Stability and Reversed-Phase Liquid Chromatographic Studies of Cyclic Boronates

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Many organic compounds of biological importance possess two or more polar functional groups in close proximity. In their chromatographic analysis, a suitable derivatization of the bifunctional groups was sometimes used to enhance the sensitivity and selectivity for detection. The value of boronic acids, as selective reagents for compounds with suitably proximal pairs of functional groups, was originally established in the carbohydrate field.1 The versatility of these reagents has been exploited in many ways, e.g. in separations based on affinity chromatography.

Transforming 1,2-diol compounds to the corresponding cyclic boronate derivatives has been more extensively used as a suitable derivatization method for gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) since 1967.2 Applications to liquid chromatography have been comparatively limited due to the susceptibility of the cyclic boronates to solvolysis. However, we have demonstrated some instances in which such derivatives show considerable stability. The first examples were cyclic boronates of the dissecondary threo-22,23-dihydroxy group present in the brassinosteroids. Several boronic acids, including naphthaleneboronic acid3, phenanthreneboronic acid4, 1-cyanoisoindole-2-m-phenylboronic acid5, dansylaminophenylboronic acid6 and ferroceneboronic acid7 have been successfully applied to the determination of brassinosteroids by reversed-phase liquid chromatography. It has been confirmed that the brassinosteroid boronate derivatives are remarkably stable, even under weakly acidic aqueous conditions.

In continuation of our liquid chromatographic research concerning the analysis of cyclic boronates, we have screened a number of dihydroxy compounds in the hope of confirming stable boronates under reversed-phase conditions. This report arises from experiments aimed at expanding the range of the resistance of boronate esters to solvolysis. In this paper we describe the utility of the boronate ester function as a derivative group for the characterization of 1,2-diol compounds by reversed-phase liquid chromatography, and briefly survey the reactivity of boronic acid derivatives with a variety of 1,2-diols.

Experimental

Chemicals

Benzenboronic acid and ferroceneboronic acid were purchased from Tokyo Kasei (Tokyo, Japan). All other reagents and solvents were of analytical-reagent grade. The following were prepared by Dr. Ekhatō:8 2α,3α-dihydroxy-5α-cholestan-6-one; 3β,22S,23S-trihydroxy-24S-ethyl-5α-cholestan-6-one; 2α,3α,22S,23S-tetrahydroxy-24S-ethyl-5α-cholestan-6-one. Ecdysterone was obtained from Mann Research Laboratories (New York, NY, USA). (1S,2S,3R,5S)-(−)-Pinanediol was from Aldrich. The other diols were available from previous studies.9,10

Preparation of boronate esters

Benzenec- or ferroceneboronic acid (1.1 molar proportion per vicinal diol) in a dry pyridine-acetonitrile mixture (1:9 by volume) was added to the substrate (100 µg) in dry pyridine (10 µl); the mixture was then heated at 70°C for 10–30 min. After cooling, several microlitres of the resulting solution were injected into the analytical column.

Liquid chromatography

A Shimadzu Model LC-6A chromatograph equipped with a Shimadzu Model SPD-6A UV-spectrophotometric detector or a Shimadzu Model L-ECD-6A electrochemical detector using a glassy carbon working electrode was employed. An Asahikagub column, ODP-50 (5 µm particle diameter, 4.6 mm i.d.×150 mm, Asahi Chemical Industry Co., Ltd., Tokyo), was used in the reversed-phase mode at 45°C. Samples were injected into the column using a Rheodyne Model 7125 rotary valve syringe-loading injector. The mobile phase for

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separating boronate esters was a mixture of acetonitrile and water in most instances containing 20 mM potassium dihydrogen phosphate (pH 4.5). A mixture of acetonitrile and water containing 0.1 M sodium perchlorate was used for the electrochemical detection. The boronate esters were monitored at 265 nm. At this wavelength, the calibration curve for pinanediol ferroceneboronate was linear in the range from 120 pmol to 2.4 nmol. The detection limit was approximately 40 pmol ($S/N = 3$).

**Results and Discussion**

**General features of cyclic boronates of 1,2-diols**

The most remarkable advantages for investigating cyclic boronates by reversed-phase HPLC are: 1) to confirm the stability of the boronates in an aqueous media, 2) to enable the recognition of partial molecular structures, e.g. the cis-diol component with a steric hindrance or conformational rigidity and 3) to perform highly sensitive detection of the diols as the boronates with fluorophore or electrophore. The disadvantages include the poor hydrolytic stability exhibited by many derivatives and the ease of solvolysis observed in multiple derivatization procedures in which the boronate group may be partially or completely eliminated. The stability of the boronate derivatives to TLC and the other hydrolysis conditions is variable, depending on local stereochemistry of both the bifunctional group and the individual boronic acid used to prepare the derivative.

The diol compounds examined are shown in Fig. 1. The cyclic boronate derivatives of the 1,2-diols were prepared on an analytical scale from 100 µg amounts of samples, generally by heating with a slight excess of the reagent in dry pyridine/acetonitrile at 70°C. In some

![Figure 1: Structures of diols and polyols](image-url)
instances, the boronate formation reactions could be monitored by TLC on silica gel with the mobile phase ethyl acetate/hexane. The isolated yield of pinacol ferroceneboronate was 95%.

Most of the cyclic boronate derivatives including both primary/secondary and secondary/secondary glycol components (e.g. butanediol (1), mephenesin (6)) were found to be hydrolyzed during attempted liquid chromatography under the reversed-phase conditions, although the analysis of their ferroceneboronates has previously succeeded using GC and GC-MS.

However, several of the diols studied gave stable ferroceneboronates which were detectable in reversed-phase liquid chromatographic analysis under the aqueous acetonitrile mobile phase conditions (Table 1).

The residence to solvolyis of stable boronates was independent on the substituents of the individual boronic acid derivatives used to prepare the corresponding boronate derivatives. A group of ferroceneboronates was also studied by reversed-phase HPLC in the presence of a phosphate buffer (pH 4.5); the retention data are noted in Table 1. In addition, the diol ferroceneboronates remained stable in ethyl acetate solution for at least several months.

**Cyclic boronates of steroidal compounds**

Several steroids containing one or two vicinal diol groups were examined regarding the formation and liquid chromatography of boronate derivatives; the products were characterized by mass spectrometry, employing electron impact, chemical ionization and fast atom bombardment (FAB) methods (data not shown).

In cases where the 2α,3α- and 22,23-diol groupings were both present, it was found that it is possible to prepare mono-boronates as well as bis-boronates. We previously reported on the formation of bis-(2α,3α; 22,23-boronates of brassinosteroids.

The tetrone (13), 2α,3α,22S,23S-tetrahydroxy-24S-ethyl-5α-cholestan-6-one (13), 2β,22S,23S-trihydroxy-24S-ethyl-5α-cholestan-6-one (11), (3) 22S,23S-dihydroxy-24S-ethyl-5α-cholestan-2-en-6-one (12). Conditions: Asahipak ODP-50 column (4.6 mm i.d.×15 cm long); mobile phase, acetonitrile/water (v/v 9:1) containing 0.1 M NaClO₄; flow rate, 0.7 ml/min; temperature, 45°C; detection, 265 nm.

The tetrolone (13), 2α,3α,22S,23S-tetrahydroxy-24S-ethyl-5α-cholestan-6-one, yielded mono- and big benzeneboronates using 1.1 and 2.2 equivalents of boronic acid, respectively. The 2α,3α-boronate group was not stable to the reversed-phase HPLC conditions: both esters were eluted as 22,23-monoboronates. The separation of ferroceneboronates of three steroidal 22,23-dihydroxy compounds is shown in Fig. 2. As expected, the order of elution reflects their polarities, according to the diminishing number of free hydroxyl groups.

The final steroid examined was the hexolone (14), ecdysterone. Satisfactory mass spectra were obtained for mixtures of mono- and bis-benzeneboronates and ferroceneboronates by FAB-MS. The steroid yielded a ferroceneboronate ester for which no significant ions were observed in EI, though FAB ionization gave a clear ion at m/z 653 (MH⁺) as the bis-ferroceneboronate. It is known that the 2β,3β-diol group does not form cyclic boronates very readily. However, the 20,22-diol group in ecdysterone affords more stable boronates (cf. also 20,22-dihydroxycholesterol). In this study the benzene and ferroceneboronate of acyesterone could be detected as the 20,22-mono-ester under the reversed-phase liquid chromatographic conditions (Fig. 3).

**Stability of cyclic boronates**

The stability could be ascribed to a steric resistance to...
solvolysis (e.g. for the ditertiary glycols, pinacol (2) and bicyclohexyl diol (4), and the secondary/tertiary glycol, pinanediol (3)), and to a conformational rigidity (for the disecondary glycols, indane-cis-1,2-diol (5), and 16β-epiestriol (10)). Recoveries of each boronate injected to the column were estimated to almost 95% by spectrophotometric measurements. The retention data were as broad as expected, except for the remarkably high retention of the bicyclohexyl diol derivative. As described above, the steroidal tetrolone (13) yielded a mono-ester of the disecondary 22,23-diol system, presumably because it was sufficiently stabilized by the adjoining groups, the isobutyl group conformation being restricted by the proximity of the steroidal skeleton. The instability of the 2α,3α-diol boronates to HPLC as well as the unfavorable equilibrium in boronate complexation have already been observed as unpublished results.

The reversed-phase liquid chromatographic analysis of a variety of cyclic boronates was demonstrated as a first example of a series of stable boronates which are resistant to hydrolysis.

In conclusion, these present data strongly suggest that stable boronates to hydrolysis occur widely in many kinds of diol compounds. The liquid chromatographic analytical method for the cyclic boronates may contribute to studies concerning the identification of the diol component with a conformational rigidity or stereochemical hindrance in biological and pharmaceutical samples, and also to their screening in unknown compounds.

References


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