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Syntheses and NMR Studies of Pyruvic Acid 4,6-Acetals of some Methyl Hexopyranosides

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The pyruvic acid 4,6-acetals of the methyl glycosides of α- and β-D-glucose, α- and β-D-mannose, and α- and β-D-galactose which correspond to naturally occurring residues in polysaccharides, have been synthesized and their ^{1}H and ^{13}C NMR spectra fully assigned. The acetalation was accomplished by reacting the 4,6-di-*O*-trimethylsilyl-derivative with ethyl pyruvate under trimethylsilyl–trifluoromethanesulfonate promotion. In most cases a mixture of the *R*- and the *S*-isomer of the acetal was obtained, which could be separated by silica gel chromatography. Spectra from some compounds with the unnatural acetal, i.e., the one with an axial methyl group, were also assigned. For compounds corresponding to the natural residues significant ^{1}H NMR chemical shift changes (>0.05 ppm) were obtained for signals from protons at different positions of the ring depending on the compound in question. The largest changes were obtained for the linkage positions, up to ~0.5 ppm. Large differences between glucose and mannose on the one hand and galactose on the other, were observed. For ^{13}C NMR signals large downfield shifts were observed for signals from C-4 and C-6, up to 6 ppm, and large upfield shifts were observed for signals from C-3 and C-5 with shifts of up to 10 ppm for the latter. The values will be used to extend the monosaccharide database in the computer program CASPER, with which computer-assisted structural analysis of oligo- and poly-saccharides can be accomplished.

Pyruvic acid is a component found in many microbial capsular polysaccharides.[1] It is commonly found acetalically linked to the 4,6-position of a hexose and in the known examples the methyl group is always equatorial. Pyruvic acid has also been found as a 2,3-, 3,4- and a 5,6-acetal, generally linked to a pair of *cis*-hydroxy groups. Occasionally it has been found in lipopolysaccharides, e.g., in those from *Shigella dysenteriae* type 6 (Ref. 2) and *Escherichia coli* serotype O-149 (Ref. 3). The acetals are probably formed in Nature from enol ethers of pyruvic acid, which are also intermediates in the formation of ethers of lactic acid, for which *R*- and *S*-isomers have been found.[1] Pyruvic acid acetals have also been found in agar, on some *C*-glycosidic glycopeptidolipids from *Mycobacterium*,[4,5] and in the teichoic acid from *Brevibacterium iodinium*.[6]

NMR spectra of pyruvated sugars have been studied only to a limited extent. It has been shown that the carbon signal from an equatorial acetal methyl group of a 4,6-pyruvate appears at ~26 ppm and that of the corresponding axial at ~17 ppm.[7] It is thus a simple task to determine the configuration of the acetalic carbon of a 4,6-acetal of pyruvic acid. For 3,4-acetals of galactose residues the chemical shift difference is small for ^{13}C signals but appreciable for ^{1}H signals. For a number of polysaccharides it has been established that for all 4,6-acetals investigated the methyl group was equatorial and that for all galactose 3,4-acetals the methyl group was *endo*.[8] For some pyruvic acid acetals linked to D-galacto- and L-fuco-pyranose derivatives it was established that the 1,3-dioxolanes have their acetal carbon signals at 107.5–109.5 ppm and that the 1,3-dioxanes have the corresponding signals at 100.5–102.4 ppm.[9] It has also been demonstrated that the C-3 signal of 3,4-pyruvates appears typically at around 81 ppm, with a difference of ~0.7 ppm between the *endo*- and the *exo*-isomer. The configuration of the acetal carbon of the 2,3-acetal in the capsular polysaccharide from *Streptococcus pneumoniae* type 4 was performed by NOE spectroscopy.[10] Analysis of pyruvic acid acetals has also been performed by methanolysis and reductive cleavage methods followed by MS.[11]

The NMR chemical shifts of monosaccharides is a part of the database in the computer program CASPER.[12] The program may be used for the analysis of oligo- and poly-saccharides; the input data are NMR-spectra and data from sugar and methylation analyses and a ranked list of structural alternatives is obtained from the analysis. The monosaccharides now available to the program are hexoses, 6-deoxyhexoses, hexosamines and hexuronic acids. In order to extend the monosaccharide database to include other sugars we have now synthesized the naturally occurring 4,6-pyruvates of the α- and the β-anomer of methyl D-gluco-, D-galacto-, and D-manno-pyranoside and completely assigned their ^{1}H and ^{13}C NMR spectra. Some of the unnatural compounds were also characterized.

Results and discussion

For the synthesis of 4,6-pyruvates several approaches have been used, most of which, however, gave fairly low yields. We have used a recent method in which 4,6-di-*O*-trimethylsilyl ethers of the sugars are reacted with pyruvic acid.[13] Thus, the methyl 4,6-benzylidene glycosides were benzoylated using benzoyl chloride and pyridine. Hydrolysis of the benzylidene acetals was then accomplished with 70% aqueous acetic acid. The resulting 4,6-diols were then trimethylsilylated and treated with ethyl pyruvate and trimethylsilyl trifluoromethanesulfonate, to give mixtures of the *R*- and the *S*-isomers of the acetal. The acetalation gave yields between 9 and 86%. It was observed that trimethylsilyl-derivatives that were purified by chromatography on silica gel column gave higher yields and cleaner reaction mixtures.

For all compounds except the β-*gluco*- and the α-*manno*-derivative both the *R*- and the *S*-isomer were obtained in measurable yields (see the Experimental). Separation of the *R*/*S*-mixtures could generally be achieved by silica gel chromatography. For the β-*galacto*-derivative, however, reversed-phase HPLC had to be used. In the final step the benzoic and pyruvic acid esters were hydrolyzed with sodium hydroxide in a water–ethanol mixture.

Stereochemistry and NMR spectroscopy of **1–9**. The ^{1}H NMR chemical shifts of **1–9** are given in Table 1. The acetalation of O-4 and O-6 affects the pyranose ring of the

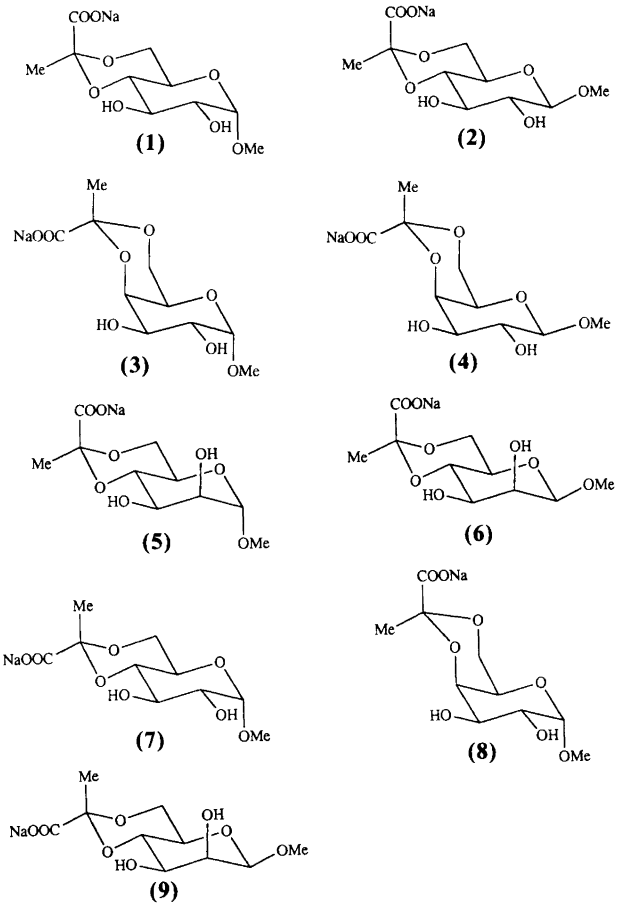

Table 1. ^{1}H NMR chemical shifts and chemical shift differences[a] obtained at 70 °C relative to internal TSP (δ 0.00).

Substance	H-1	H-2	H-3	H-4	H-5	H-6_a	H-6_b	Me	OMe
Me α-Glc-4,6-Py-*equat.*[b]	4.79	3.59	3.75	3.41	3.65	3.69	3.96	1.45	3.41
(**1**)	−0.02	0.03	0.07	0.00	0.01	−0.07	0.09		−0.02
Me β-Glc-4,6-Py-*equat.*	4.44	3.33	3.68	~3.46	~3.46	3.73	4.05	1.47	3.54
(**2**)	0.07	0.05	0.18	0.06	0.00	−0.01	0.13		−0.04
Me α-Gal-4,6-Py-*equat.*	4.87	3.94	3.88	4.22	3.68	3.89	4.06	1.45	3.41
(**3**)	0.02	0.10	0.07	0.23	−0.21	0.13	0.30		−0.02
Me β-Gal-4,6-Py-*equat.*	4.33	3.61	3.67	4.17	3.55	3.94	4.08	1.46	3.56
(**4**)	0.02	0.09	0.03	0.24	−0.13	0.16	0.30		−0.02
Me α-Man-4,6-Py-*equat.*	4.75	3.97	3.90	3.79	3.66	3.76	3.94	1.46	3.40
(**5**)	−0.02	0.03	0.13	0.12	0.05	−0.02	0.04		−0.02
Me β-Man-4,6-Py-*equat.*	4.62	4.04	3.81	3.71	3.37	3.78	4.03	1.47	3.53
(**6**)	0.08	0.06	0.21	0.11	0.02	0.04	0.11		0.00
Me α-Glc-4,6-Py-*axial*	4.84	3.67	3.79	3.68	3.71	3.88	4.01	1.62	3.42
(**7**)	0.03	0.11	0.11	0.27	0.07	0.12	0.14		−0.01
Me α-Gal-4,6-Py-*axial*	4.85	~3.93	~3.93	4.42	3.72	3.92	4.28	1.59	3.42
(**8**)	0.00	0.09	0.12	0.43	−0.17	0.16	0.52		−0.01
Me β-Man-4,6-Py-*axial*	4.63	4.08	3.81	3.99	3.39	3.93	4.06	1.63	3.54
(**9**)	0.09	0.10	0.21	0.39	0.04	0.19	0.14		0.01
Me α-D-Glc	4.81	3.56	3.68	3.41	3.64	3.76	3.87		3.43
Me β-D-Glc	4.37	3.28	3.50	3.40	3.46	3.74	3.92		3.58
Me α-D-Gal	4.85	3.84	3.81	3.99	3.89	3.76	3.76		3.43
Me β-D-Gal	4.31	3.52	3.64	3.93	3.68	3.78	3.78		3.58
Me α-D-Man	4.77	3.94	3.77	3.67	3.61	3.78	3.90		3.42
Me β-D-Man	4.54	3.98	3.60	3.60	3.35	3.74	3.92		3.53

[a] Chemical shift differences are calculated by subtraction of the chemical shifts of the corresponding methyl glycoside.[15]
[b] 4,6-Py-*equat./axial* refers to the orientation of the acetal methyl group.

residue in different ways. The substitution itself changes the electronic distribution and thereby the NMR chemical shifts. Secondly, the hydroxymethyl group is locked in a position where normally it is not found, i.e., as the rotamer with O-6 1,3-parallel to O-4. For glucose and mannose this rotamer does not exist in significant amounts and for galactose it is the least populated rotamer. The interaction of the oriented electron lone-pairs on O-4 and O-6 with 3-H and 5-H may also influence chemical shifts.

In the ^{1}H NMR spectrum of **1–6** significant chemical shift differences up to 0.5 ppm relative to the unsubstituted methyl hexosides, are observed. The substituted positions, 4 and 6, generally show the largest changes, up to 0.30 ppm for the 6-H signal in the α-*galacto*-derivative but 0.0 ppm for the 4-H signal in the α-*gluco*-derivative. The variation between anomeric pairs is small for glucose and mannose, but considerable for galactose. The signal from 3-H is shifted between 0.07 and 0.21 ppm for the *gluco*- and the *manno*-derivative and less for the *galacto*-derivative. This is possibly an effect of the directed electron lone-pairs on O-4. Signals for 5-H for the *gluco*- and the *manno*-derivative are shifted less than 0.05 ppm, the *galacto*-derivatives however, have their values shifted −0.21 (α) and −0.13 ppm (β), respectively. For the derivatives with an axial methyl group, **7–9**, all signals from substituted positions, i.e., 4 and 6, are significantly shifted, up to 0.52 ppm. Signals for 3-H and 5-H are shifted similarly to the derivatives with an equatorial methyl group.

For ^{13}C NMR signals (Table 2) significant changes are observed for all ring carbons. The largest shifts are observed for the C-5 signals and are all negative, i.e., shifted upfield, between −8 and −10 ppm. For C-3, the signals are also shifted upfield with values between −1 and −3 ppm. The signals for C-4 are shifted downfield, ~6 ppm, for glucose and mannose, but only ~2 ppm for galactose. The signals for C-6 are shifted ~3 ppm. For all derivatives the signals for the acetal carbons are found in the range 101.5–102.9.

For derivatives **7–9** large upfield shifts for the C-5 signal are observed, similar to those for **1–6**, and also smaller upfield shifts for the C-3 signal. Signals for C-4 are shifted downfield but significantly less so, from ~0 to 3 ppm. The acetalic carbon has its resonance a few ppm upfield compared with derivatives **1–6**.

The implementation of values in the CASPER program could be made in two different ways. The basic idea in the program is to take δ-values for monosaccharides and then, for a certain type of linkage, chemical shift differences (glycosylation shifts) are added. These have been demonstrated for a large number of model substances. For oligosaccharide elements not studied approximations are made from known compounds. Thus, values for the hexose 4,6-acetals could be used as such, as new 'monosaccharides', or the pyruvic acid acetal could be treated as a substituent with its induced chemical shift differences stored. The latter approach allows the simulation of values for any 4,6-pyruvated sugar residue. On the other hand, when little is known about the effects of

Table 2. ^{13}C NMR chemical shifts and chemical shift differences[a] obtained at 70 °C relative to internal acetone (δ 31.00).

Substance	C-1	C-2	C-3	C-4	C-5	C-6	Me	OMe	C–	CO
Me α-Glc-4,6-Py-*equat.*[b]	100.70	72.62	71.10	76.86	62.94	65.13	25.49	55.94	102.10	175.61
(1)	0.51	0.39	−3.00	6.18	−9.58	3.46		0.01		
Me β-Glc-4,6-Py-*equat.*	104.75	74.73	73.83	76.56	66.75	64.92	25.49	58.05	102.24	175.64
(2)	0.62	0.74	−2.95	5.87	−10.03	3.10		0.17		
Me α-Gal-4,6-Py-*equat.*	100.64	68.65	68.65	71.94	62.84	65.48	25.97	55.92	101.26	176.20
(3)	0.29	−0.52	−1.81	1.75	−8.70	3.42		−0.04		
Me β-Gal-4,6-Py-*equat.*	104.18	71.19	72.62	71.67	66.92	65.51	26.00	57.66	101.51	176.37
(4)	−0.54	−0.45	−1.17	2.05	−9.00	3.67		−0.22		
Me α-Man-4,6-Py-*equat.*	102.43	71.10	68.73	74.43	64.05	64.97	25.60	55.57	102.43	175.83
(5)	0.68	0.25	−2.83	6.64	−9.40	3.05		0.02		
Me β-Man-4,6-Py-*equat.*	102.48	71.38	71.13	74.38	67.35	64.92	25.62	57.81	102.48	175.80
(6)	0.59	0.28	−2.80	6.49	−9.75	2.90		0.20		
Me α-Glc-4,6-Py-*axial*[b]	100.94	72.54	71.19	73.73	63.84	62.67	17.68	56.02	99.72	176.07
(7)	0.75	0.31	−2.91	3.05	−8.68	1.00		0.09		
Me α-Gal-4,6-Py-*axial*	100.75	68.73[c]	68.60[c]	69.93	63.28	63.28	16.81	55.97	98.70	176.53
(8)	0.40	−0.44	−1.86	−0.26	−8.26	1.22		0.01		
Me β-Man-4,6-Py-*axial*	102.64	71.27	71.00[c]	71.05[c]	68.10	62.51	17.78	57.86	100.05	176.18
(9)	0.75	0.17	−2.93	3.16	−9.00	0.49		0.25		
Me α-D-Glc	100.19	72.23	74.10	70.68	72.52	61.67		55.93		
Me β-D-Glc	104.13	73.99	76.78	70.69	76.78	61.82		57.88		
Me α-D-Gal	100.35	69.17	70.46	70.19	71.54	62.06		55.96		
Me β-D-Gal	104.72	71.64	73.79	69.62	75.92	61.84		57.88		
Me α-D-Man	101.75	70.85	71.56	67.79	73.45	61.92		55.55		
Me β-D-Man	101.89	71.10	73.93	67.89	77.10	62.02		57.61		

[a] Chemical shift differences are calculated by subtraction of the chemical shifts of the corresponding methyl glycoside.[15]
[b] 4,6-Py-*equat./axial* refers to the orientation of the acetal methyl group. [c] May be interchanged.

pyruvation, the simulations may be inaccurate. This study shows, e.g., that glucose and mannose derivatives are fairly similar but with differences of up to 0.5 ppm for some carbon signals. Galactose, on the other hand, differs substantially from these, in particular for the C-4 signal. In our present program version we have therefore implemented the pyruvated sugars as 'monosaccharides'.[14]

Experimental

General. Yields in the syntheses were not optimized. The compounds **1–9** were purified until more than 95% pure as indicated by ^{13}C NMR spectroscopy. The identities of intermediates was ascertained by analysis of ^{13}C NMR spectra in which the correct number of signals at the expected chemical shifts was observed. The stereochemistry of the acetalic carbon atom was determined from the acetal methyl δ-values.

NMR spectroscopy. NMR spectra of solutions of the compounds, as the sodium salts, in deuterium oxide were recorded at 70 °C using a JEOL GSX-270 instrument. Chemical shifts are reported in ppm, using sodium 3-trimethylsilylpropanoate-d_4 (δ_H 0.00) and acetone (δ_C 31.00) as internal references. Double quantum filtered COSY, relayed COSY and C,H-COSY experiments were used to assign signals and performed according to standard pulse sequences. The assignments were obtained combining the results from the two-dimensional spectra, including couplings patterns in the cross peaks. The observed couplings indicated that the pyranose rings assume their normal conformation, i.e., $^4C_1$.

*Methyl 2,3-di-*O*-benzoylhexopyranosides.* The 4,6-benzylidene derivatives of α- and β-D-glucopyranoside, α- and β-D-galactopyranoside, and α- and β-D-mannopyranoside (10–15 g) were dissolved in pyridine (20–50 ml), kept at 0 °C and benzoyl chloride (20–25 ml) was added. After 5 min the reaction was complete and the mixture was poured onto ice and when a solid product was obtained it was separated by filtration. Crystallization of this from ethanol or chromatography on silica gel (toluene–ethyl acetate 5:1) yielded the pure product (>90%). Hydrolysis in 70% aqueous acetic acid at 60 °C for 2–3 h gave the 2,3-di-*O*-benzoyl derivatives. Chromatography on a column of silica gel (30 × 5 cm) in toluene–ethyl acetate 1:1 gave the pure products (>90%).

*Methyl 2,3-di-*O*-benzoyl-4,6-di-*O*-trimethylsilylhexopyranosides.* Trimethylsilyl chloride (15 ml, 0.12 mmol) was added to a solution of the diol (~4 g, 1 mmol) in pyridine (20 ml) and the mixture was stirred at room temperature for 5–10 min and the solvents evaporated off. Rapid purification on a silica gel column (10 × 5 cm) using toluene–ethyl acetate mixtures as the eluants yielded the product (>90%).

Table 3.

Substance	*R* : *S*	Total yield (%)
Me α-D-Glc*p*-4,6-Py	17 : 83	86
Me β-D-Glc*p*-4,6-Py	0 : 100	13
Me α-D-Gal*p*-4,6-Py	51 : 49	55
Me β-D-Gal*p*-4,6-Py	60[a] : 40[a]	9
Me α-D-Man*p*-4,6-Py	0 : 100	28
Me β-D-Man*p*-4,6-Py	38 : 62	60

[a] Estimated from TLC.

*Methyl 2,3-di-*O*-benzoyl-4,6-*O*-(1-carboxyethylidene)-hexopyranosides.* The trimethylsilyl derivative (2–4 g, 1–2 mmol) was dissolved in dry methylene chloride (20–25 ml) and ethyl pyruvate (1–2 ml, 2–4 mmol) was added. The solution was stirred at room temperature for 2–5 h. When no more starting material could be detected (TLC), or indications of anomerization occurred, the reaction mixture was diluted with dichloromethane, shaken with saturated $NaHCO_3$ and the organic phase washed with water. After evaporation of solvents, the mixture was separated on a silica gel column using hexane–ethyl acetate mixtures. The separation was successful for all mixtures except for the β-D-galactopyranoside derivatives, which were separated after deesterification. The amounts of the *R*- and the *S*-forms obtained are given in Table 3.

*Methyl 4,6-*O*-(1-carboxyethylidene)hexopyranosides.* The benzoylated acetal (100–800 mg) was dissolved in a mixture of ethanol–water (3:1) and the pH was adjusted to a meter reading of 13 using 0.1 M sodium hydroxide solution. The solution was kept at room temperature for 2–3 h until no more starting material remained (TLC). The solution was neutralized with 0.1 M hydrochloric acid, concentrated and desalted on a Bio-Gel P2 column, eluted with water. The yields varied between 40 and 80%. The *R*/*S*-mixture of the β-D-galactopyranose derivative was partially resolved on the column of Bio-Gel P2 and completely on a C_{18} reversed-phase HPLC column.

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