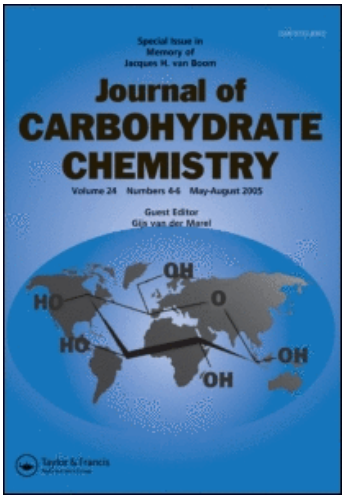

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A HIGHLY SENSITIVE METHOD TO IDENTIFY THE D,L-CONFIGURATIONS OF MONOSACCHARIDES BASED ON (-)-TBMB CARBOXYLIC ACID AND HPLC

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ABSTRACT

D,L-Isomers of monosaccharides were coupled with a fluorescent chiral derivatizing agent, (-)-2-*tert*-butyl-2-methyl-1,3-benzodioxole-4-carboxylic acid [(-)-TBMB carboxylic acid], to afford a pair of diastereoisomers. The two were well separated with HPLC (reverse phase conditions; ODS, CH_3CN:H_2O:*iso*PrOH) within 60 min and could be detected by fluorescence of the TBMB group at the few pico-molar level.

INTRODUCTION

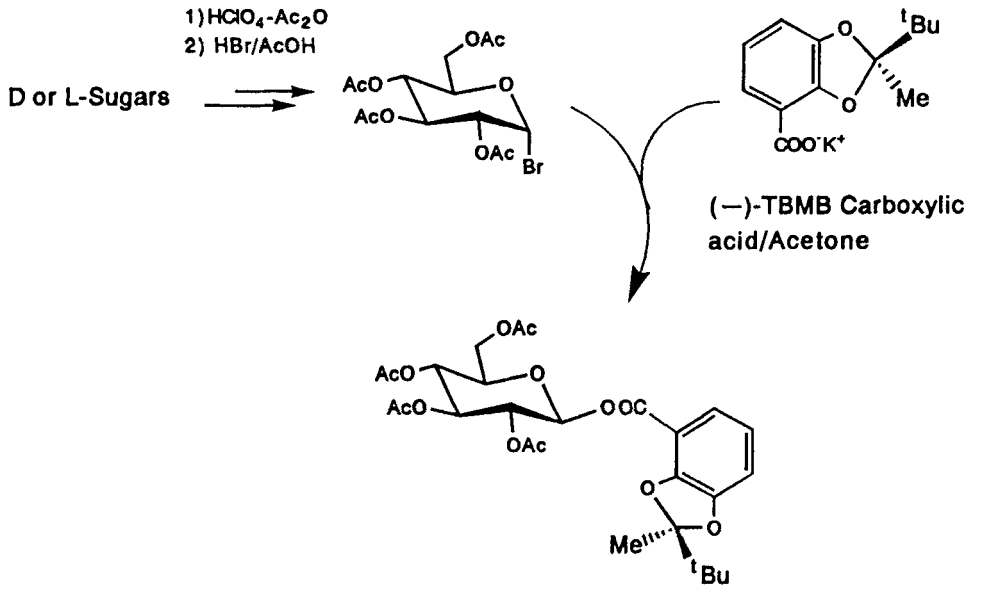
Chemical or enzymic syntheses of sugars with unnatural L-configuration are of interest to us because these sugars may have different biological properties from the corresponding D-isomers, for example in their interactions with lectins or antibodies. Moreover, some monosaccharides like galactose, fucose, arabinose and rhamnose are found as naturally occurring L-sugars. Thus, identification of the D,L-configuration should be important when these sugars are isolated in nature. This is, however,

difficult especially when the amount of isolated sugars is not enough for the measurements of optical rotations nor enzymic degradations. Development of more sensitive methods to identify the D and L-isomers, therefore, are important.

Several methods have been developed[1-8] after the gas chromatographic method of Vliegenthart *et al.*.[3,4] Recently, we reported an ^1H NMR method[8] using a chiral derivatizing agent, (-)-TBMB carboxylic acid[9-11] (SCHEME) in which strong signals from the *tert*-butyl and methyl groups of the derivatized sugar provided diagnostic signals for discriminating D-sugars from L-sugars. In the present paper, we would like to show that D,L-monosaccharides can be separated by HPLC after derivatization with this agent to form a pair of diastereoisomers. Taking advantage of TBMB carboxylic acid as a fluorescence marker will enable us to detect and discriminate between D,L-isomers of monosaccharides in a highly sensitive manner.

RESULTS AND DISCUSSIONS

In our previous NMR study,[8] we reported a general route to derivatize monosaccharides with (-)-TBMB carboxylic acid (SCHEME) Here, the derivatization procedure was modified for micro analysis by HPLC. The typical procedure is as follows; reducing sugar (*ca.* 0.01 mg, *ca.* 50 nmol) was treated at room temperature with 0.1 mL of acetic anhydride containing a catalytic amount of $HClO_4$ (*ca.* 0.01 %). After 15 min, 0.1 mL of EtOH was added, and the mixture was kept at room temperature for 30 min. K_2CO_3 (1 mg) and 0.1 mL of CH_2Cl_2 were added, and the mixture was centrifugated. The supernatant was collected and concentrated *in vacuo*. The residue was dissolved in 0.1 mL CH_2Cl_2 and treated with 0.2 mL of 33% HBr/AcOH (commercially available) for 1h. The mixture was concentrated *in vacuo* below 60 ℃ and dissolved in acetone (0.1 mL). To the solution was added an excess amount of (-)-TBMB carboxylic acid and an equimolar amount of potassium hydrogern carbonate. The mixture was warmed at 60 ℃ for 1 h. A portion of the solution was applied to silica gel TLC coated on a aluminium sheet (5 cm x 5 cm, available as DC-Alufolien from Merck) and developed with toluene-ethyl acetate (2/1).



SCHEME

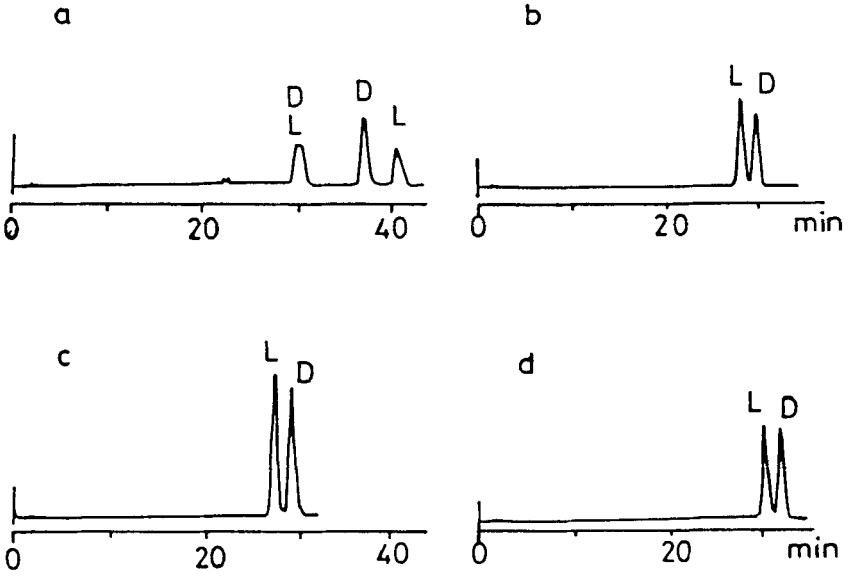


FIGURE. HPLC separations of D,L-isomers of rhamnose (a), glucose (b), galactose (c) and fucose (d). (HPLC conditions are cited under the Table).

Table Reverse Phase HPLC Separations of 1-*O*-(-)-TBMB-D- and L-Sugars.

Sugars	Rt(min)	Configurations	α [a]	Rs [b]
Xylose	28.3	L (*1R*)		
	29.8	D (*1S*)	1.05	1.39
Arabinose	24.8	D (*1R*)		
	25.7	L (*1S*)	1.04	0.93
Glucose	28.5	L (*1R*)		
	30.1	D (*1S*)	1.06	1.33
Galactose	27.5	L (*1R*)		
	29.3	D (*1S*)	1.07	1.69
Fucose	30.6	L (*1R*)		
	32.4	D (*1S*)	1.06	1.32
Mannose	27.1	D (*1R*)		
(1,2-*trans*)	28.5	L (*1S*)	1.04	1.05
Mannose	22.3	L (*1R*)		
(1,2-*cis*)	22.8	D (*1S*)	1.02	0.45
Rhamnose	36.9	D (*1R*)		
(1,2-*trans*)	40.3	L (*1S*)	1.09	2.00
Rhamnose	30.0	L (*1R*)		
(1,2-*cis*)	30.0	D (*1S*)	1.00	0.00

Condition: ODS column (150 mm x 4.6 mmϕ), solvents: CH_3CN:H_2O:isPrOH = 4:4:1, Flow rate = 0.8 mL/min.

a. Separation factor = (retention volume of one diastereoisomer - void volume of the column)/(retention volume of another diastereoisomer - void volume of the column).

b. Resolution factor = 2 x (distance between the peaks of two diastereoisomers)/(sum of band width of the two peaks).

A fluorescent band (Rf = *ca.* 0.5) was cut off from the sheet, the silica gel extracted with CH_3CN, and a sample from the extract injected onto an HPLC column (ODS column, 4.6 mm ϕ x 15 cm; CH_3CN:H_2O:isoPrOH = 4:4:1) for analysis.

The coupling reaction yielded 1,2-*trans* pyranosides exclusively for Glc, Gal, Fuc, Xyl and Ara. Man and Rha with an *axial* OAc-2 group gave a mixture of 1,2-*trans* and *cis* pyranosides in *ca.* 2:1 ratio. The isomers could be easily separated on silica gel TLC at the final stage. When racemic TBMB carboxylic acid and D-glucose were used for the reaction, a 1:1 mixture of the two diastereoisomers of 1-*O*-[(+)- and (-)-TBMB carbonyl]-D-glucose derivatives were obtained. This means that there is no D,L-discrimination by the chirality of (-)-TBMB carboxylic acid in the present coupling reaction.

HPLC results in the FIGURE and from the summarized data in the Table indicated that D,L-isomers of TBMB carbonylated sugars were well separated in 20-45 min under the conditions described above. Although the D,L-separation of 1,2-*cis* anomers of mannose and rhamnose was insufficient, the main 1,2-*trans* anomers were well separated enough to carry out the D,L-sugar identification.

As expected, the elution order of the D,L-isomers was governed by the anomeric configuration; (*1R*)-isomer was always eluted faster than the (*1S*)-isomer (Table), and this rule is practically useful for the assignment of the D,L-configuration since the coupling reaction affords mainly 1,2-*trans* isomers.

CONCLUSIONS

An HPLC method was developed to determine the D,L-configurations of monosaccharides which involves a coupling reaction with (-)-TBMB carboxylic acid and per-*O*-acetyl pyranosyl bromides to give diastereoisomeric 1-*O*-TBMB carbonylated sugars which could be separated by HPLC. The minimum amount of sugars for the fluorescence detection could be reduced to a few pico mole using an HPLC column, thus allowing highly sensitive discrimination between the D,L-sugar isomers. This approach will become a complementary way to the NMR spectroscopy method reported previously.[8]

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