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NMR spectroscopic analysis of the borate diol esters of methyl apiofuranosides

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Abstract

The borate diol esters formed by reacting methyl β-D- and methyl β-L-apiofuranosides with boric acid were studied by ^{11}B, ^{1}H and ^{13}C NMR spectroscopy, and by FABMS. Methyl β-D-apiofuranoside was shown to form more stable borate diol diesters and a monoester than those of methyl β-L-apiofuranoside. The borate diol diesters of methyl β-D-apiofuranoside are present as two diastereomers in approximately equal molar ratios.

Keywords: Boron; Borate esters; Methyl apiofuranosides; MS; NMR; Rhamnogalacturonan II

1. Introduction

Apiose, 3-*C*-hydroxymethyl-D-*glycero*-tetrose, is one of the components of rhamnogalacturonan II (RG-II), which is a low-molecular-weight, structurally complex pectic polysaccharide released from the primary cell walls of plants by treatment with *endo*-α-(1→4)-polygalacturonase [1]. The results of recent studies have demonstrated that in the primary cell walls of plants two chains of RG-II are crosslinked by a 1:2 borate diol ester to form a dimer (dRG-II–B) [2–8]. dRG-II–B is formed in vitro by treating monomeric RG-II (mRG-II) with boric acid. Divalent cations, including Pb^{2+}, Sr^{2+} and Ba^{2+}, promote dimer formation in vitro [4]. The apiosyl residues in RG-II are the most likely sites of borate esterification [4,5,9]. We have recently demonstrated that the apiosyl residue of the 2-*O*-Me-Xyl-containing side chain of RG-II is the site of borate esterification irrespective of whether dRG-II–B originated from the plant cell wall or was formed in vitro [10]. Thus, the naturally occurring and in vitro formed dimers are likely to have similar structures. Moreover, when borate binds OH-2 and OH-3 of two apiosyl residues, boron is a chiral atom and two diasteromers are formed: bis[methyl 3-*C*-(hydroxymethyl)-β-L-*threo*-furanoside]-(*S*)-2,3:2′,3′- and (*R*)-2,3:2′,3′-borate. ^{11}B NMR spectroscopy cannot distinguish two diasteromers in dRG-II–B because the chemical shift differences between two diasterotopic ^{11}B signals are smaller than the line width of the ^{11}B signals. The ^{1}H and ^{13}C NMR spectra of the dRG-II-

Abbreviations: RG-II, rhamnogalacturonan II; dRG-II–B, dimeric RG-II–boron complex; NBA, *p*-nitrobenzyl alcohol.

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B are complex, and borate-induced chemical shifts cannot be assigned. Thus, we have synthesized methyl apiofuranosides to use as model compounds to study the stereochemistry of the borate–apiose complex [11]. We now report on the structural analysis of borate–methyl apiofuranoside complexes by NMR spectroscopy and by FABMS.

2. Experimental

General methods.—Methyl 3-*C*-(hydroxymethyl)-β-D-*erythro*-tetrofuranoside (methyl β-D-apiofuranoside, **1**) and methyl 3-*C*-(hydroxymethyl)-β-L-*threo*-tetrofuranoside (methyl β-L-apiofuranoside, **2**) were synthesized and separated as previously described [11]. The borate complexes were prepared by mixing an equal volume of 0.1 M boric acid and 0.1 M methyl apiosides in D_2O. The pH of the solution was adjusted with ammonia solution in D_2O or 5 N NaOH solution. The total volume of the sample was 500 mL. The samples were equilibrated for 30 min prior to NMR spectroscopy.

NMR spectroscopy.—^{11}B NMR spectra were recorded at 25 °C with a Jeol Alpha 500 FTNMR spectrometer operating at 160 MHz with 0.1 M boric acid as the external reference [7]. ^1H and ^{13}C spectra were recorded at 25 or 4 °C with a Bruker DRX 600 NMR spectrometer at 600 MHz with acetone as the internal standard (δ 2.330) and 150 MHz with MeOH-d_4 as the internal standard (δ 49.30), respectively.

FABMS analysis. — Negative - ion - mode FABMS was recorded with a Jeol HX 110A mass spectrometer operated at an accelerating voltage of 10 kV [12]. *p*-Nitrobenzyl alcohol (NBA) was used as the matrix, and xenon gas was used as the bombarding gas.

3. Results and discussion

Interaction of borate anion with methyl β-D-apiofuranoside (**1**).—The extent of borate diol ester formation between **1** (L = 0.1 M) and boric acid (B = 0.1 M) at pH 4–12 was determined by using ^{11}B NMR spectroscopy. Borate diester formation of **1** occurred at pH 5.1. The amount of borate-esterified **1** increased with increasing pH. At pH 8.0, the signal corresponding to boric acid disappeared. Two signals at δ −8.0 (83 Hz) and δ −12.7 (53 Hz) were detected at pH 8.0, which correspond to the five-membered-ring esters of a borate diol diester ($B–L_2$) and a borate diol monoester (B–L) [13], respectively. The relative ratio of borate diol diester and monoester at pH 8.0, determined by integration of the ^{11}B signals, was 17:3. This ratio did not change until pH 12.0.

Negative-ion-mode FABMS of the methyl β-D-apiofuranoside–borate complex formed at pH 8.0 showed an intense peak at m/z 335 (M^-) that corresponds to two methyl apiosyl residues and one boron, and a weak peak at m/z 511 ($M + Na + NBA^-$). When **1** was reacted with $H_3^{10}BO_3$, an intense peak at m/z 334 (M^-) was observed. These results established that one mole of boron crosslinks two moles of methyl β-D-apiofuranoside.

To distinguish borate diol monoesters and diesters, ^1H and ^{13}C NMR spectroscopy was applied. When diol diesters form between borate and **1**, two diasteromers (*S* and *R* isomers, **4** and **5**, respectively) should be present. The ^1H NMR spectrum of the borate complex at pH 8.0 contained three signals in the anomeric proton region, in addition to the anomeric proton of **1**. From the signal intensity of the anomeric protons and of the ^{11}B signals, the small doublet at δ 4.902 was assigned to H-1 of B–L (methyl 3-*C*-(hydroxymethyl)-β-L-*threo*-tetrofuranose 2,3-borate, **3**). Two broad singlets at δ 4.889 and 4.899 were assigned to H-1 of the two diastereomers of $B–L_2$, bis[methyl 3-*C*-(hydroxymethyl)-β-L-*threo*-tetrofuranose)-(*S*)-2,3:2′,3′ and -(*R*)-2,3:2′,3′-borates] (**4**) and (**5**), respectively. The relative ratio of **4** and **5** was ∼1:1. The ^1H NMR chemical shifts of the three borate esters were completely assigned by DQF-COSY and 2D-NOESY (Fig. 1, Table 1). Nevertheless, the borate-binding sites could not be established by ^1H NMR spectroscopy.

The ^{13}C NMR chemical shifts of **3**–**5** were completely assigned by HMQC and HMBC (Table 2). The ^{13}C chemical shifts of C-2 and C-3 in **3**, **4** and **5** were ∼6–7 ppm downfield, suggesting that C-2 and C-3 of the apiosides

are the borate esterification sites. A chemical shift difference of between 3 and 10 ppm upon borate esterification has been reported for the ^{13}C nuclei. Moreover, the diasteromers **4** and **5** could not be distinguished by 2D-NOESY and HMBC.

As described earlier, a small amount of borate diesters formed at pH 5.1. It was difficult to assign the ^{1}H and ^{13}C chemical shifts. The borate esters formed at pH 8.0 were completely assigned. However, it is not certain whether stereochemically similar

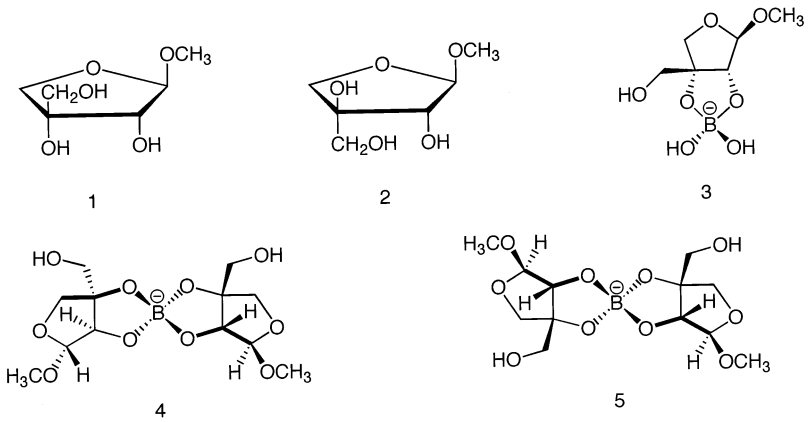

Fig. 1. Structure of compounds **1**–**5**.

Table 1
^{1}H NMR chemical shifts of methyl β-D-apiofuranoside (**1**) and its borate diol esters (**3**–**5**) at pH 8.0 and 25 °C

Compound	Relative proportion [a] (%)	Chemical shift (ppm) [b]						
		H-1	H-2	H-3′a [c]	H-3′b [c]	H-4a [c]	H-4b [c]	OCH_3
1	35	4.968 (3.7) [d]	3.936	3.637 (s)	3.637	3.891 (10.3)	4.044	3.441
3	5	4.902 (0.7)	4.019	3.655 (11.7)	3.608	3.847 (10.0)	3.800	3.339
4 [e]	29	4.889 (bs)	4.005	3.581 (11.8)	3.647	3.870 (10.0)	3.813	3.333
5 [e]	31	4.899 (bs)	4.005	3.584 (11.8)	3.647	3.838 (10.0)	3.805	3.334

[a] Obtained by ^{11}B NMR spectroscopy.
[b] Relative to external acetone (δ 2.230) in D_2O.
[c] Protons of the hydroxymethyl group and protons attached to C-4 are numbered as H-3′a and H-3′b, and H-4a and H-4b, respectively.
[d] Values in parentheses are coupling constants.
[e] The assignments for compounds **4** and **5** are interchangeable.

Table 2
^{13}C NMR chemical shifts of methyl β-D-apiofuranoside (**1**) and its borate diol esters (**3**–**5**) at pH 8.0 and 25 °C

Compound	Chemical shift (ppm) [a]					
	C-1	C-2	C-3	C-3′ [b]	C-4	OCH_3
1	110.20	77.30	80.08	64.23	74.32	56.78
3	110.70	83.42	86.92	65.38	75.08	55.08
4 [c]	110.18	83.25	87.09	65.13	74.86	55.06
5 [c]	110.28	83.42	86.94	65.13	74.90	55.06

[a] Values are referenced to the ^{13}C signal of external methanol-d_4 (49.70 ppm).
[b] Carbon of the hydroxymethyl group is numbered as C-3′.
[c] The assignments of **4** and **5** are interchangeable.

apoiside–borate dimers formed at pH 8.0 and 5.1.

O'Neill et al. [4], have reported that monomeric RG-II and boric acid react most rapidly at pH 3.8 in the presence of Pb^{2+}, Ba^{2+} and Sr^{2+}. When $Pb(OAc)_2$ was added to a mixture of **1** and boric acid at pH 3.8, no borate esterification was observed. Indeed, the pH-dependent borate esterification of **1** was not affected by the presence of Pb^{2+}. These results provide additional evidence that the conformation and structure of RG-II itself promotes borate diol diester formation [10].

*Interaction of borate anions with methyl β-*L*-apiofuranoside* (**2**).—^{11}B NMR spectroscopy showed that borate ester formation of **2** occurred only at pH > 8.3. About 30% of borate bound **2** to form two borate monoesters (B–L); that is, five-membered-ring esters at δ −13.9 (70 Hz) and six-membered-ring esters at δ −18.3 (80 Hz) [13]. At pH 12, the broad peak of free borate anion (δ −6.5) disappeared and a small signal at δ −9.8 (75 Hz), which corresponds to $B–L_2$, was observed. The exchange between boric acid and the borate ester complex of **2** was slow on the ^{11}B NMR time scale, but the exchange was very fast on the ^{1}H NMR time scale, even at 4 °C. Thus, there were no clear correlations of the signals of the borate complexes in the DQF-COSY spectrum. Consequently, the binding sites of borate in **2** could not be determined. Nevertheless, the results indicate that the *threo* hydroxyl groups in **2** do not favor the formation of stable borate esters.

4. Conclusions

Methyl β-D-apiofuranoside formed more stable borate esters. Bis[methyl 3-*C*-(hydroxymethyl)-β-L-*threo*-tetrofuranose]-(*S*)-2,3:2′,3′- and -(*R*)-2,3:2′,3′-borates occurred predominantly in almost equal molar ratio of the two diastereomers, **4** and **5**. Although ^{13}C and ^{1}H NMR spectroscopy established that two diastereomers (*S* and *R*) are formed, it was not possible to assign signals to each isomer. Borate ester formation occurred above pH 8.0 in **2** that has the *threo* configuration. B–L was predominant, although some $B–L_2$ was present.

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