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Note

N.m.r. spectra of aldobiuronic and aldotriuronic acid derivatives related to 4-*O*-methyl-D-glucurono-D-xylans

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4-*O*-Methyl-D-glucurono-D-xylans, a group of polysaccharides characteristic of land plants[1–3], consist of a main chain of (1→4)-linked β-D-xylopyranosyl residues, some of which are substituted at O-2 by single groups of 4-*O*-methyl-α-D-glucopyranosyluronic acid. Partial acid hydrolysis of these polysaccharides afforded[4] an aldobiuronic acid, namely, 2-*O*-(4-*O*-methyl-α-D-glucopyranuronosyl)-D-xylose[4,5] (**1**), as well as higher-molecular-weight oligosaccharides. Chemical synthesis[6] of **1** and its methyl ester methyl β-glycosides[7], as well as related aldotriuronic acid derivatives previously reported by Hirsch *et al.*[8], furnished useful model compounds for chemical and physical studies of this class of polymers, some of which are characterized here in terms of a detailed description of their ^{1}H- and ^{13}C-n.m.r. spectra. The compounds examined were methyl 2-*O*-(methyl 4-*O*-methyl-α-D-glucopyranosyluronate)-β-D-xylopyranoside (**2**), methyl 2-*O*-(methyl 4-*O*-methyl-β-D-glucopyranosyluronate)-β-D-xylopyranoside (**3**), methyl 2-*O*-(methyl 4-*O*-methyl-α-D-glucopyranosyluronate)-4-*O*-β-D-xylopyranosyl-β-D-xylopyranoside (**4**), and methyl *O*-(methyl 4-*O*-methyl-α-D-glucopyranosyluronate)-(1→2)-*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranoside (**5**).

A detailed analysis of the ^{1}H- and ^{13}C-n.m.r. spectra of compounds **2–5** in D_2O solution was made possible by the use of 2-dimensional techniques. A 400-MHz, ^{1}H-spectrum of each was recorded, and all of the signals and splitting patterns were identified by ^{1}H-homocorrelation spectroscopy at 200 MHz; these latter parameters were then found, by simulation, to closely reproduce each spectrum at 400 MHz. ^{13}C-Chemical shift parameters of ring carbon atoms were unambiguously determined by ^{13}C–^{1}H-heterocorrelation spectroscopy at 100 MHz

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(^{13}C) and were in good agreement with assignment sequences previously reported[9], except for a few changes of shift values. The overall data are collected in Tables I and II, and a representative 2-D,^{1}H-homocorrelation spectrum, that of compound **5**, is reproduced in Fig. 1. Assignment for two closely-spaced pairs of ^{13}C signals for compound **3** may be interchangeable, as well as those for some of the anomeric methoxyl and 4-*O*-methyl ^{1}H-resonances.

In general, the n.m.r. parameters obtained for **2, 4**, and **5** are in close accord with those expected for oligosaccharides comprised of sugars having the α-D-*gluco* and β-D-*xylo* configurations. This point was of concern especially because the (1→2)-glycosidic bond is associated with relatively wide variations in ^{13}C-chemical shifts as evident, for example[10], from an analysis of chemical-shift patterns in relation to conformational stability. Consequently, it is noteworthy that the ^{13}C parameters for the 2-*O*-substituted methyl β-D-xylopyranoside residue of biose **2**, in relation to those[11] of methyl β-D-xylopyranoside (**7**), are close to the parameters[12] for the 2-*O*-substituted methyl β-D-glucopyranoside residue of methyl β-kojibioside (**8**) in relation to those of methyl β-D-glucopyranoside (**9**) (Table III). Hence, it is evident that the chemical shift differences ($\Delta\delta$) for each carbon atom, associated with the introduction of the α-(1→2) linkage, is approximately the same in **2** as in **8**. An analogous comparison of the ^{13}C data for the β-D-xylopyranosyl end group of trisaccharide **4** with those for the 2-*O*-substituted one in **5** gives a pattern of $\Delta\delta$ values (Table III) differing only slightly from that of **2**; in both instances, the net effect of substitution at O-2 is a moderate deshielding of the group of C-5,6 ($\Sigma\Delta\delta$ of 4.4–3.9, Table III).

Data for the compounds containing the methyl 4-*O*-methyl-α-D-glucopyranosyluronate group (**2, 4** and **5**) also show an overall consistency with expectation. The ^{13}C-chemical shifts (Table II), which are nearly the same for the corresponding carbon atoms in all three compounds, are only slightly displaced from those of methyl (methyl α-D-glucopyranosid)uronate[13], allowing for the characteristically large deshielding of C-4 due to the *O*-methyl substituent. The ^{1}H-chemical shifts for these end groups also are closely consistent relative to each other. Noteworthy, however, is the long range coupling between H-1′ and -5′ of **2** (^{4}J 0.015 Hz), and H-1″ and -5″ of **4** (^{4}J 0.025 Hz) which, although small, were readily detected by the 2-D experiments; this extra splitting was not observed, however, for the glycosylinonic acid group of **5** under the same experimental conditions but was shown by a DQF COSY experiment[14] at 40°, whereas at 20° this specific correlation was not detected. A previous report[15] on a 2-D COSY spectrum of **4** did not describe such a long-range coupling.

An analysis[16] of 2-D n.m.r. spectra of the aldotriuronic acid corresponding to **5**, *i.e.*, **6** (in admixture with its α anomer), provided ^{13}C- and ^{1}H-chemical-shift data that closely parallel most of the parameters listed here for **5**. This indicated that, overall, the conformations of the free acid **6** and methyl ester **5** are essentially the same.

The patterns of both the ^{1}H- and ^{13}C-chemical shifts (Tables I and II) of the

TABLE I

^{1}H-N.M.R. DATA[a] FOR COMPOUNDS **2–6**

Compound	*Unit*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*OMe-1*	*OMe-4*	*MeCO*
2	β-D-Xyl*p*OMe	4.47	3.32	3.52	3.63	3.97 3.32[b]	3.55		
			(7.74)	(9.40)	(9.40)	(5.35, 10.75, 11.65)[c]			
	Me 4-OMe-α-D-GlcA*p*	5.28	3.62	3.83	3.37	4.69		3.48	3.87
			(3.80)	(9.54)	(9.46)	(10.20, $J_{1,5}$ = 0.015)[d]			
3		4.42	3.43	3.63	3.63	3.95 3.32	3.47		
			(7.28)	(8.78)	(8.40)	(4.48, 9.60, 11.71)			
		4.71	3.37	3.63	3.38	4.07		3.46	3.85
			(8.03)	(9.46)	(9.27)	(9.89)			
4	β-D-Xyl*p*OMe	4.46	3.33	3.64	3.76	4.08 3.37	3.52		
			(7.60)	(9.03)	(9.03)	(5.20, 10.0, 11.84)			
	β-D-Xyl*p*	4.45	3.25	3.42	3.62	3.96 3.30			
			(7.77)	(9.17)	(9.17)	(5.45, 10.95, 11.57)			
	Me 4-OMe-α-D-GlcA*p*	5.23	3.60	3.81	3.35	4.69		3.47	3.84
			(3.65)	(9.85)	(9.30)	(10.0, $J_{1,5}$ = 0.025)			
5		4.34	3.28	3.58	3.79	4.15 3.45	3.55		
			(7.81)	(9.40)	(9.38)	(5.43, 10.34, 11.86)			
		4.62	3.37	3.51	3.64	3.98 3.31			
			(7.85)	(9.34)	(9.28)	(5.56, 10.96, 11.54)			
		5.31	3.61	3.82	3.36	4.74		3.49	3.86
			(3.91)	(9.72)	(9.39)	(10.9)			
6[e]	β-D-XylOH	4.46	3.13	3.45	3.68	3.99 3.32			
	β-D-Xyl*p*	4.50	3.28	3.39	3.53	3.86 3.19			
	4-OMe-α-D-Glc*p*A	5.19	3.48	3.70	3.22	4.53		3.40	

[a]For solutions in D_2O. δ Values relative to the signal of $Me_3Si(CH_2)_2SO_3Na$ as reference; coupling constants in parentheses in Hz; δ and *J* values were obtained by iterative fitting of the experimental spectrum recorded at 400 MHz with the ITRCAL program. [b]The lower value corresponds to $J_{4,5a}$ and the higher to $J_{4,5b}$. [c]$J_{4,5a}$, $J_{4,5b}$, $J_{5a,5b}$ for methyl β-D-xylopyranoside or β-D-xylopyranosyl unit. [d]$J_{4,5}$ and $J_{1,5}$ for methyl (4-*O*-methyl-α-D-glucopyranosiduronate) unit. [e]Ref. 16. δ Values relative to the signal of internal acetone referred to Me_4Si [δ_{Me_4Si} (acetone) 2.12].

TABLE II

^{13}C-CHEMICAL SHIFTS[a] (δ) FOR COMPOUNDS **2–6**

Compound	*Unit*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*OMe-1*	*OMe-4*	*OMe-5*
2	β-D-Xyl*p*OMe	105.6	79.1	75.3	70.7	66.2	58.5		
	Me 4-OMe-α-D-Glc*A*p	99.3	72.1	73.2	82.5	70.7		61.0	54.4
3		103.7	82.4	76.1	70.1	65.7	58.0		
		104.1	74.1	75.7	82.3	74.2		61.2	54.4
4	β-D-XylOMe	105.6	79.2	73.4	78.2	63.9	58.6		
	β-D-Xyl*p*	103.6	74.1	76.0	70.5	66.5			
	Me 4-OMe-α-D-Glc*p*A	99.7	72.2	73.3	82.7	70.8		61.1	54.3
5		105.2	74.1	75.1	77.8	64.0	58.2		
		102.9	78.4	75.5	70.7	66.1			
		99.4	71.6	73.4	82.6	70.7		61.0	54.3
6[b]		97.21	74.67	74.68	76.96	63.63			
		102.29	77.68	75.04	70.19	65.58			
		98.51	71.61	72.98	82.33	70.26			

[a] δ Values reported relative to the signal of acetone (δ 30.4 relative to the signal of Me_4Si). [b] Ref. 16.

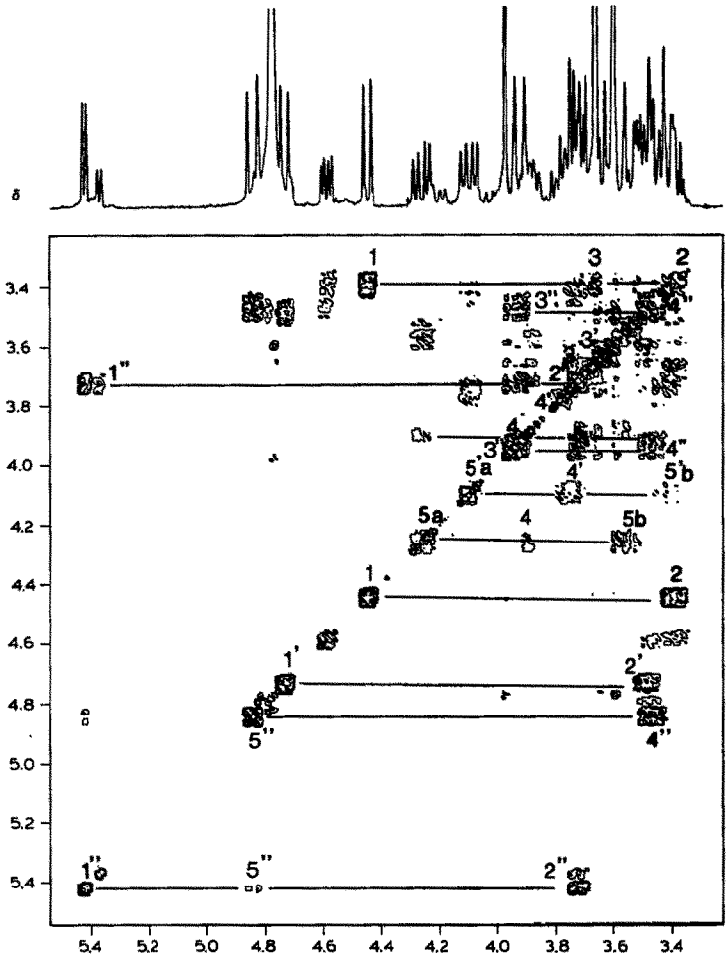


Fig. 1. DQF COSY Homonuclear correlation with double quantum filter[14] of trisaccharide **5** in D_2O recorded at 300 MHz and 40°. The spectrum resulted from a 256 × 512 data matrix.

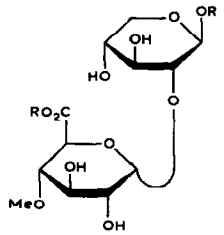
2-*O*-substituted methyl β-D-xylopyranoside residue of **3**, the glycosyluronic acid end group of which possesses the β-D configuration, differed substantially from those already seen for **2**. Relative to methyl β-D-xylopyranoside (**7**), all of the carbon atoms except C-2 are more strongly shielded (Table III). Hence, despite the strong deshielding of C-2 ($\Delta\delta$ +7.4) associated with the presence of the β-(1→2) linkage, the net effect ($\Sigma\Delta\delta$) on the carbon atoms of this residue is an overall increase in shielding. Among the protons of **3** (Table I), H-2 and -3 are particularly strongly deshielded in comparison with those of **2**. More striking, however, are the reduced values of $^3J_{H,H}$ throughout. This indicated that the ring conformation differs appreciably from that in **2**, and also in methyl β-D-xylopyranoside, owing

TABLE III

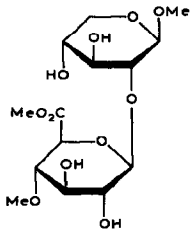
A COMPARISON OF ^{13}C-CHEMICAL-SHIFT CHANGES[a] ASSOCIATED WITH THE LINKING OF α- OR β-GLYCOSYL END GROUPS TO O-2 OF β-D-XYLOPYRANOSIDES AND SOME OTHER GLYCOPYRANOSIDES

Linkage	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	CH_3	$\Sigma\Delta\delta^b$
α-(1→2)								
$\Delta\delta$ (**2**[c] − **7**[d])	+0.5	+5.1	−1.6	+0.3	−0.1		+0.2	+4.4
$\Delta\delta$ (**5**[e] − **4**[f])	−0.7	+4.3	+0.5	+0.2	−0.4			+3.9
$\Delta\delta$ (**8**[g] − **9**[h])	+0.5	+4.4	−1.5	−0.4	−0.2	+0.1	+0.1	+3.0
β-(1→2)								
$\Delta\delta$ (**3**[i] − **7**[d])	−2.4	+7.4	−1.8	−1.3	−1.6		−1.3	−1.0
$\Delta\delta$ (**10**[j] − **11**[k])	−3.1	+7.8	−1.8	−1.5	−2.3			+0.9

[a]Expressed as $\Delta\delta$, the difference between the chemical shifts of the indivual carbon atoms of the 2-*O*-substituted β-D-xylopyranoside (or other) residue, and those of the corresponding, unsubstituted compound or end group of the compound. [b]Sum of $\Delta\delta$ values[10,11]. [c]δ_c of methyl β-D-xylopyranoside residue of **2** (Table II). [d]δ_c of methyl β-D-xylopyranoside[13] (**7**). [e]δ_c of β-D-xylopyranosyl end group of **5** (Table II). [f]δ_c of β-D-xylopyranosyl end group of **4** (Table II). [g]δ_c of methyl β-D-glucopyranoside residue[12] of **8**. [h]δ_c of methyl β-D-glucopyranoside (**9**). [i]δ_c of methyl β-D-xylopyranoside residue of **3** (Table II). [j]δ_c of methyl α-L-arabinopyranoside residue[17] of **10**. [k]δ_c of methyl α-L-arabinopyranoside (**11**).



1 R = H
2 R = Me



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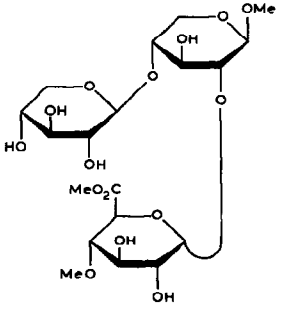

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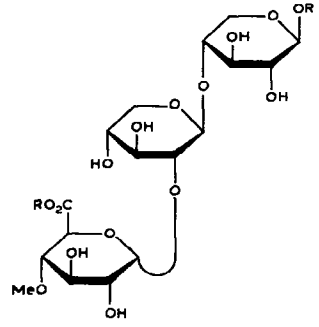

5 R = Me
6 R = H

7 Methyl β-D-xylopyranoside
8 Methyl β-kojibioside
9 Methyl β-D-glucopyranoside
10 Methyl 2-*O*-β-D-glucopyranosyl-α-L-arabinopyranoside
11 Methyl α-L-arabinopyranoside
12 β-Sophorose

presumably to the presence of a destabilizing interaction between the reducing and nonreducing residues of **3**.

These observations for **3** found a close analogy in the n.m.r. data reported[17] for methyl 2-*O*-β-D-glucopyranosyl-α-L-arabinopyranoside (**10**). Thus, a comparison of the ^{13}C-chemical shifts for the glycoside residue of **10** with those for methyl α-L-arabinopyranoside (**11**) demonstrated a pattern of $\Delta\delta$ values (**10** − **11**) (Table III) similar to that obtained by comparing **3** and **6**. Hence, the present finding accords well with the conclusion[17] that the conformation of the α-L-arabinopyranoside residue of **10** is displaced towards the $^1C_4$ (L) chair, from the $^4C_1$ (L) form favored by the unsubstituted glycoside **11**. It is worth noting that ^{13}C-n.m.r. data[12] for the reducing residue of β-sophorose (**12**) do not exhibit[10] an analogous net deshielding relative to β-D-glucose ($\Sigma\Delta\delta$ 5.5). Nevertheless, the presence of an inherently destabilizing factor in **12** may be inferred from the observation[18] that the proportion of this anomer, at equilibrium in water, is far smaller than that of the α-D-anomer (37:63), whereas the converse holds for most other D-glucose disaccharides.

EXPERIMENTAL

The ^1H-n.m.r. spectra were recorded with a Bruker WH-400 and with a Varian XL-200 spectrometer, at room temperature for solutions in D_2O, and the chemical shifts (δ) are reported from the signal of sodium 4,4-dimethyl-4-silapentane-1-sulfonate as reference (internal reference in two cases and substitution reference in others). The COSY experiments were performed at room temperature with the XL-200 and XL-300 spectrometers (90-t1-90-t2 and 90-t1-45-t2). The sequence was run with 512 data points for each FID (providing a digital resolution of 2 Hz), and with 256 increments during the evolution time (t1). Four transients were acquired on each FID. Zero filling in the t1 dimension and pseudo echo-shaping multiplication were applied to the data. In the case of **5**, the DQF COSY experiment[14] was recorded at 40° with a XL 300 spectrometer. The hetero-correlation, 2D experiments were recorded with the Bruker WH-400 spectrometer using 128 increments during the evolution time (t1). The spectra were acquired with a 1.5-Hz digital resolution in the detection domain and 3.8-Hz digital resolution in the evolution domain. Sine Bell Multiplication was applied in both domains.

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