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Note

Gas chromatographic separation of the methyl ether methylglycopyranoside series hexose, 6-deoxyhexose and pentose acetates

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The derivatives used most widely for determining the positions of glycosidic linkages in sugar residues in polysaccharides are partially methylated alditol acetates[1]. They offer information on glycosidic linkages by means of their mass spectra, while the kind of monosaccharide epimer can be determined by its gas–liquid chromatographic (GLC) retention index (R_t). Derivatives of this type have some limitations. Some of them, owing to the overlapping of GLC peaks, have been overcome by using ethyl ethers instead of methyl ethers[2] or by additional separation using thin-layer chromatography[3]. However, the alditol acetates can be applied only with the aldose- and ketose-containing polysaccharides. A general method for investigating substances containing hexosamine, N-acetylneuraminic acid (NANA) and uronic acid, involves methanolysis. This reaction has the advantage over hydrolysis of preventing the extensive destruction of neutral sugars[4,5]. The double number of methyl ethers in the methanolysis products is likely to complicate their chromatographic separation owing to the formation of α- and β-anomers. On the other hand, each sugar is characterized by four GLC values, *viz.*, two R_t values for the anomers, and their partial peak areas (R_a).

To increase the volatility, partially methylated sugars are converted into trimethylsilyl ethers or acetylated derivatives. The latter seem preferable for two reasons. Firstly, the mass spectra of this type of derivative of aldoses[6], hexosamine[7–10], NANA[11,12] and uronic acids[13] have been extensively studied. Secondly, the acetate derivatives are capable of being stored. Also, one can readily obtain samples of methyl ether methylglycosides by partial methylation of the methylglycoside required[5,14,15]. However, the available R_t data of some methyl ethers, methyl α- and β,L-Ara[16], α- and β,D-Glc[17], β,D-Xyl[15] and α,D-Gal[5] acetate are not sufficient for the wide use of these derivatives in analysing the methylation of aldose-containing polysaccharides. This paper presents retention indices for the required set of methyl ether acetates of the following methylglycosides: α- and β,D-Glc, α- and β,D-Gal, α,D-Man, α- and β,D-Qui, α- and β,L-Fuc, α,D-Rha, α- and β,D-Xyl, α- and β,L-Ara and α,D-Lyx.

EXPERIMENTAL

A method of preparing the required set of methyl ether methylglycosides has already been described[14]. The GLC separation was performed on Pye Unicam 104 GLC and LKB 9000s GLC–MS instruments. In determining R_t values we used columns, liquid phases and operating conditions as follows: 200 × 0.4 cm I.D. glass column containing 3% QF-1 (A) and 3% NPGS (B) on Chromosorb W (100–120 mesh). Columns A and B were temperature programmed at 5°/min from 110° to 230°. Argon was used as the carrier gas at a flow-rate of 60 ml/min. The GLC–MS system was operated with 1% liquid phase column and with helium as the carrier gas at a flow-rate of 25 ml/min. The R_t scale was O-methyl-2,3,4-O-trimethyl-β,*D*-Xyl; 1000- aldononitrile of D-Gal acetate.

Three types of measurement were made. To a first approximation an R_t value was determined by injecting into the column a set of methyl ethers of each methylglycoside, dissolved in chloroform secondly, to determine the relative R_t, two sets of methyl ethers of the same type of methylglycoside (*e.g.*, hexosides) were injected into the column simultaneously in different proportions. Thirdly, if the peaks of the methyl ethers coincided, they were identified by studying the mass spectra taken over the GLC peak contour.

RESULTS AND DISCUSSION

The R_t data are given in Tables I–VIII according to the monosaccharide ring methylation pattern. In each table, the list of sugars is divided into epimer groups as the weight of the C-5 substituent increases. Within each group, the epimers listed follow one another as the R_t value of the α-anomer increases, followed by the β-

TABLE I

RETENTION INDICES OF Me 2,3,4-OMe GLYCOPYRANOSIDES

C-5 substituent	*Anomer*	*Sugar*	*QF-1*	*NPGS*
H	α	Xyl	031	039
	β	Xyl	000	000
	α	Lyx	055	057
	α	Ara	116	128
	β	Ara	112	128
CH_3	α	Qui		000
	β	Qui		030
	α	Rha	016	008
	α	Fuc	085	078
	β	Fuc	074	063
CH_2OMe	α	Glc	183	190
	β	Glc	125	123
	α	Man	183	190
	α	Gal	200	230
	β	Gal	200	225
CH_2OAc	α	Glc	407	417
	β	Glc	352	360
	α	Man	407	436
	α	Gal	442	494

TABLE II

RETENTION INDICES OF Me 2,3-OMe_2 GLYCOPYRANOSIDE ACETATES

C-5 substituent	*Anomer*	*Sugar*	*QF-1*	*NPGS*
H	α	Ara	202	254
	β	Ara	220	254
	α	Xyl	270	265
	β	Xyl	220	203
	α	Lyx	282	286
CH_3	α	Fuc	200	193
	β	Fuc	172	181
	α	Qui	257	209
	β	Qui	222	170
	α	Rha	287	222
CH_2OMe	α	Gal	352	399
	β	Gal	324	371
	α	Glc	436	436
	β	Glc	385	371
	α	Man	448	449
CH_2OAc	α	Gal	539	589
	β	Gal	502	560
	α	Glc	620	640
	β	Glc	585	584
	α	Man	639	640

TABLE III

RETENTION INDICES OF Me 2,4-OMe_2 GLYCOPYRANOSIDE ACETATES

C-5 substituent	*Anomer*	*Sugar*	*QF-1*	*NPGS*
H	α	Ara	230	242
	β	Ara	336	323
	α	Lyx	294	286
	α	Xyl	398	350
	β	Xyl	305	286
CH_3	α	Rha	260	193
	α	Fuc	340	286
	β	Fuc	289	237
	α	Qui	365	286
	β	Qui	280	214
CH_2OMe	α	Man	436	416
	α	Gal	495	494
	β	Gal	448	449
	α	Glc	557	518
	β	Glc	466	436
CH_2OAc	α	Man	654	640
	α	Gal	722	697
	β	Gal	675	668
	α	Glc	769	743
	β	Glc	681	655

anomer. This method of presentation of R_t is convenient for the identification of epimers when the methylation pattern, and sometimes that of anomers, is determined from the mass spectrum. The β-anomer of the mannose series and thirteen compounds

TABLE IV

RETENTION INDICES OF Me 3,4-OMe_2 GLYCOPYRANOSIDE ACETATES

C-5 substituent	*Anomer*	*Sugar*	*QF-1*	*NPGS*
H	α	Lyx	168	160
	α	Xyl	199	167
	α	Ara	353	355
CH_3	α	Rha	125	115
	α	Qui	182	160
	α	Fuc	287	237
CH_2OMe	α	Man	284	262
	α	Glc	375	314
	α	Gal	426	424
CH_2OAc	α	Man	524	505
	α	Glc	614	584
	α	Gal	675	647

TABLE V

RETENTION INDICES OF Me 2-OMe GLYCOPYRANOSIDE ACETATES

C-5 substituent	*Anomer*	*Sugar*	*QF-1*	*NPGS*
H	α	Ara	412	400
	β	Ara	500	460
	α	Lyx	508	445
	α	Xyl	518	450
	β	Xyl	430	385
CH_3	α	Rha	470	364
	α	Qui	479	385
	β	Qui	395	311
	α	Fuc	486	404
	β	Fuc	491	358
CH_2OMe	α	Man	624	589
	α	Glc	644	622
	β	Glc	565	535
	α	Gal	648	622
	β	Gal	565	571
CH_2OAc	α	Man	790	766
	α	Gal	803	783
	β	Gal	728	734
	α	Glc	814	797
	β	Glc	732	734

in the set of methyl ethers[14] were not investigated as they were not formed under the conditions of methanolysis used. The last conclusion was confirmed when determining the positions of glycoside linkages of sugars in a lipopolysaccharide from *Yersinia pseudotuberculosis*[18,19].

Numerical values of the retention indices were measured as a first approximation to within ±10 for methyl ethers of the three selected methylglycosides, *i.e.*, β,D-Xyl; α,L-Fuc and α,D-Man. The relative R_t values of epimers in each group were then determined by GLC, making measurements to within ±5 on pairs of single type sugar ether sets. For example (Table III), the R_t value equal to 340 for methyl

TABLE VI

RETENTION INDICES OF Me 3-OMe GLYCOPYRANOSIDE ACETATES

C-5 substituent	*Anomer*	*Sugar*	*QF-1*	*NPGS*
H	α	Lyx	398	378
	α	Xyl	454	400
	β	Xyl	512	458
	α	Ara	464	460
	β	Ara	387	400
CH_3	α	Rha	353	284
	α	Fuc	365	331
	β	Fuc	405	338
	α	Qui	450	351
	β	Qui	542	338
CH_2OMe	α	Man	517	505
	α	Gal	517	528
	β	Gal	620	642
	α	Glc	614	589
	β	Glc	684	645
CH_2OAc	α	Man	710	697
	α	Gal	705	715
	β	Gal	792	797
	α	Glc	803	772
	β	Glc	866	821

TABLE VII

RETENTION INDICES OF Me 4-OMe GLYCOPYRANOSIDE ACETATES

C-5 substituent	*Anomer*	*Sugar*	*QF-1*	*NPGS*
H	α	Lyx	476	400
	α	Xyl	486	405
	β	Xyl	466	429
	α	Ara	486	439
CH_3	α	Rha	445	306
	α	Qui	470	335
	β	Qui	460	358
	α	Fuc	502	385
CH_2OMe	α	Man	601	528
	β	Glc	616	584
	β	Gal	686	642
CH_2OAc	α	Man	829	743
	α	Glc	859	792
	β	Glc	836	797
	α	Gal	866	788
	β	Gal	894	853

2,4-O-dimethyl-3-O-acetyl-α,L-fucopyranoside lies within the range 330–350, but methyl 2,4-O-dimethyl-3-O-acetyl-β,D-quinovopyranoside (R_t = 355) elutes after the former from column A, the R_t value being higher by 15 ± 5.

The double number of methyl ethers of each monosaccharide reduced the possibility of complete separation. For this reason, epimer groups have some unseparated pairs, as shown in Table IX. However, the problem of whether one or two isomers

TABLE VIII

RETENTION INDICES OF Me GLYCOPYRANOSIDE ACETATES

C-5 substituent	*Anomer*	*Sugar*	*QF-1*	*NPGS*
H	α	Xyl	622	506
	β	Xyl	630	550
	α	Lyx	617	523
	α	Ara	617	565
	β	Ara	602	518
CH_3	α	Rha	562	436
	α	Qui	570	443
	β	Qui	590	480
	α	Fuc	582	461
	β	Fuc	605	525
CH_2OMe	α	Man	710	640
	α	Gal	722	665
	β	Gal	745	733
	α	Glc	740	670
	β	Glc	732	705
CH_2OAc	α	Man	882	818
	α	Gal	888	828
	β	Gal	894	905
	α	Glc	899	853
	β	Glc	902	898

TABLE IX

POORLY SEPARATED PAIRS OF ISOMERIC METHYL ETHER METHYL GLYCOPYRANOSIDE ACETATES

Positions of OMe groups	*Anomer*	*Pairs of isomeric sugar*	*Difference in R_t values on different columns*	
			QF-1	*NPGS*
2, 3, 4, 6	α	Glc	0	0
	α	Man		
2, 3, 4, 6	α	Gal	0	5
	β	Gal		
2, 3, 4	α	Ara	4	0
	β	Ara		
2, 6	α	Glc	4	0
	α	Gal		
2	β	Gal	4	0
	β	Glc		
4	β	Gal	7	4
	β	Glc		
—	α	Rha	8	7
	α	Qui		
—	β	Gal	8	7
	β	Glc		

constitute a particular GLC peak can be solved by finding out the second separated anomers for the pair under suspicion. For example, GLC–MS analysis of the acetylated methanolysis products of any polysaccharide reveals three peaks of the Me 2,6-OMe_2–3,6-OAc_2–hexopyranosides which lie within the R_t ranges 525–555, 560–580 and 610–630 on the NPGS column. Differences between them are 12 ± 5

and 35 ± 5. Thus, α anomers of glucose and galactose derivatives compose the third peak, the first and second peaks are β anomers of glucose and galactose derivatives, respectively, as shown in Table V.

In conclusion, Tables I–VIII, giving the retention indices of methyl ether methylglycoside acetates, provide a reliable means of determining the epimers of the sugar series studied in the GLC–MS analysis. Out of 285 pairs of epimer sets, there are only eight unseparated pairs.

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