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Note

High-performance liquid chromatography of hexopyranosides and hexofuranosides

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Several reports have appeared on the analysis of mono-, di- and oligosaccharides by high-performance liquid chromatography (HPLC)[1–5]. The method was recently developed to separate the oligosaccharides and glycopeptides in the liver and the urine of patients of lysosomal storage disorders[6]. The separation of mono- and oligosaccharides and their anomers was recently studied by Kahle and Tesařík[7]. In the study of glycosides, Ward and Pelter[8] showed that methyl β-D-glucopyranoside-2,3,6-tribenzoate was eluted before the α-anomer in the analysis of naturally-occurring organic compounds by HPLC. Lehrfeld[9] separated anomers of monosaccharides, oligosaccharides, and methyl D-glucopyranoside as their perbenzoylated derivatives, and the α anomer of methyl D-glucopyranoside was eluted before the β anomer. Recently, the HPLC separation of acetyl derivatives of flavone and flavanol glycosides was reported[10].

The present investigation has been undertaken in order to separate hexopyranoside and hexofuranoside derivatives, and numerous alkyl and aryl D-glucosides, D-galactosides, D-mannosides, D-glucosiduronic acids, and their acetyl and benzoyl derivatives in pyranose or furanose form were chromatographed.

EXPERIMENTAL

All separations were carried out on a Waters Assoc. (Milford, MA, U.S.A.) instrument with a Model M-600A solvent delivery system, Model U6K sample injector, Model R401 differential refractometer, and Model M440 UV detector. Unless otherwise stated, the differential refractometer was used for detection. HPLC was performed on a Waters Assoc. μBondapak-carbohydrate column (30 cm × 4 mm I.D.), and Radial-Pak B column (10 cm × 8 mm I.D.). All operations were carried out at room temperature. Acetonitrile–water (9:1) was used for hexosides as a mobile phase. Benzene–ethyl acetate (9:1) was used for acetyl derivatives and benzene–chloroform (85:15) and benzene–ethyl acetate (99:1) were used for benzoyl derivatives as a mobile phase. The flow-rate was 2.0 ml/min.

Acetylation of hexoside was performed with acetic anhydride in pyridine[11]. Benzoylation of hexoside was performed with benzoyl chloride in pyridine[12].

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RESULTS AND DISCUSSION

Hexosides

Table I shows the retention times of the examined hexopyranosides and hexofuranosides in acetonitrile–water (9:1) on the μBondapak-carbohydrate column. Hexopyranosides and hexofuranosides were clearly dissolved. The furanosides of D-

TABLE I
RETENTION TIMES OF HEXOSIDES ON A μBONDAPAK-CARBOHYDRATE COLUMN
Mobile phase: acetonitrile–water (9:1). p = Pyranose, f = furanose.

Aglycon	*Ring form*	*Anomer*	*Retention time (min)*	*Ref.*
D-*Glucoside*				
Methyl	p	α	7.4	13
	p	β	7.2	13
	f	α	5.3	14
	f	β	5.5	14
Ethyl	p	β	6.0	15
	f	β	4.3	16
Phenyl	p	α	4.0	17
	p	β	3.8	17
	f	β	3.4	18
m-Tolyl	p	α	3.8	19
	p	β	3.6	19
	f	β	3.1	18
Guaiacyl	p	β	4.1	19
	f	β	3.3	18
2-Naphthyl	p	α	3.7*	19
	p	β	3.5*	19
	f	β	3.1*	20
D-*Galactoside*				
Methyl	p	α	7.4	21
	p	β	8.1	22
	f	β	5.0	23
Ethyl	p	α	5.6	21
	p	β	6.4	24
	f	β	4.4	25
Phenyl	p	α	3.9	26
	p	β	3.9	27
	f	β	3.1	28
m-Tolyl	p	α	3.6	29
	p	β	3.6	30
	f	β	3.0	31
Guaiacyl	p	α	4.0	29
	p	β	4.4	29
	f	β	3.1	31
D-*Mannoside*				
Methyl	p	α	4.9	32
	f	α	4.7	32
D-*Glucosiduronic acid*				
2-Naphthyl	p	β	1.6	33
	f	β	1.8	20

* UV detection.

glucoside, D-galactoside, and D-mannoside were always eluted before the corresponding pyranosides; the pyranoside of D-glucosiduronic acid eluted before the corresponding furanoside. The order of retention of methyl D-glycosides was α-f < β-f < β-p < α-p for the methyl D-glucosides, and β-f < α-p < β-p for the methyl D-galactosides. The β anomer of D-glucopyranoside was always eluted before the α anomer; the α anomer of D-galactopyranoside was eluted before the β anomer or at the same time. In the separation of carbohydrate on the μBondapak-carbohydrate column, acetonitrile–water has been used as a mobile phase[1,3]. For the separation of hexopyranoside and hexofuranoside, a good separation was obtained with acetonitrile–water (9:1). With acetone–water (98:2) as mobile phase a broad peak was obtained. With methanol–water or ethanol–water as the mobile phase, it was not possible to separate hexosides.

Acetyl derivatives

Table II shows the retention times of acetyl and benzoyl derivatives of D-hexosides. Compared with the data shown in Table I, some differences in elution order were observed. The elution order of methyl D-glycosides was α-f < β-f < α-p < β-p for the methyl D-glucoside, α-p < β-p < β-f for the methyl D-galactoside, and α-f < α-p < β-p for the methyl D-mannoside. Furanosides were eluted before pyranosides for alkyl D-glucosides, and the α anomer of aryl D-glucopyranoside was eluted before β-furanoside. The elution order of aryl D-glucoside was α-p < β-f < β-p, with exception for guaiacyl D-glucoside. The elution order of alkyl and aryl D-galactoside was α-p < β-p < β-f. It is interesting that the α and β anomer of pyranoside have shorter retention times than β-furanoside. The separation of D-hexopyranosides and D-hexofuranosides was not so effective as that obtained on the μBondapak-carbohydrate column, except for methyl D-mannoside. The α and β anomers of hexopyranosides, however, could be better separated by conversion to the acetyl ester. With dichloromethane–ethyl acetate (99:1, 95:5) or benzene–chloroform (97:3) as the mobile phase, the separation of hexoside isomers was unsuccessful.

Benzoyl derivatives

As shown in Table II, the separation of hexopyranosides and hexofuranosides was not so effective as that obtained on a μBondapak column, except for methyl D-mannoside. However, four isomers of methyl D-glucoside were most clearly resolved from each other with benzene–ethyl acetate (99:1) under all conditions studied in this work. The order of retention of methyl D-glucosides was α-p < α-f < β-f < β-p. α Anomers of methyl D-glucopyranoside and D-glucofuranoside were eluted before the corresponding β anomers. This result is in agreement with the observation made by Lehrfeld[9] in the study of benzoylated carbohydrates on a Corasil II column that methyl α-D-glucopyranoside was eluted before methyl β-D-glucopyranoside. The α and β anomers of alkyl D-glucopyranoside and D-galactopyranosides were more clearly separated than those of the acetyl derivatives. The elution order of aryl D-glucoside was α-p < β-f < β-p, with exception for 2-naphthyl D-glucoside. The elution order of alkyl and aryl D-galactoside was α-p < β-f < β-p, with exception for *m*-tolyl D-galactoside. With dichloromethane–diethyl ether (95:5) or dichloromethane–*n*-propanol (95:5, 8:2) as the mobile phase, the separation of hexoside isomers was unsuccessful.

TABLE II
RETENTION TIMES OF ACETATES AND BENZOATES OF HEXOSIDES ON A RADIAL-PAK B COLUMN
Mobile phase: A = benzene–ethylacetate (9:1); B = benzene–chloroform (85:15); C = benzene–ethylacetate (99:1). p = Pyranose; f = furanose.

Aglycon	*Ring form*	*Anomer*	*Retention time (min)*		
			Acetate	*Benzoate*	
			A	*B*	*C*
D-*Glucoside*					
Methyl	p	α	6.5	4.9	5.6
	p	β	7.3	6.5	7.6
	f	α	6.3	5.7	6.6
	f	β	6.4	6.0	7.2
Ethyl	p	β	6.4		
	f	β	5.8		
Phenyl	p	α	4.0	3.1	3.5
	p	β	5.0	3.5	3.7
	f	β	4.6	3.2	3.6
m-Tolyl	p	α	3.9	3.1	3.4
	p	β	4.6	3.8	4.2
	f	β	4.3	3.3	3.6
Guaiacyl	p	β	6.6		
	f	β	6.7		
2-Naphthyl	p	α	3.8	3.0	3.4
	p	β	4.7	3.2	3.5
	f	β	4.3	2.9	3.0
D-*Galactoside*					
Methyl	p	α	6.0	5.0	5.7
	p	β	6.8	6.0	6.6
	f	β	7.4	5.4	6.0
Ethyl	p	α	5.2	4.4	4.8
	p	β	6.1	5.4	6.1
	f	β	6.5	4.9	5.3
Phenyl	p	α	4.0	3.1	3.5
	p	β	4.7	3.9	4.4
	f	β	5.1	3.3	3.7
m-Tolyl	p	α	3.6	3.1	3.3
	p	β	4.5	3.6	3.8
	f	β	4.9	3.1	3.3
Guaiacyl	p	α	5.3	5.4	5.9
	p	β	6.5	5.8	6.6
	f	β	7.7	5.6	6.3
D-*Mannoside*					
Methyl	p	α	6.8	6.1	7.0
	p	β	9.5		
	f	α	6.0	5.8	6.4

CONCLUSION

Hexopyranosides and hexofuranosides can be separated on a μBondapak-carbohydrate column with acetonitrile–water (9:1) as the mobile phase. The α and β

anomers of hexopyranosides can be separated by conversion to their acetyl or benzoyl esters, and subsequent analysis on a Radial-Pak B column. Four isomers of methyl D-glucoside were well separated from each other by conversion to their benzoy. ester, and subsequent analysis on a Radial-Pak B column.

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