


FIG. 3. Postulated representations of the principal fragments from the mass spectrometric breakdown of n-butylboronates derived from β-hydroxyphenethylamines.

Table 2. *GLC retention indices of n-butylboronates of β-hydroxy-β-arylethylamines on* 1% *OV-17*

Parent compound	Temp. °C	Retention Index
β-Hydroxyphenethylamine	140	1799
Norpseudoephedrine	140	1774
Phenylpropanolamine	140	1776
Pseudoephedrine	140	1782
Ephedrine	140	1796
Octopamine	170	2218
4-Deoxynoradrenaline	170	2203
Synephrine	170	2185
Phenylephrine	170	2171
Normetanephrine	190	2315
Metanephrine	190	2270
Noradrenaline	190	2478
Adrenaline	190	2438
3,4-Dihydroxynorephedrine	190	2450
Isoprenaline	190	2512

In the compounds studied, the substituents on the benzene ring are retained in fragments of type V, where the hydroxy-amine side-chains are reduced to a common moiety (C_2H_2N), and of types VI, VIa and VIb, which are hydrocarbon fragments. These relatively prominent ions readily indicate the combined molecular weights of the substituents on the benzene ring (cf. Reisch & others, 1968). Certain other fragments arise from the breakdown of hydroxyl and methoxyl substituents on the benzene ring. Thus synephrine gives an ion at m/e = 216 due to loss of ·OH. Metanephrine gives a similar ion at m/e = 246 and also one at m/e = 232 due to loss of ·OMe.

As noted above, the spectra of n-butylboronates contain, sometimes as major ions, fragments dependent on the presence of the n-butyl substituent. Thus, the ion of type X is the base peak in the spectra of β-hydroxyphenethylamine n-butylboronate, synephrine n-butylboronate and adrenaline bis-n-butylboronate. When this fragment is predominant, the two daughter ions VIII and IX can also be observed. The fragment I appears to arise by loss of Et from the butyl side-chain.

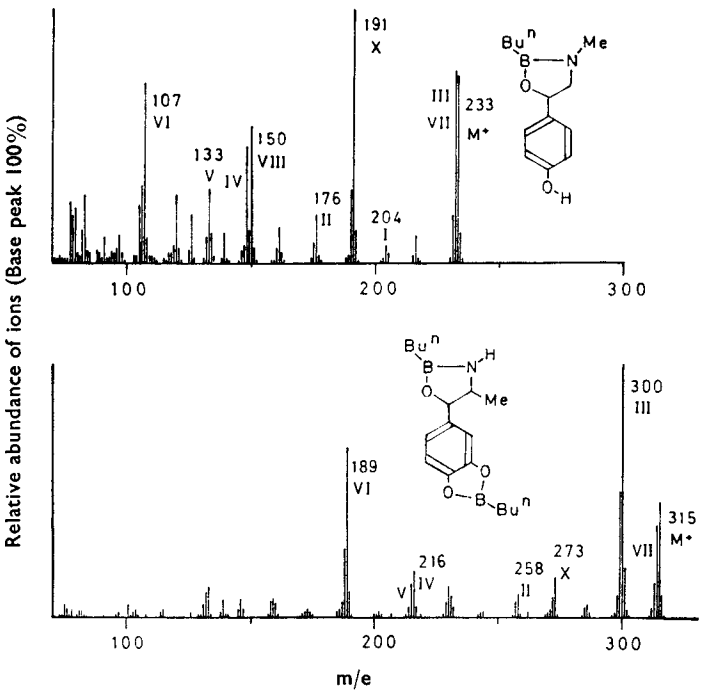


FIG. 4. Mass spectra of n-butylboronates of synephrine and 3,4-dihydroxynorephedrine. Samples were introduced into the mass spectrometer through a 10-ft column packed with 1% OV–17 on Gas Chrom Q. Conditions of measurement were as stated in the Experimental section.

Representative results are depicted in Fig. 4, in which the mass spectra of 3,4-dihydroxynorephedrine bis-n-butylboronate and synephrine n-butylboronate are given and the fragment types indicated.

Isoprenaline n-butylboronate gave only two major fragments. The first (m/e =328) is presumably due to loss of Me from the isopropyl group on the nitrogen atom. The other predominant peak (m/e = 244) is most likely due to further loss of Bu^nBO (Fig. 5).

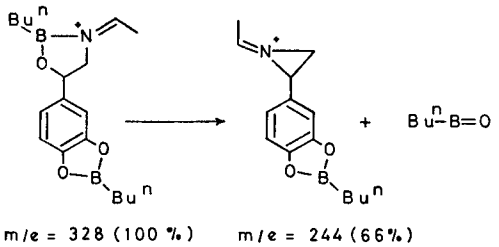


FIG. 5. A postulated fragmentation in the mass spectrometric breakdown of isoprenaline n-butylboronate.

This transition is verified by a metastable peak at m/e = 181·8.

Within the group of compounds studied, substituents in the benzene ring appear to have little effect on fragmentation, which is accordingly insensitive to positional isomerism in the ring. Consequently, n-butylboronates of octopamine and 4-deoxynoradrenaline, which have a free phenolic group at the *para-* and *meta*-position respectively, cannot be effectively distinguished by their mass spectra. Their retention times are, however, different (Table 2).

Conversely, the n-butylboronates of ephedrine and β-hydroxyphenethylamine, which cannot be separated under the conditions used, can be detected in the presence of one another by virtue of their different mass spectra. This is illustrated in Table 3, which shows how the peak positions of the two compounds can be located by means of a multiple scanning technique by measuring the heights of the respective base peaks for each scan.

Table 3. *The effect of multiple scanning GC-MS, for a mixture of ephedrine and β-hydroxyphenethylamine as n-butylboronates* (10 *ft column*, 1% *OV*-17, 130°).

Retention Index of scan	Height of peak (mm) in mass spectrum	
	m/e = 161	m/e = 216
1784	—	30
1786	—	74
1788	1	85
1791	7	57
1793	9	30
1796	9	19
1798	8	10

Gas liquid chromatography

The GLC properties of the boronates of β-hydroxy-amines (Anthony & others, 1969) and 1,2- and 1,3-diols (Brooks & others, 1968) have been examined previously in this laboratory. In the series of β-hydroxyphenethylamines studied, unsatisfactory peaks were obtained in the presence of free phenolic groups, especially for the derivatives of primary amines.

The problem of distinguishing between the diastereoisomers (−)-ephedrine (1*R*,2*S* configuration) and (+)-ψ-ephedrine (1*S*,2*S* configuration) by GLC has been considered frequently in the literature. The methods so far reported (Brochmann-Hansen & others, 1962; Beckett & others, 1965) have been based on chemical conversion of the

isomers by reaction with acetone to the corresponding oxazolidines. Although these two hydroxy-amines, as their boronates, cannot be distinguished by mass spectrometry, we have obtained separation of the n-butylboronates by GLC with a moderately polar column. The difference in retention behaviour was enhanced by using boronates with substituents bulkier than Bu^n on the boron atom. This is illustrated in Table 4.

Table 4. *The effect of different groups on the boron atom* (R″ *in Fig. 1b*) *in resolving the diastereoisomers ephedrine and ψ-ephedrine as their boronate derivatives by GLC*

R″	Temp. (°C)	Retention Index (I) Ephedrine	ψ-Ephedrine	ΔI
Methyl	90	1513	1509	4
n-Butyl	140	1796	1782	14
t-Butyl	130	1680	1669	11
Cyclohexyl	150	2080	2064	16
Phenyl	170	2258	2238	20

Conclusion

Qualitative analysis of catecholamines and related β-hydroxy-amines after reaction with n-butylboronic acid is possible by the combined gas chromatography-mass spectrometry technique. The boronic acid reacts under mild conditions both with the β-hydroxy-amine group to form a 1,3,2-oxazaborolidine ring and with the catechol grouping to form a 1,3,2-dioxaborole ring.

Mass spectrometry gives the molecular weight, indicates the mass of substituents at positions 2 and 4 of the oxazaborolidine ring, and gives the combined molecular weights of substituents on the benzene ring. Diastereoisomers on the oxazaborolidine ring and positional isomers on the benzene ring can be distinguished by GLC by use of a moderately polar stationary phase.

The reaction of n-butylboronic acid with β-hydroxy-amines as described above is not complete, but occurs without a catalyst. The selectivity of the reagent affords a clear distinction by GLC between catecholamines and their methylated analogues (e.g. adrenaline and metanephrine), and between compounds with and without a β-hydroxy-amine grouping.

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