Journal of Chromatography, 204 (1981) 201–205
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,262

QUANTITATIVE ANALYSIS OF DIASTEREOISOMERIC ALKANOLAMINES AS CYCLIC BORONIC ACID DERIVATIVES

G. MUNRO* and L. R. ROWE
Glaxo Group Research Ltd., Ware, Herts. (Great Britain)
and
M. B. EVANS
The Hatfield Polytechnic, Hatfield, Herts. (Great Britain)

SUMMARY

Cyclic boronic derivatives formed by the interaction of bi-functional compounds and organo-boronic acids are commonly used to facilitate the gas–liquid chromatographic (GLC) analysis of diols, amino-alcohols and hydroxy compounds with adjacent primary amide moieties. When the phenylalkanolamines salmefamol and labetalol were converted to their bis-boronate derivatives irreproducible quantitative results were obtained by GLC. Because these results raise serious doubts concerning the suitability of boronic acid derivatives for quantitative analysis the factors affecting the reproducibility of the quantitative data have been investigated.

INTRODUCTION

Salmefamol, a long acting bronchodilator[1], and labetalol, a new type of anti-hypertensive drug[2] which both possess in their molecules two asymmetric centres, normally exist as diastereoisomeric mixtures, consequently a sensitive method for the determination of the isomeric composition of both drugs was required for quality control purposes.

The separation of diastereoisomers is highly dependent upon molecular structure[3,4], in particular it has been shown that separation can be enhanced by incorporation of part of the molecule into a cyclic system or the introduction of bulky groups on or near to asymmetric centres.

Cyclic boronic esters formed by interaction of bi-functional compounds and organo-boronic acids are commonly used to facilitate the gas–liquid chromatographic (GLC) analysis of diols[5], amino alcohols[6] and hydroxy compounds with adjacent primary amide moities[7]. The present communication is concerned with the conversion of both salmefamol and labetalol into rigid five-membered rings containing boron.

EXPERIMENTAL

Reagents

Organo-boronic acids and organo-boron dihalides were obtained from commercial suppliers and used as supplied.

0021-9673/81/0000–0000/$02.50 © 1981 Elsevier Scientific Publishing Company

Equipment

Gas chromatograms were obtained using a Perkin-Elmer F-30 gas chromatograph equipped with glass columns and flame ionisation detection. The columns, 2 or 4 m × 1.75 mm I.D., were packed with mixtures of stationary phase and support phase (for details see the text). Nitrogen was used as carrier gas. Solutes for analysis were introduced as dilute solutions by means of SGE micro-syringes via a serum cap.

Preparation of derivatives

Compound (10 mg) and methyl, *n*-butyl or phenyl boronic acid (100 mg) in anhydrous dimethylformamide (0.5 cm^3) were allowed to stand at approximately 20°C for 20 min. Aliquots of 1.0 mm^3 were taken for GLC analysis.

RESULTS AND DISCUSSION

When salmefamol and labetalol were converted to rigid five-membered rings containing boron, excellent separation of the individual diastereoisomers was observed. However the reproducibility of the isomer ratios was found to be poor, as illustrated by the repetitive analysis in Table I, and the total yield of derivative was inconsistent[8]. As these results raise serious doubts concerning the suitability of boronic acid derivatives for quantitative analysis, factors affecting the reproducibility of quantitative data have been investigated.

Possible causes of the irreproducibility of boronate analyses could include:

(1) Different rates of reaction between the individual diastereoisomers and the boronic acid.

(2) Thermal instability of one or both of the derivatives.

(3) Hydrolysis of one or both of the derivatives prior to or during chromatography.

(4) Reaction with excess reagent to form involatile products.

(5) Chemisorption of the solute due to reaction with organo-boronic acid bound to the stationary phase.

Differential reaction rates, thermal instability and hydrolysis were shown to be unlikely because satisfactory quantitative results were obtained on freshly packed columns (see Table II), furthermore heating derivatives in solution provided no evidence of decomposition and the addition of the water scavenger, isopropenylacetate, produced no improvement of results obtained from serial injection.

The possible effects of excess boronic acid deposited onto the column packing upon the chromatography of boronate esters were investigated by the use of:

(1) Cyclic boron derivatives synthesised by alternative routes.

(2) Columns prepared from diatomaceous supports, deactivated diatomaceous supports and non-diatomaceous support phases with a range of liquid phases.

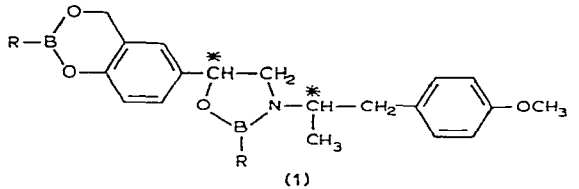
(3) Columns equipped with *n*-butyl boronic acid pre-columns.

The attempt to study cyclic boron derivatives synthesised by alternative routes which would ensure that there was no excess of reagent present, was made by utilising three boron dihalides, methyl boron dichloride, *n*-butyl boron dibromide or phenyl boron dibromide. Such boron halides are known to be more reactive than organoboronic acids[9]. Unfortunately despite the use of a wide range of anhydrous solvents such as pyridine, dimethylformamide, acetonitrile, acetone, chloroform, methylene

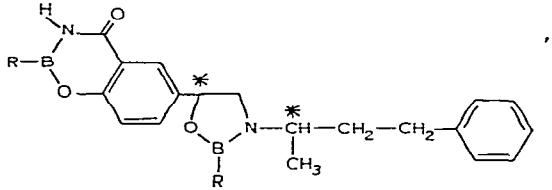
TABLE I

THE RESULTS OF SERIAL INJECTIONS OF THE BIS-BORONATE DERIVATIVES OF SALMEFAMOL (1) AND LABETALOL (2)

Column: 2 m × 1.75 mm I.D. glass column packed with 5% OV-101 coated onto 100–120 mesh Gas-Chrom Q AW HMDS.



(1)



(2)

The ratio is defined as the % (w/w) of isomer 1 : % (w/w) of isomer 2 where % (w/w) isomer 1 (I_1) =

$$100\left[\frac{\text{integrated area of the peak due to isomer 1}}{\text{integrated area of the peaks due to both isomers 1 and 2}}\right]$$

and % (w/w) isomer 2 (I_2) =

$$100\left[\frac{\text{integrated area of the peak due to isomer 2}}{\text{integrated area of the peaks due to both isomers 1 and 2}}\right].$$

Isomer 1 = the first eluted isomer; isomer 2 = the second eluted isomer.

Bis-boronate derivative	*Ratio of % (w/w) I_1:% (w/w) I_2*		
	Injection A	*Injection B*	*Injection C*
Salmefamol	57.5:42.5	62.7:37.3	68.8:31.2
Labetalol	50.6:49.4	53.1:46.9	56.7:43.3

dichloride and toluene, no stable derivatives were formed even when the reaction vessel was placed in an ice-bath. Solutions of sample and reagent quickly discoloured and the reaction vessel gaskets and teflon seals were rapidly decomposed, although initially the gas chromatograms of these solutions exhibited two peaks corresponding to those in the satisfactory chromatograms obtained following reaction between salmefamol or labetalol with the appropriate organo-boronic acid. The derivatives giving rise to these peaks were clearly unstable. Subsequent injections were devoid of any peak except that associated with the reagents used. Confirmation that the column was still performing satisfactorily was obtained by making an injection of a bis-boronate of salmefamol which had been prepared by the usual procedure. Good results were initially obtained, thus the use of boron dihalides was discontinued but the fact that the GLC columns did not appear to deteriorate as rapidly when the boron dihalides were used instead of the organo-boronic acids is circumstantial

TABLE II

COMPARISON OF THE ISOMER RATIOS* FOR SALMEFAMOL AND LABETALOL

Obtained by using freshly prepared *n*-butyl boronic acid derivatives injected onto a freshly prepared GLC column (5% w/w, OV-101 coated on 100–120 mesh Gas-Chrom Q AW HMDS), and the silylation–acylation procedure described by Munro and co-workers[8,10,11]

Compound	*n-Butyl boronic acid derivative* I_1:I_2	*Silylation–acylation* I_1:I_2
Salmefamol (1; R = *n*-butyl)	51.5:48.5	50.7:49.3
Labetalol (2; R = *n*-butyl)	51.5:48.5	51.2:48.8

* The ratio of % (w/w) of isomer 1 to % (w/w) of isomer 2 as defined in Table I.

evidence to implicate the organo-boronic acids in the anomolous quantitative results which had been obtained previously.

To further test the effects of excess boronic acid deposited onto the column packing upon the chromatography of boronic acid esters, fresh columns were prepared. Two of these contained 5% (w/w) OV-101 on 100–120 mesh Gas-Chrom Q AW HMDS. The third contained 5% OV-101 coated onto an unsilanised 80–120 mesh Diatomite S support. A sample of the *n*-butyl boronate of salmefamol was prepared and extracted into a methylene dichloride solution containing *n*-triacontane and *n*-tetratriacontane as internal standards. This solution was examined using a deactivated Gas-Chrom Q column and peak area ratios for the boronate ester diastereoisomers determined relative to the *n*-alkane standards. The column was then removed from the gas chromatograph and 5 mg of *n*-butyl boronic acid was added as a pre-column. Analysis of the boronate *n*-alkane calibration solution was repeated using identical instrumental conditions.

Whereas the deactivated OV-101 column gave satisfactory results, the *n*-butyl boronic acid contaminated and the diatomite S columns gave chromatograms with

TABLE III

THE RESULTS OF INJECTING THE *n*-BUTYLBORONATE DERIVATIVES OF SALMEFAMOL AND LABETALOL

I_1 and I_2 are the first and second eluted isomers respectively C_A and C_B are the preceding and succeeding hydrocarbons. Columns: glass columns packed with 5% w/w OV-101 coated onto 100–120 mesh Gas-Chrom Q AW HMDS (column 1); unsilanised, non-acid-washed 80–100 mesh Diatomite S (column 2); 100–120 mesh Gas-Chrom Q AW HMDS with 5 mg *n*-butyl-boronic acid added at the injection port end (column 3).

Compound	*Ratio of peak areas*								
	Column 1			*Column 2*			*Column 3*		
	$\frac{I_1}{C_A}$	$\frac{I_2}{C_A}$	$\frac{C_A}{C_B}$	$\frac{I_1}{C_A}$	$\frac{I_2}{C_A}$	$\frac{C_A}{C_B}$	$\frac{I_1}{C_A}$	$\frac{I_2}{C_A}$	$\frac{C_A}{C_B}$
Salmefamol	4.49	4.74	1.1	No isomers eluted		1.1	No isomers eluted		1.1
Labetalol	2.70	2.79	0.71	0.2	0.21	0.79	No isomers eluted		0.76

alkane but no boron derivative peaks (see Table III). These results suggest that the presence of either excess reagent or free surface silanols is sufficient to render unsatisfactory the gas chromatographic examination of the *n*-butyl boronic acid derivative of salmefamol. Subsequent experiments revealed that the methyl and phenyl boronic acid derivatives of salmefamol and the *n*-butyl methyl and phenyl boronic acid derivatives of labetalol all behaved similarly.

As the elution of the *n*-alkanes would not be expected to be effected by the change of support or by the presence of *n*-butyl boronic acid/anhydride these results clearly demonstrate the occurrence of on-column reaction.

Further studies to elucidate the nature and mechanism of the on-column reaction which occurs between cyclic boron derivatives and gas chromatography columns are currently in progress and will be reported elsewhere.

REFERENCES

1 D. Hartley, D. Jack, L. H C. Lunts and A. C. Ritchie, *Nature (London)* 219 (1968) 861.
2 D. A. Richard, J. Tuckman and B. N. C. Pritchard, *J. Clin. Pharm.*, 3 (1976) 849.
3 C. J. Simpson, *J. Chromatogr. Sci.*, 10 (1972) 425.
4 R. L. Sterne, B. L. Karger, W. J. Keane and H. C. Rose, *J. Chromatogr.*, 39 (1969) 17.
5 C. J. W. Brooks and J. Watson, in C. L. A. Harbourne (Editor), *Gas Chromatography 1969*, Institute of Petroleum, London, 1969.
6 G. M. Anthony, C. J. W. Brooks and B. S. Middleditch, *J. Pharm. Pharmacol.*, 22 (1970) 205.
7 S. J. Gaskall and C. J. W. Brooks *J. Chromatogr.*, 122 (1976) 415.
8 G. Munro, *C.N.A.A. Ph.D. Thesis*, The Hatfield Polytechnic Hatfield, 1979.
9 H. Steinberg, *Organoboron Chemistry*, Wiley, New York, 1964.
10 G. Munro, J. H. Hunt, L. R. Rowe and M. B. Evans, *J. Pharm. Pharmacol.*, 28 (1976) 27.
11 G. Munro, J. H. Hunt, L. R. Rowe and M. B. Evans, *Chromatographia*, 11 (1978) 440.