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Note

Gas chromatography of aromatic boronic acids: on-column derivatization

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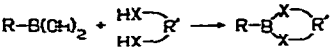
Substituted benzeneboronic acids are of considerable importance as reactive ligands for affinity chromatography[1] and as derivatizing reagents for use in gas chromatography (GC) with electron-capture detection[2]. Since simple boronic acids, such as butaneboronic acid, are well known as derivatizing agents for bifunctional organic compounds[3], it is reasonable to expect simple bifunctional compounds to be useful derivatizing reagents for the more complex boronic acids. A general scheme for the reaction is given (Scheme 1). Other workers[4] have briefly reported the derivatization of electronegative aromatic boronic acids with pinacol (2,3-dihydroxy-2,3-dimethylbutane). For the purpose of analysis by GC–mass spectrometry (MS), we preferred the use of propane-1,3-diol or 1,2-dihydroxybenzene as derivatizing agent for substituted benzeneboronic acids[5] Both of these methods[4,5] involved derivatization prior to GC analysis. This note describes a simple on-column derivatization of boronic acids with propane-1,3-diol. The new procedure has a number of advantages over the conventional one. For example, it is shown that volatile components in a mixture of boronic acids can be examined separately by injection of the underivatized mixture, followed later by an injection of the derivatizing reagent for subsequent characterization of the boronic acid(s) present



X = O, NH
R' = $(CH_2)_{2 \text{ or } 3}$ $(C(CH_3)_2)_2$ ∝
Scheme 1.

A third method for analysis of boronic acids by GC has been reported[6]. It entails dehydration and trimerization of boronic acids to boroxines (trioxatriborans), a reaction which can also be brought about in the gas chromatograph if the injector port is maintained at an elevated temperature[4,6]. This older procedure has not been used because many substituted benzeneboronic acids after cyclization afford more than one GC peak[4] and because boroxines of substituted benzeneboronic acids have very long retention times[5]. Also, the method is not suitable for mixtures since condensation between different boronic acid components would lead to complex boroxines.

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EXPERIMENTAL

Materials

The boronic acids described herein were synthesised in this department in respect of its work on reactive ligands for affinity chromatography[1] Their synthesis will be described elsewhere The exception to this is 2-dimethylaminomethylbenzeneboronic acid which was kindly supplied by Professor G Wüiff Propane-1,3-diol was obtained from Fisons Scientific. All solvents used were redistilled before use

The propane-1,3-diol reagent comprised 50 mmol of the diol in 100 ml pyridine–ethyl acetate (1.1)

Gas chromatography

A Pye-Unicam 104 series gas chromatograph with flame-ionization detector was used The glass column (5 ft × 4 mm I D) was packed with 3% OV-17 on Gas-Chrom Q, 100–120 mesh The oven temperatures used were between 140 and 210°C (see below) and the injector temperature was set to be as near as possible to that of the oven Argon (40 ml/min) was used as carrier gas

Gas chromatography–mass spectrometry

The instrument used was a VG Micromass 7070F coupled to a Pye-Unicam 204 gas chromatograph and a Finnigan Incos data system The GC column and conditions were as above except that helium was used as carrier gas The GC–MS interface was a single-stage, all-glass jet separator, maintained at 250°C Ion source conditions accelerating voltage, 4 kV, electron beam energy, 70 eV; emission current, 200 μA; ion source temperature, 200–250°C.

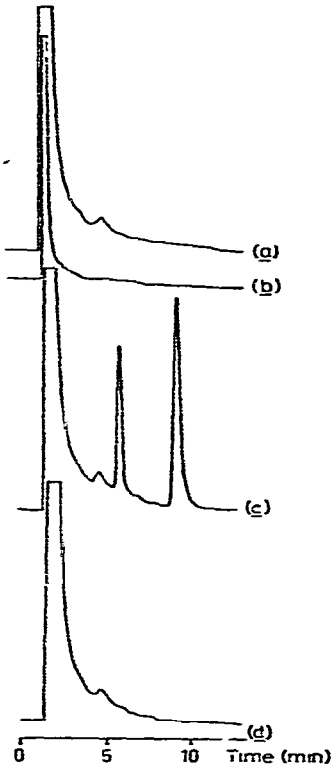
Methods

The aromatic boronic acid sample was dissolved in a suitable solvent (usually ethyl acetate or methanol) and an aliquot (ng–μg range) injected onto the GC column. After elution of the solvent, the propane-1,3-diol reagent (2 μl) was injected to derivatize and hence elute the boronic acid(s) The time between the two injections was varied between *ca* 2 and 30 min (see below)

Conventional "bench" preparation of cyclic boronate esters uses the same derivatizing agent and has been described elsewhere[5]

RESULTS AND DISCUSSION

A typical analysis is shown in Fig. 1 for a mixture of benzene- and 4-methylbenzeneboronic acids. The first trace is that obtained for the propane-1,3-diol reagent only (*i e* a blank) and the second, showing no peaks other than solvent, for the underivatized boronic acid mixture After 10 min, the derivatizing agent was injected and trace c was obtained Two well-shaped peaks were observed for the two cyclic boronate derivatives, the small peak eluting soon after the solvent also being present in the blank (an impurity in the derivatizing reagent) The identity of the eluting species in all cases cited here was checked by combined GC–MS[5]. To check that derivatization was complete, a further injection of the diol reagent was performed and this showed no cyclic boronate ester peaks (Fig 1d) In fact, even when the column



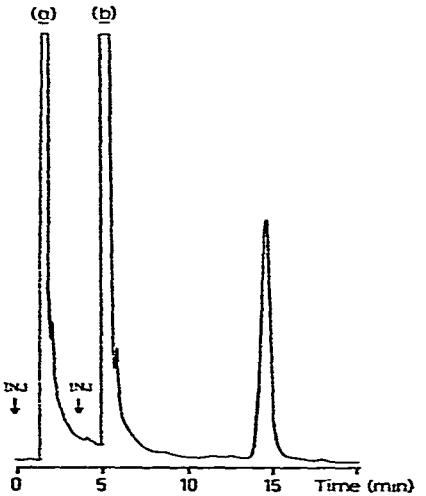


Fig. 1. GC analysis of a mixture of benzene- and 4-methylbenzeneboronic acid. (a) Propane-1,3-diol in ethyl acetate–pyridine only, (b) underivatized mixture of boronic acids in ethyl acetate, (c) injection of propane-1,3-diol in ethyl acetate–pyridine to effect on-column derivatization, and (d) repeat injection of propane-1,3-diol in ethyl acetate–pyridine. All injections were consecutive with a 10-min delay between each. GC conditions: 3% OV-17 at 140°C; injector temperature, 150°C.

Fig. 2. GC analysis of 4-methyl-3-nitrobenzeneboronic acid. Injection points (INJ), of underivatized acid in methanol (a) followed by propane-1,3-diol derivatizing reagent (b), are shown GC conditions. 3% OV-17; column and injector temperature, 200°C.

was overloaded with a boronic acid, on-column derivatization was complete and no memory effects were observed.

Isolated boronic acids occur as mixtures of the acid and the trimeric cyclic anhydride (boroxine). Also, it has been reported that benzeneboronic acid undergoes conversion to triphenylboroxine in the injection port of a gas chromatograph[4-6]. Therefore, it seems likely that the material adsorbed at the top of the column prior to derivatization is a mixture of the boronic acid itself and its boroxine, the composition depending on the temperature of the injector heater and the chemical structure of the acid. Since the diol derivatizing agent reacts with both species to give the same cyclic boronate ester, this situation does not interfere with the analysis. The temperatures of the injection port and a packed OV-17 column required to form triphenylboroxine from benzeneboronic acid and then to elute it are 270 and 200°C respectively[6], whereas the temperatures of the injector and oven in the present study were 150 and 140°C respectively, reflecting the greater volatility of propane-1,3-diol benzenebo-

ronate. If the boronic acid itself or the boroxine derived from it were to spread or elute slowly through the column before on-column derivatization were effected, broad peak shapes would be obtained with retention times significantly less than those of the authentic cyclic boronate derivatives. Since this problem would be most severe with the most volatile boronic acid of the aromatic family, a comparison was made of the GC behaviour of pre-formed propane-1,3-diol benzeneboronate[5] and the same derivative formed on-column. Under conditions giving a retention time of 5.7 min for the pre-formed derivative, the retention time in the on-column experiment was 5 5 min. Whilst this slight reduction in retention time was observed (relative retention time 0 965), there was no observable band-spreading with both peaks having widths at half-height of 17.7 sec

The potential problem of efficient trapping on the top of the column was examined also for 4-methyl-3-nitrobenzeneboronic acid With the inlet port and column at 200°C, the retention time of the cyclic ester derivative was measured to be 10.1 min when the reagent solution was injected 6 min after the boronic acid, 9.7 min when the delay between injections was 12 min, and 9.5 min with a delay as long as 30 min The peak width at half-height was 33 sec in each case with no significant tailing Again, some movement of the boronic acid and/or its boroxine through the column is indicated but no loss of resolution occurs. In this short study, GC conditions were not optimized. In particular, lowering the temperature of the injector was not investigated but would be expected to reduce or eliminate the variation in retention time Reproducibility would be enhanced by injecting the derivatizing agent a set time after the boronic acid injection.

For conventional formation of cyclic boronate esters, the range of suitable solvents is limited to ethyl acetate, dimethylformamide, pyridine, tetrahydrofuran

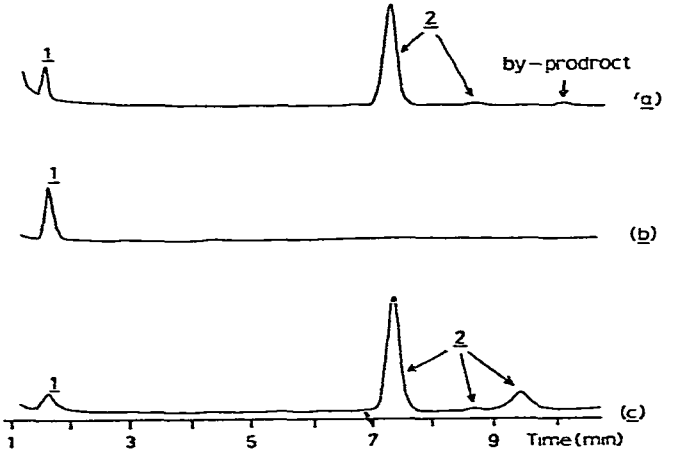
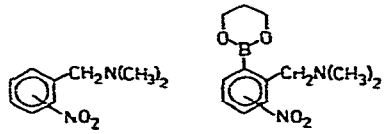


Fig. 3 Analysis by GC–MS of the mixture resulting from nitration of 2-dimethylaminomethylbenzeneboronic acid using conventional derivatization (a) and on-column derivatization (b) injection of underivatized mixture and (c) injection of derivatizing agent The inlet to the ion source was opened in each case immediately after elution of the solvent Note that all three isomeric products (compounds 2) were observed only with the on-column procedure (*cf.* traces a and c) and that compound 1 was present prior to derivatization (trace b) GC conditions· 3% OV-17 at 180°C for 2 min, then temperature increased to 220°C at 8°/min, injector temperature, 250°C (a) or 175°C (b, c)

and chloroform[3]. Some of the more polar benzeneboronic acids are hardly, or not at all, soluble in these solvents and so could not be derivatized. However, the restriction on solvents is removed by use of the on-column reaction and even alcohols can be used. To illustrate this, on-column derivatization of a sample of 4-methyl-3-nitrobenzeneboronic acid in methanol is shown in Fig. 2 with a 3.3 min delay between injection of the methanolic solution and the derivatizing agent The peak width at half-height (38 sec) was increased slightly over that obtained (33 sec) when using ethyl acetate as solvent.



1 2

Two further advantages of the on-column procedure became clear during a study by GC–MS of a mixture resulting from nitration of 2-dimethylaminomethylbenzeneboronic acid. Conventional derivatization followed by GC–MS analysis afforded two major peaks corresponding to isomers of compounds 1 and 2, and two minor peaks assigned to a second isomer of compound 2 and a by-product due to further reaction of compound 2 with the diol (Fig. 3a). First, we required to know if compound 1 was formed during derivatization or was present in the nitration product. Injection of the underivatized mixture showed that compound 1 was already present (or formed on the column) as seen in Fig. 3b. This illustrates that volatile components can be characterized separately. Subsequent injection of the derivatizing agent afforded the trace shown in Fig 3c The two isomers of compound 2 were readily observed, the by-product was not detected, and a third isomer of 2 (not observed in the pre-formed boronate mixture) was seen to elute at the end of the analysis. We propose that this last cyclic boronate ester, when formed during conventional derivatization, reacts further to give, in part or whole, the observed by-product. During on-column derivatization, contact between substrates and reagents is transitory, thereby drastically reducing the chances of further reaction occurring. The fact that compound 1 is also present after on-column derivatization indicates that it is formed during derivatization as well as being present in the original mixture and/or being a thermal product of the boronic acids (see above).

CONCLUSION

The method of on-column derivatization reported herein has been employed successfully with several aromatic boronic acids and mixtures thereof. It is now used routinely in this laboratory. The advantages of the procedure are: (1) speed and simplicity of analysis, (2) use of any solvent commensurate with gas chromatography, including those unsuitable for the "bench reaction", (3) separate characterization of volatile compounds in mixtures of boronic acids is possible since long delays between injection of sample and derivatizing agent are tolerated without loss of chromatographic efficiency, and (4) difficulties with cyclic boronates that are prone to further

reaction upon conventional derivatization are removed The disadvantages are that (1) some solvent tailing is inevitable with pyridine and propane-1,3-diol in the reagent mixture and (2) slight variation in retention times is observed. Standardization of the procedure, improvements to the derivatizing reagent solution and optimization of GC conditions are expected to reduce or eliminate disadvantages

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