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Note

The study of the relationship between retention and structure on D-mannose and its derivatives with high-performance anion-exchange chromatography

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High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) has proved to be a very useful method for the compositional and structural analysis of mono- and oligo-saccharides derived from glycoproteins because of the advantages of allowing rapid and direct quantitation of either underivatized or derivatized samples and the ability to characterize samples through coelution with samples of known structures or through retention time comparison [1–6]. But for unknown samples, standard references are needed, which may hinder the use of HPAEC-PAD because the required standard carbohydrates are often expensive and sometimes unavailable. The study of the relationship between carbohydrate structure and retention time on an anion-exchange separation column may help to interpret the chromatogram of an unknown carbohydrate sample [7], especially when the related standard references are unavailable. This research could also provide information on the reaction activity of different hydroxyl groups for some synthetic intermediate products in the process of carbohydrate syntheses.

Most publications on carbohydrate analysis by HPAEC-PAD focus on the separation and characterization of oligosaccharides derived from glycoproteins [8–13], and efforts have also been made to illustrate the relations between retention and structures of carbohydrates. Paskach et al. [14] studied ninety-three sugars and sugar alcohols of one to four monosaccharide units with HPAEC on quaternary ammonium resins under

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alkaline conditions. The effects of column, molecular structure, acidic dissociation constant, chain length and substituent on capacity factors were discussed, and the response of pulsed amperometric and differential refractive index detectors were also investigated. Lee [7] determined the elution volume of 18 monosaccharides by using isocratic elution with 16 mM NaOH and discussed the differences in elution volumes and structures of these sugars. Koizumi et al. [15] analyzed positional isomers of methyl ethers of D-glucose and concluded that the reduction in retention time resulting from *O*-methylation follows the order of 2-OH > 3-OH > 6-OH ≥ 4-OH > 1-OH.

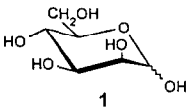
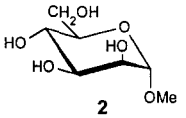
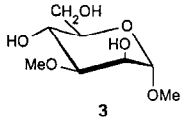
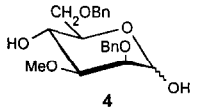
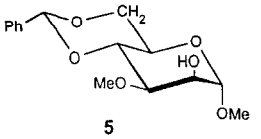
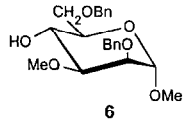
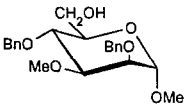
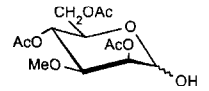
The goal of this study is to demonstrate the relationship between structure and retention time for the D-mannose and its derivatives by determining the difference in retention time on a Dionex CarboPac PA-1 column. The influence of the number and position of the OH groups and some other substituent groups on the retention time of D-mannose and its derivatives are investigated. The samples, as well as the synthetic intermediates, are studied to see if there are any interrelationships between elution positions and reaction activities.

Detection of electrochemically active and inactive compounds.—The structures of all compounds used in the study are shown in Table 1. For the detection of electrochemically active samples, the PED-II detector was used along with the detection conditions described in the Experimental section. Compounds **1**, **2**, **3** and **8** were found to have electrochemical activity and could be detected by the PED-II detector. Compound **4** had both electrochemical activity and UV absorption and could be detected by both detectors. Other samples could only be detected by the VDM-II detector because of the lack of electrochemical activity. This is in agreement with their respective molecular structures. The compounds that have either a 1-OH or 6-OH group can be oxidized on the surface of the gold electrode in high-pH eluent and can therefore be detected by the electrochemical detector. The compounds that have either a benzyl group or a benzal group can be detected only by the UV spectrophotometric detector. However, compound **7** does not have electrochemical activity although there is a 6-OH group in the molecule. This may be caused by the influence of the benzyl group in positions **2** and **4**.

Definition of the maximum absorption for all samples.—To detect samples at maximum sensitivity which do not have electrochemical activity using a UV spectrophotometric detector, the absorption profiles of all samples were determined, and the results are shown in Fig. 1. We demonstrate in Fig. 1 that, for most of the compounds, the optimum absorption occurs about at 205 nm. However, in our experiment, the analysis wavelength of the VDM-II detector was set at 214 nm because the background absorption of the eluent was so serious that the detector could not be offset to zero when a shorter wavelength was used. From Fig. 1 we also find that compounds **4**, **5**, **6** and **7** have strong absorption at 214 nm and are suitable to be detected by the VDM-II detector.

Influence of mannose and its derivative structures on their retention times.—Carbohydrates behave as weak acids because they possess multiple hydroxyl groups. In high-pH eluents such as 10–200 mM sodium hydroxide, they are partially or completely anionic, and can be retained and separated on an anion-exchange column. The anomeric hydroxyl group is more acidic than any of the other hydroxyl groups on a reducing sugar. For methyl α,β-D-glucopyranoside, each of the hydroxyl groups has a somewhat

Table 1
Structures and retention times of all samples compounds **1–8**

Structure [a]	Retention time (min) [b]
 **1**	4.58
 **2**	1.42
 **3**	1.31
 **4**	12.40
 **5**	2.16
 **6**	4.80
 **7**	5.30
 **8**	3.07

[a] Ac = acetyl; Bn = benzyl; Ph = phenyl.
[b] Eluent: 40 mM NaOH, isocratic; column: CarboPac PA-1, 4.5×250 mm.

different pK_a value [16]. Roberts et al. [17] reported the acidity of hydroxyl groups in methyl α- or β-D-glucopyranoside to be 2-OH > 6-OH > 3-OH > 4-OH when NaOH is ≤ 0.1 M. It is understandable that masking or transformation of some of the hydroxyl groups should greatly influence the elution positions [7]. The results in Table 1 show that without a large substituent group, such as a benzyl or benzal group, the position and

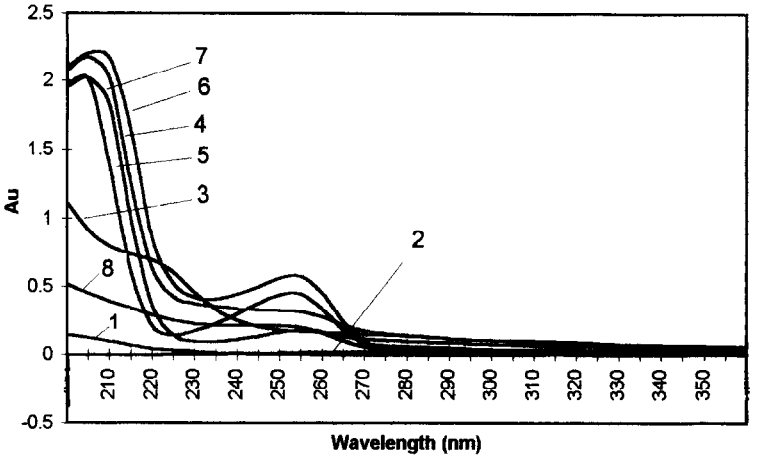


Fig. 1. Absorption graphs of all samples. Concentrations of all samples are 500 mg/L, Shimadzu UV-120-02 spectrophotometer with 1 cm quartz cells.

number of hydroxyl groups in a sugar are two major factors influencing the retention time of the sugar. The order of retention times of compounds **1**, **2** and **3** was as follows: $1 > 2 \geq 3$. This means the retention time decreases with decreasing numbers of hydroxyl groups. In comparing the retention times of compounds **1–3**, we find that the 1-OH group plays a more important role in the separation of monosaccharides than OH groups in other positions. Masking of the 1-OH group with a methyl group (compounds **2** and **3**) results in a pronounced reduction in retention time. But the retention time of compound **4** is very long, although it possesses only two hydroxyl groups. Considering the 3D structure of **4**, the two benzyl groups at the 2- and 6-positions are spread into space, making the molecule larger than that of the other samples. First, a larger molecule may retain longer than a smaller one on the column. Secondly, the voluminous benzyl group is hydrophobic and may have an affinity to the resin matrix, which itself is medium-high in hydrophobicity, thus increasing the retention time. Thirdly, the acidity of the 1-OH group may be strengthened due to the presence of 3-*O*-methyl and 2,6-di-*O*-benzyl groups. This can be verified by our synthetic experiments in which we found that the reaction activity of a 1-OH group could be increased dramatically when the 2,6-OH groups were masked with benzyl groups (unpublished results).

For compounds **5**, **6**, **7** and **8**, the order of elution is **7** > **6** > **8** > **5**. The structures of compounds **7** and **6** are similar, and the difference in retention time may be considered in two aspects. First, the acidity of the 4- and 6-OH groups is different. Although the 6-OH is a primary hydroxyl group and the 4-OH is a secondary one, the difference in acidity in D-mannose is very small. Secondly, the presence of a 3-*O*-methyl group renders the 4-OH less accessible to the resin matrix, but the 6-OH is more easily accessible to the resin matrix because of their molecular structures. Although compound **8** possesses a 1-OH group, its retention time is shorter than that of either compound **6** or **7** due to the presence of hydrophilic 2-, 4- and 6-OAc groups. In addition, the molecular volume of compound **8** is smaller than that of either compound **6** or **7**. For compound **5**,

there is neither a 1-OH nor a hydrophobic benzyl group in molecule; therefore, the retention time is shorter than that of either compound **6**, **7** or **8**.

The retention times of all compounds follow the order of **4** > **7** > **6** > **1** > **8** > **5** > **2** > **3**. The cause of the extreme increase in retention time for sample 4 has been discussed above. The retention times of samples 6 and 7 are shorter than that of **4** because of the lack of a 1-OH group, but longer than that of 1 due to the presence of a benzyl group. Comparing the retention times of 8 and the latter three compounds (**2**, **3**, and **5**), we can also determine the importance of a 1-OH group. Although compounds **2** and **3** have more OH groups than **8**, and **5** has a benzal group, they are all retained less strongly because they lack a 1-OH group.

Generally, the reactivity of different hydroxyl groups in D-mannose may be reflected in the retention time. As described above, 1-OH is very important in retaining these samples on the column. Comparing compounds **6** and **7**, we find that the 6-OH is more important than 4-OH in retaining them on the column. Koizumi et al. [15] reported the contribution of each hydroxyl group of D-glucose and its derivatives to retention time, and the result indicated that the presence of a free 1-OH group reduced the effect of differences in acidities of the other hydroxyl groups, and other effects, such as hydrophobic interactions, might prevail. We find that the influences of 1-OH in D-mannose and D-glucose on retention times are very similar. Some substituent groups, especially the benzyl group, can dramatically change the retention time of the sample.

2. Experimental

Instrumentation.—A Dionex Model DX-300 ion chromatograph equipped with a 110-μL sample loop, Dionex CarboPac PA Guard column (3 × 25 mm) and CarboPac PA-1 column (4 × 250 mm) were employed. The detectors were a Dionex pulsed electrochemical detector Model-II (PED-II) and a Dionex variable wavelength detector Model-II (VDM-II). The former was operated in its integrate amperometry mode.

Because some of the samples are short of electrochemical activity on the Au electrode, the Dionex Model PED-II and Dionex Model VDM-II detectors were connected in tandem to detect sequentially the electrochemically active and inactive compounds. The experiments were run at a 1.0 mL/min eluent flow-rate using a pre-degassed solution of 40 mM sodium hydroxide. For the VDM-II detector, the wavelength used for analysis was set at 214 nm, and for the PED-II detector, the pulse potentials and duration were as follows: time = 0 s, E = +0.05 V; 0.48 s, +0.05 V; 0.49 s, +0.70 V; 0.61 s, +0.70 V; 0.62 s, −0.70 V; 0.69 s, −0.70 V; integration: begin, 0.28 s, end, 0.48 s. A gold electrode served as the working electrode. All the operations were controlled by a Dionex AI-450 chromatography workstation. Output data from the two detectors were recorded and stored in the computer.

Reagents.—Sample: All the samples except D-mannose were synthesized according to refs. [18,19].

Sodium hydroxide solution (50% w/w) was prepared using 10 MΩ deionized water and handled according to the procedures in ref. [20].

Procedure.—About 5 mg of each sample was dissolved with 1.5 mL of methanol and diluted to 10 mL with deionized water, and a 110 mL sample solution (~ 50 mg of sugar) was injected into the ion chromatography system to determine the retention time.

To define the maximum UV absorption for all samples, the absorption graphs for all samples were determined by using a Shimadzu UV-120-02 spectrophotometer with 1-cm quartz cells.

Acknowledgements

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