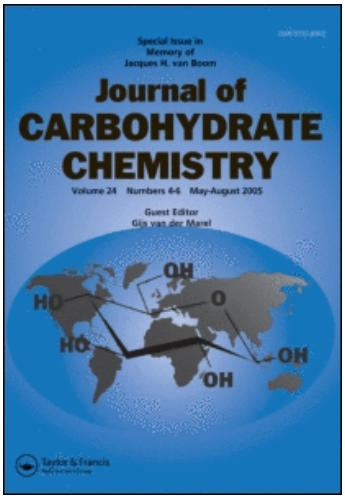

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Reaction Mass Spectrometry of Saccharides Using Areneboronic Acids as Reagents

Hou-Jun Yang[a]; Yao-Zu Chen[b]
[a] Department of Chemistry, Tsinghua University, Beijing, PRC [b] National Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou, PRC

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REACTION MASS SPECTROMETRY OF SACCHARIDES USING ARENEBORONIC ACIDS AS REAGENTS

Hou-Jun Yang*[a] and Yao-Zu Chen[b]

[a] Department of Chemistry, Tsinghua University, Beijing 100084, PRC
[b] National Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou 730000, PRC.

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ABSTRACT

1-Naphthylboronic acid and 2,4-dimethylphenylboronic acid reacted stereospecifically with hydroxyl groups of saccharides on the probe tip of a fast atom bombardment apparatus to form characteristic negatively-charged boronate ions as determined by mass spectrometry. From the relative abundances of these characteristic ions, the epimers of mono- and disaccharides may be distinguished. This approach is offered as a simple and effective means for identifying sugars. The collisional activation mass spectra of these characteristic ions are also discussed.

INTRODUCTION

Boronic acids and their derivatives react stereospecifically with polyhydroxy compounds and these reactions have been used for stereochemical analysis of sugars in solution.[1-7] In our previous papers, the application of trimethylborate as a reagent gas in chemical ionization (CI) mass spectrometry for differentiation between saccharide stereoisomers and for determination of acyclic glycol configuration have been reported.[8,9] For thermolabile and high polar compounds, such as saccharides and glycols, the fast atom bombardment (FAB) ion source is more suitable than a CI source. Rose and his co-workers found that *in situ* reactions of boronic acids with polyhydroxy compounds may provide information on the configuration of these compounds by negative ion FAB mass spectrometry.[10] They studied the negative ion FAB spectra of four aldopentoses using 4-toluneboronic acid as a reagent. In the present work, to improve the stereospecificity of this reaction, two reagents with greater steric bulk, 1-naphthylboronic acid (NB) and 2,4-dimethylphenylboronic acid (DMPB), were used in obtaining negative ion FAB

mass spectra of cyclohexanediols and mono– and disaccharides. It was found that both reagents reacted stereospecifically with these polyhydroxy compounds to form characteristic negatively charged boronate ions, from the relative abundances of which, the stereoisomers of *cis*– and *trans*–cyclohexanediol, mono– and disaccharides may be distinguished. The collisional activation mass spectra of these characteristic boronate ions are also discussed.

RESULTS AND DISCUSSION

Negative Ion FAB Mass Spectra of Cyclohexanediols

To investigate the stereochemical effects in the reaction between areneboronic acids and saccharides, some cyclohexanediols were initially examined. There is no significant difference between the normal negative ion fast atom bombardment mass spectra of *cis*– and *trans*–1,2– cyclohexanediol. However, when 1–naphthylboronic acid (NB) or 2,4–dimethylphenylboronic acid (DMPB) was present in the ion source, the relative abundance of the sample cyclic boronate ion $[M_s+M_r-H-2H_2O]^-$ formed by the reaction of the *cis*–diol M_s with the reagent M_r was much higher than that of the *trans*–isomer (Table 1). This can be ascribed to the greater steric strain in the latter isomer. As for 1,4–cyclohexanediol, the two hydroxyl groups are too remote to form the bridged cyclic boronate ion, and the characteristic ion $[M_s+M_r-H-2H_2O]^-$ appeared neither in the negative ion fast atom bombardment (NIFAB) mass spectrum of *trans*–1,4–diol nor in that of *cis*–1,4–diol. However, the relative abundances of acyclic boronate ions $[M_s+M_r-H-H_2O]^-$ formed from *cis*–1,4–diols were quite different from that of corresponding ions formed from *trans*–1,4–isomer (Table 1).

Negative Ion FAB Mass Spectra of Monosaccharides

In the NIFAB mass spectra of monosaccharides using areneboronic acids as reagents, the characteristic ions are cyclic boronate ions $[M_s+M_r-H-2H_2O]^-$ and $[M_s+2M_r-H-4H_2O]^-$ formed through condensation of two saccharide hydroxyl groups with the reagents. As with the cyclohexanediols, a vicinal–*cis* arrangement of hydroxyl groups is favorable for condensation. Given that the number of vicinal–*cis* hydroxyl group arrangements is different for each saccharide stereoisomer, it would be expected that among these isomers, a difference in relative abundance from the cyclic boronate ions should be observed. Thus, by comparison of the relative abundances of these characteristic ions, the stereoisomeric saccharides may be distinguished (Table 2). As an example, the pathway for formation of cyclic boronate ions from D–mannose is postulated (Scheme).

D–Mannopyranose can react with NB through C_4–OH and C_6–OH or C_2–OH and C_3–OH to form six– or five–membered ring boronate ion $[M_s+M_r-H-2H_2O]^-$ at m / e 315. The m / e 315 product can then react with a second molecule of reagent to yield the $[M_s+2M_r-H-4H_2O]^-$ ion at m / e 451. When DMPB was applied, the characteristic ion $[M_s+2M_r-H-4H_2O]^-$ at m / e 407 even appeared as the base peak.

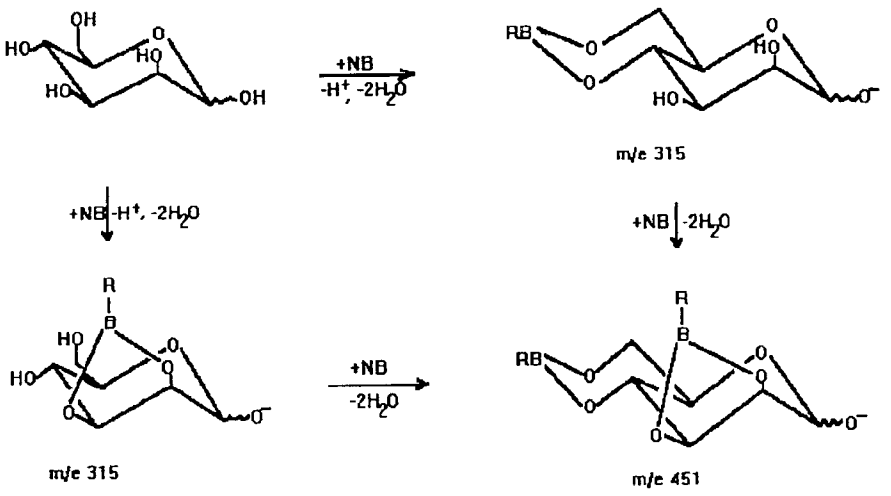
Table 1. Major characteristic ions in NIFAB mass spectra of cyclohexanediols with areneboronic acids as reagents

Reagents	Samples		$[M_s-H]^-$	$[M_s+M_r-H-H_2O]^-$	$[M_s+M_r-H-2H_2O]^-$	$r=\frac{[M_s+M_r-H-2H_2O]^-}{[M_s+M_r-H-H_2O]^-}$
NB	cis-cyclohexanediol-1,2	m / z(%)	115(21.5)	269(14.8)	251(23.0)	1.6
	trans-cyclohexanediol-1,2	m / z(%)	115(30.5)	269(6.7)	251(0.0)	0.0
	cis-cyclohexanediol-1,4	m / z(%)	115(19.0)	269(19.2)	251(0.0)	0.0
	trans-cyclohexanediol-1,4	m / z(%)	115(33.5)	269(26.8)	251(0.0)	0.0
DMPB	cis-cyclohexanediol-1,2	m / z(%)	115(16.0)	247(12.7)	229(22.4)	1.8
	trans-cyclohexanediol-1,2	m / z(%)	115(19.5)	247(11.8)	229(0.0)	0.0
	cis-cyclohexanediol-1,4	m / z(%)	115(34.0)	247(28.8)	229(0.0)	0.0
	trans-cyclohexanediol-1,4	m / z(%)	115(27.0)	247(45.3)	229(0.0)	0.0

Table 2. NIFAB reaction mass spectral data of monosaccharides using areneboronic acids as reagents

Reagents	Samples	$[M-H]^-$	$[M_s+M_r-H-2H_2O]^-$	$[M_s+2M_r-H-4H_2O]^-$	$r=\frac{[M_s+2M_r-H-4H_2O]^-}{[M_s+M_r-H-2H_2O]^-}$
NB	D-mannose	179(25.0)	315(100)	451(42.0)	0.4
	D-glucose	179(27.5)	315(100)	451(29.9)	0.3
	D-galactose	179(7.0)	315(100)	451(12.0)	0.1
	D-fructose	179(39.0)	315(100)	451(32.5)	0.3
	L-sorbose	179(31.0)	315(100)	451(21.7)	0.2
	D-arabinose	149(30.0)	285(100)	421(20.0)	0.2
	D-xylose	149(47.0)	285(100)	421(0.0)	0.0
	L-rhamnose	163(31.5)	299(100)	435(0.0)	0.0
DMPB	D-mannose	179(22.0)	293(100)	407(129.7)	1.3
	D-glucose	179(9.0)	293(100)	407(38.2)	0.4
	D-galactose	179(0.0)	293(100)	407(29.0)	0.3
	D-fructose	179(21.5)	293(100)	407(39.8)	0.4
	L-sorbose	179(5.0)	293(100)	407(25.2)	0.25
	D-arabinose	• 149(64.0)	263(100)	377(41.4)	0.4
	D-xylose	• 149(43.5)	263(100)	377(29.1)	0.3
	L-rhamnose	163(16.5)	277(100)	391(0.0)	0.0

• Both the m / z values of $[M_s-H]^-$ and $[M_r-H]^-$ are 149 in this case

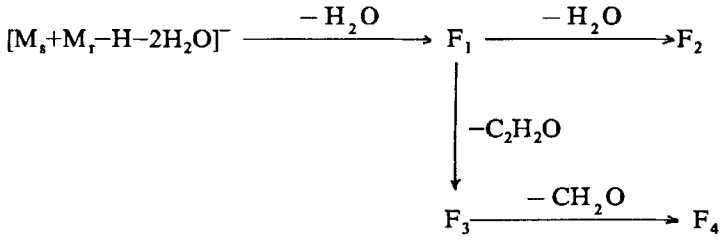


Scheme Reactions of D-mannose with NB in NIFAB MS

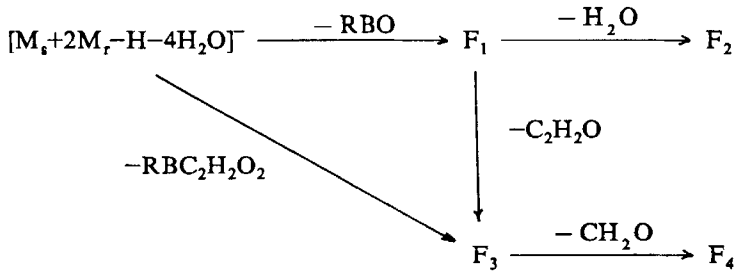
Collisional Activation Mass Spectra of Characteristic Ions of Monosaccharides

The collisional activation (CA) mass spectral data from characteristic ions $[M_s+M_r-H-2H_2O]^-$ and $[M_s+2M_r-H-4H_2O]^-$ of some monosaccharides are summarized in Table 3. The fragmentation modes of these characteristic ions are as follows:

For $[M_s+M_r-H-2H_2O]^-$:



For $[M_s+2M_r-H-4H_2O]^-$:



R = 1–naphthyl or 2,4–dimethylphenyl

Table 3. CA data of $[M_s+M_r-H-2H_2O]^-$ and $[M_s+2M_r-H-4H_2O]^-$ formed by monosaccharides

	parent ion	daughter ions m / z(%)				parent ion	daughter ions m / z(%)			
	$[M_s+M_r-H-2H_2O]^-$	F_1	F_2	F_3	F_4	$[M_s+2M_r-H-4H_2O]^-$	F_1	F_2	F_3	F_4
D–mannose+NB	315(100)	279(22)	279(15)	255(73)	225(23)	451(100)	297(21)	279(32)	255(100)	225(30)
D–glucose+NB	315(100)	297(40)	279(30)	255(56)	225(27)	451(100)	297(24)	279(44)	255(10)	225(19)
D–galactose+NB	315(100)	297(15)	279(0)	255(51)	225(23)	451(100)	297(10)	279(35)	255(24)	225(0)
D–arabinose+NB	285(100)	267(20)	249(0)	225(21)	—	421(100)	267(41)	249(0)	225(0)	—
L–rhamnose+NB	299(100)	281(26)	263(0)	239(33)	—	435(100)	—	—	—	—
D–mannose+DMPB	293(100)	275(16)	257(0)	233(68)	203(12)	407(100)	275(15)	257(28)	233(100)	203(30)
D–glucose+DMPB	293(100)	275(26)	257(15)	233(33)	203(14)	407(100)	275(45)	257(100)	233(17)	203(15)
D–galactose+DMPB	293(100)	275(13)	257(10)	233(47)	203(14)	407(100)	275(20)	257(53)	233(20)	203(13)
D–arabinose+DMPB	263(100)	245(14)	227(0)	203(20)	—	377(100)	245(31)	227(13)	203(0)	—
L–rhamnose+DMPB	277(100)	259(28)	241(0)	217(29)	—	391(100)	—	—	—	—

Table 4. NIFAB reaction mass spectral data of disaccharides using areneboronic acids as reagents

Reagents	Samples	$[M_s+M_r-H-2H_2O]^-$	$[M_s+2M_r-H-4H_2O]^-$	$[M_s+2M_r-H-4H_2O]^- / [M_s+M_r-H-2H_2O]^-$(%)
NB	maltose	477(14.0)	613(14.9)	106.4
	cellobiose	477(18.5)	613(15.9)	85.9
	lactose	477(50.3)	613(20.3)	40.4
	sucrose	477(63.1)	613(25.8)	40.8
DMPB	maltose	455(15.2)	569(31.2)	204.8
	cellobiose	455(28.0)	569(38.9)	138.8
	lactose	455(44.4)	569(37.7)	84.9
	sucrose	455(100)	569(25.4)	25.4

It was noteworthy that the relative abundances of the fragment ions were different for each saccharide stereoisomer. Therefore, data from CA spectra provides a complementary means to distinguish the parent saccharides.

Negative Ion FAB Mass Spectra of Disaccharides

NIFAB mass spectra of four disaccharides, using NB or DMPB as reagent, have been measured. Similar to monosaccharides, these disaccharides can be differentiated by comparison of the relative abundances of characteristic ions $[M_s+M_r-H-2H_2O]^-$ and $[M_s+2M_r-H-4H_2O]^-$ (Table 4). Both sucrose and lactose have an abundant $[M_s+M_r-H-2H_2O]^-$ ion at m / e 477 in the reaction mass spectra using NB as the reagent. In fact, $[M_s+M_r-H-2H_2O]^-$ at m / e 455 became the base peak in the reaction mass spectrum of sucrose when DMPB was used as the reagent. In comparison, maltose and cellobiose show a less abundant $[M_s+M_r-H-2H_2O]^-$ ion. Comparing the ratio of the $[M_s+2M_r-H-4H_2O]^-$ to $[M_s+M_r-H-2H_2O]^-$ ions, the order of decreasing value of ratio is: maltose > cellobiose > lactose > sucrose.

Collisional Activation Mass Spectra of Characteristic Ions of Disaccharides

The characteristic ions, $[M_s+M_r-H-2H_2O]^-$ and $[M_s+2M_r-H-4H_2O]^-$ at m / e 477 and 613, respectively, derived from reaction of lactose with NB, were selected to examine collisional activation. The lactose main fragment ions in the CA spectrum of $[M_s+M_r-H-2H_2O]^-$ appeared at m / e 417, 315 and 349, and those of $[M_s+2M_r-H-4H_2O]^-$ at m / e 315, 297 and 485. Both peaks at m / e 349 and 485 come from the corresponding parent ions by loss of a molecule of naphthalene. The daughter ion at m / e 315 was observed in the CA spectrum of $[M_s+M_r-H-2H_2O]^-$ and $[M_s+2M_r-H-4H_2O]^-$. Formation of this daughter ion may be attributed to loss of one of the sugar units.

CONCLUSIONS

The results described herein showed that negative ion fast atom bombardment in conjunction with collisional activation mass spectrometry using areneboronic acids as reagents provides a convenient and effective way to distinguish saccharide stereoisomers. The *in situ* reaction is highly stereospecific and reproducible. The application of this method to structural analysis of glycosides is in progress in our laboratory.

EXPERIMENTAL

1–Naphthylboronic acid (mp 211–2 ℃) and 2,4–dimethylphenylboronic acid (mp 198–9 ℃) were sythesized according to Refs. 11, 12. Cyclohexanediols were prepared according to Refs. 13, 14: *cis*–1,2 mp 96–7 ℃, *trans*–1,2 mp 124–5 ℃, *cis*–1,4 mp 111–2 ℃ and *trans*–1,4 mp 140–1 ℃. All saccharides used were chromatographically pure:

D–mannose	$[\alpha]_D^{20} = +14.0–14.5$	from Shanghai 2nd Chemical Factory
D–glucose	+52.5–53.0	from Shanghai 2nd Chemical Factory
D–galactose	+79.5–80.5	from Shanghai 2nd Chemical Factory
D–fructose	−88 − 92	from Shanghai 2nd Chemical Factory
L–sorbose	−42.5–43.0	from Shanghai 2nd Chemical Factory
L–rhamnose	+7.3 − 8.0	from Shanghai 2nd Chemical Factory
D–arabinose	−108	E. Merck
D–xylose	+19.0–20.0	Beijing 3rd Chemical Factory
Maltose	+130 ± 1	Institute of Biochemistry, Shanghai
Cellobiose	+33 − 36	Institute of Biochemistry, Shanghai
Lactose	+52	Xinzhong Chemical Co. Ltd. Shanghai
Sucrose	+66.4–66.6	Xian Chemical Factory, Xian

Glycerol and PEG–200 were obtained from Beijing Chemical Factory and used without further treatment. Argon (99.995%) and helium (99.995%) were obtained from Weiyuan Chemical Industry Co., Sichuan.

Method

The mass spectra were measured with a VG ZAB–HS mass spectrometer, an instrument of reversed geometry, fitted with a high field magnet and a FAB ion source and coupled to a PDP 11 / 250 data system. The fast atom gun was operated at 8KeV with a tube current of 1mA. Argon was used to provide the primary beam of atoms. The mass spectrometer was operated at a resolution of 3000 and accelerating voltage of 8KV.

The stainless steel probe tip was coated with a thin layer of the matrix PEG–200, 0.5μL of saccharide solution (100μg / μL in H_2O) and 1.5μL NB or 1μL DMPB (saturated in methanol) were added to the matrix. The probe was inserted into the mass spectrometer for immediate analysis. Each sample was run four times under the same conditions and each time the spectrum was the average of eight consecutive spectra. The standard relative deviation of these measurements was less than 10%. The mass scale was calibrated against glycerol, affording mainly a series of peaks every 92n+1 mass units.

The collisional activation spectra of characteristic ions $[M_s+M_r-H-2H_2O]^-$ and $[M_s+2M_r-H-4H_2O]^-$ were acquired by scanning the electric sector voltage from 420 to 50 V at a rate of 29 V / s. Helium was used as the collision gas at a pressure of 2×10^{-7}Torr measured at the analyser ionization gauge (background pressure 2×10^{-8}Torr).

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