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2D-N.M.R. ANALYSIS OF THE STRUCTURE OF AN ALDOTRIOURONIC ACID OBTAINED FROM BIRCH WOOD

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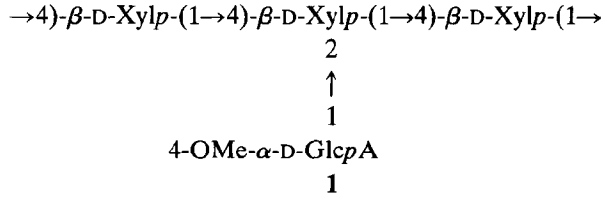
ABSTRACT

The primary structure of the trisaccharide 2′-*O*-(4-*O*-methyl-α-D-glucosyluronic acid)xylobiose, which was obtained from birch wood, has been determined by n.m.r. spectroscopy, using homonuclear and heteronuclear, shift-correlated, two-dimensional techniques.

INTRODUCTION

Pure (4-*O*-methyl-D-glucurono)-D-xylan (**1**) is obtained by alkaline extraction of birch wood. Successive partial hydrolysis with acid yields, after chromatographic separation from neutral xylo-oligosaccharides and other oligouronic acids, a



mixture of two aldotriouronic acids, for which the isomeric structures of 2′-*O*-(4-*O*-methyl-α-D-glucosyluronic acid)xylobiose (**2**) and 2-*O*-(4-*O*-methyl-α-D-glucosyluronic acid)xylobiose (**3**) may be anticipated. The structure of these trisaccharides had previously been investigated only by methylation analysis of the mixture[1].

We now report on the direct structural analysis of the main (~97%) component of the mixture, using two of the newly developed two-dimensional (2D-) n.m.r.

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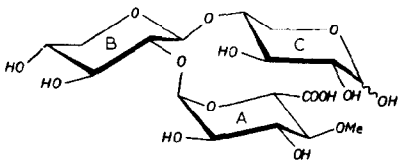

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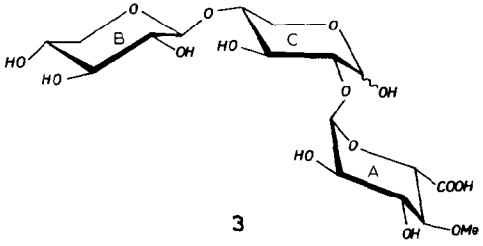

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techniques, namely, homonuclear ("COSY") and heteronuclear shift-correlated spectroscopy[2–5]. Application of these techniques facilitates the structural analysis of oligosaccharides, because they not only greatly enhance resolution, but also establish scalar connectivities between protons and between directly bonded carbons and protons, respectively.

Only a few examples of the complete analysis of oligosaccharide primary structures have been reported. The ^{1}H- and ^{13}C-n.m.r. spectra of raffinose were fully assigned in an early application of heteronuclear shift-correlated spectroscopy[5], and the primary structure of a trisaccharide conjugate, the glycolipid gangliotriaosylceramide, has been evaluated from n.m.r. data only[6]; after peracetylation, even hexasaccharides have been shown to be amenable to analysis[7].

RESULTS AND DISCUSSION

The 400-MHz ^{1}H-n.m.r. spectrum of the mixture of aldotriouronic acids is shown in Fig. 1, as well as the structure and the assignments of the main component **2**, whose proton chemical shifts are given in Table I. The spectrum was analysed by using the COSY technique. Two contour plots of the COSY spectrum are shown in Fig. 2. In these plots, each off-diagonal peak has been assigned to a vicinal J connectivity. All shifts and connectivities, except those of C3α, C4α, and C5α, are clearly observable. Connectivity sequences were established which involve all the coupled resonances in the various glycosyl residues, whose configurations were then determined, as usual, by inspection of the vicinal coupling constants. The distinction between the resonances of the reducing and non-reducing xylose residues was accomplished by relating them to the characteristic anomeric resonances.

A special feature of COSY is that singlet resonances do not give rise to any off-diagonal peaks in the symmetrised contour plots. This may be useful because it overcomes the problem, often encountered in practice, of important spectral regions being obscured by the HDO resonance. In the contour plot in Fig. 2, shifts and connectivities of A5, B1, and C1β can be observed without any perturbation, whereas, in the 1D spectrum taken at the same temperature, their signals are totally overlapped by the HDO resonance (the insert at δ 4.5 in Fig. 1 had to be recorded at a lower temperature).

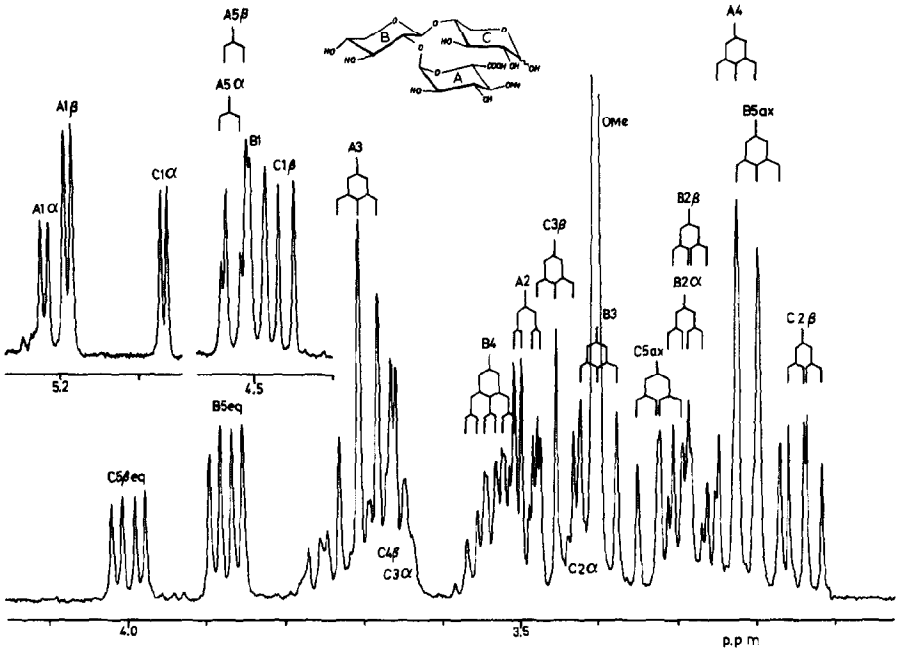

Fig. 1. Structure and 400-MHz ^{1}H-n.m.r. spectrum of trisaccharide **2**.

Having assigned all of the resonances in the spectrum, we next investigated the position of the glycosidic linkage between the glucuronic acid and the xylobiose residues. For this purpose, the nuclear Overhauser effects (n.O.e.s) observable upon saturation of the A1 resonances were measured. The results, obtained by the conventional F.t.-difference spectroscopy technique, are shown in Fig. 3. Beyond

TABLE I

PROTON CHEMICAL SHIFTS[a] FOR **2** (δ SCALE)

Atom	*β*	*α*	*Atom*	*β*	*α*
A1	5.19	5.22	C1	4.46	5.06
A2	3.48	3.48	C2	3.13	3.42
A3	3.70	3.70	C3	3.45	3.64
A4	3.22	3.22	C4	3.68	3.66
A5	4.53	4.54	C5ax	3.32	
B1	4.50	4.50	C5eq	3.99	
B2	3.28	3.29	OMe	3.40	3.40
B3	3.39	3.39			
B4	3.53	3.53			
B5ax	3.19	3.19			
B5eq	3.86	3.86			

[a]Measured relative to internal acetone, and converted to the Me_4Si scale by applying the relationship δ_{Me_4Si} (acetone) = 2.12.

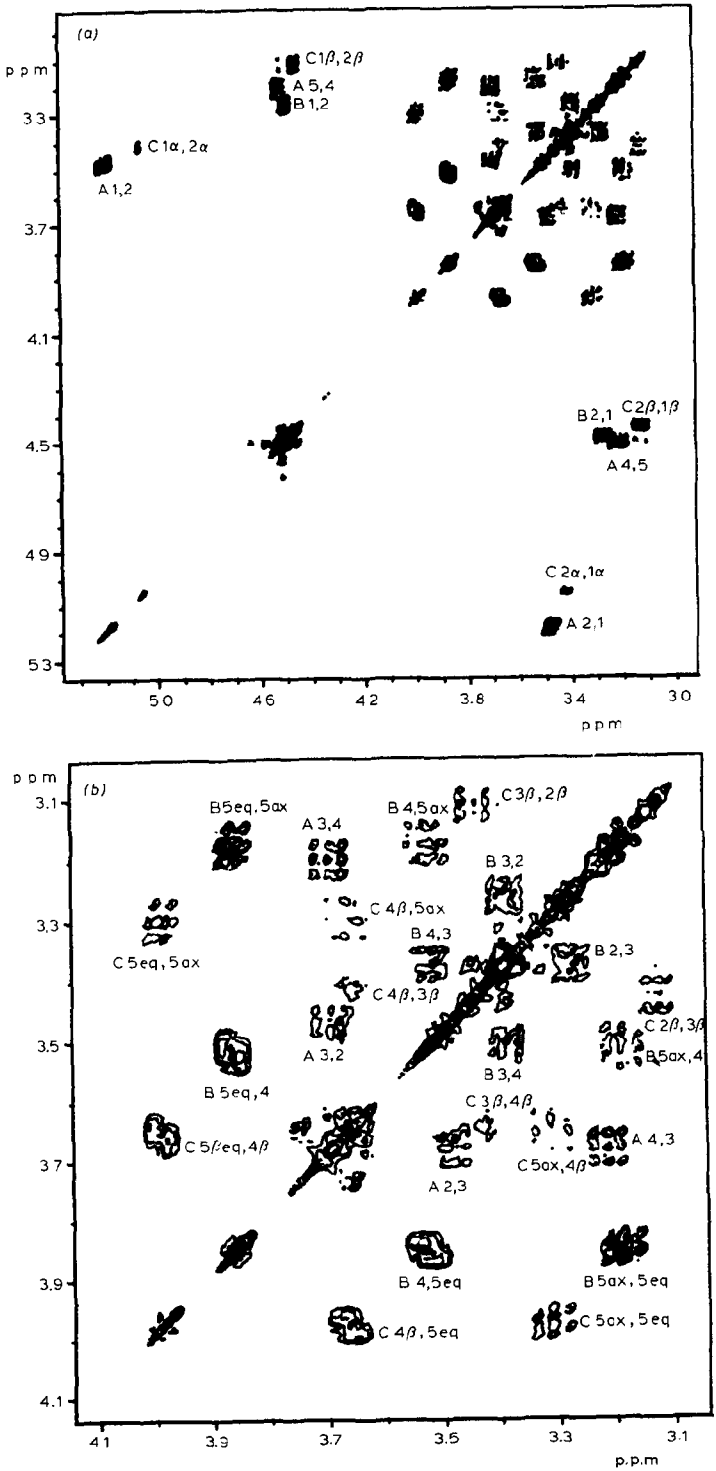

Fig. 2. Contour plot of the 2D-COSY spectrum of 2; (*a*) full spectrum, (*b*) expanded upfield region. Each peak is assigned to a scalar connectivity between two protons.

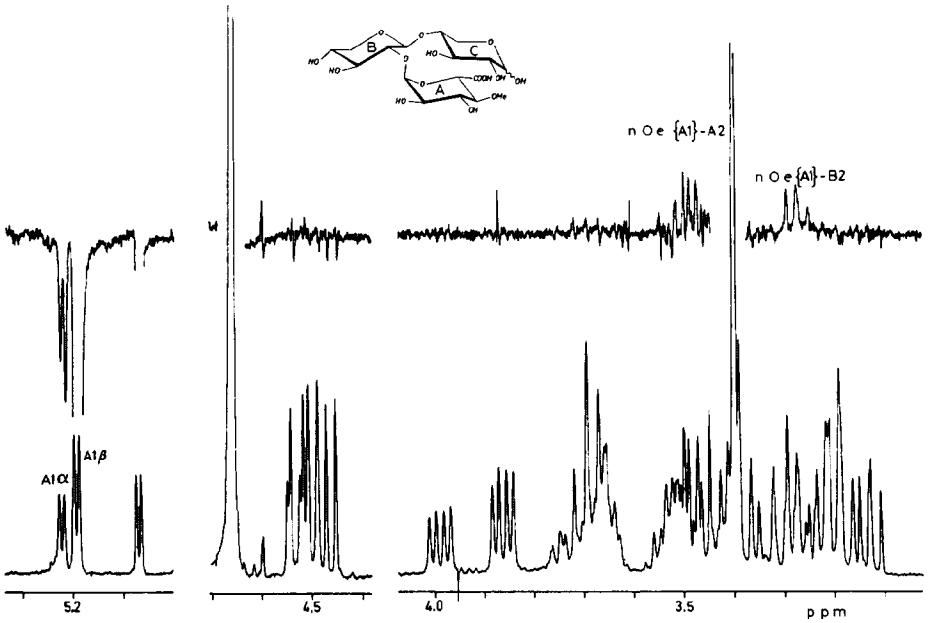

Fig. 3. Normal and F.t.-n.O.e. difference spectrum obtained by saturation of the A1 resonances.

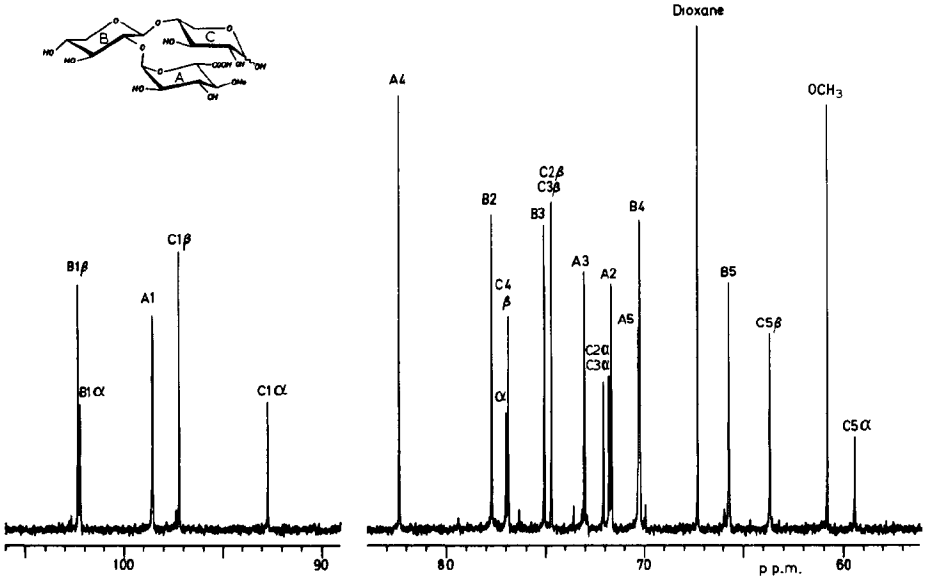

Fig. 4. Structure and 100-MHz ^{13}C-n.m.r. spectrum of **2**.

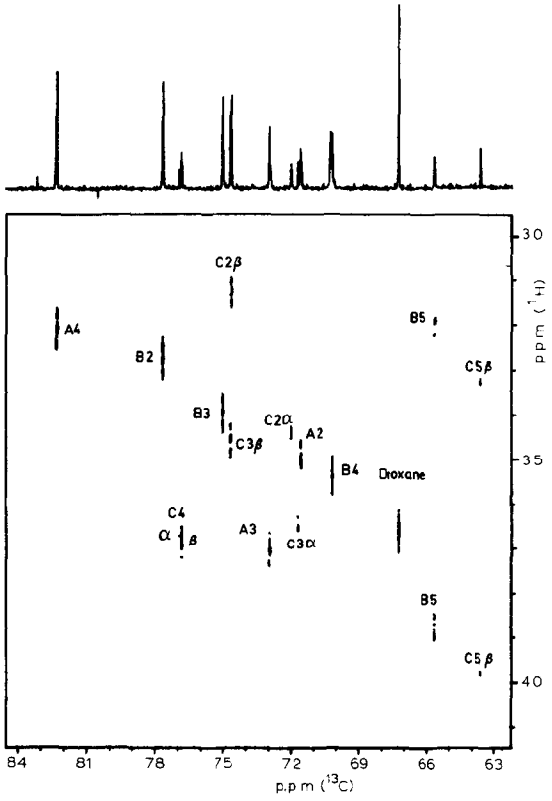

Fig. 5. Contour plot of the 2D heteronuclear shift-correlated spectrum (expanded region) of **2**. Each peak is assigned to a directly bonded carbon–proton pair.

the vicinal n.O.e. {A1}-A2, an inter-ring n.O.e. {A1}-B2 can clearly be observed, proving that the glucuronic acid is glycosidically linked to the non-reducing xylosyl group.

The proton noise-decoupled, 100-MHz, ^{13}C-n.m.r. spectrum of the mixture was also recorded (Fig. 4). A few minor signals, probably due to the trisaccharide **3**, can be seen, but assignment was not attempted. All the resonances of the main component were assigned by heteronuclear, shift-correlated 2D-spectroscopy. The expanded upfield region of the contour plot of this 2D spectrum is displayed in Fig. 5. Proton assignments were transferred to the carbon spectrum by inspection of this plot. Some proton resonances not observable or not assignable in the 1D spectrum (*e.g.*, C2α, C3α, C4α) were then well separated and easily assigned; the same is true for some carbon resonances (*e.g.*, C2β and C3β).

In Table II, the carbon shifts of **2** are compared with those of its constituent monosaccharides. There is little difference between the shifts of C2 and C3 in **2** and the corresponding shifts for D-xylose, whereas a downfield shift of 3.7 p.p.m. was observed for B2 when compared with C-2 in the methyl D-xyloside; the same is true

TABLE II

^{13}C CHEMICAL SHIFTS[a] (p.p.m.) FOR **2** AND ITS CONSTITUENT MONOSACCHARIDES[10]

Atom		*Trisaccharide* **2**		D-*Glucuronic acid*	
		β	*α*	*β*	*α*
	1	98.51	98.53		93.17
	2	71.61	71.59		72.03
A	3	72.98	72.76		73.36
	4	82.33	82.33		72.42
	5	70.26	70.26		71.39
				Methyl D-*xyloside*	
	1	102.29	102.19	105.1	
	2	77.68	77.68	74.0	
B	3	75.04	75.04	76.9	
	4	70.19	70.19	70.4	
	5	65.68	65.68	66.3	
				D-*Xylose*	
	1	97.21	92.68	97.5	93.1
	2	74.67	72.02	75.1	72.5
C	3	74.68	71.72	76.8	73.9
	4	76.96	77.68	70.2	70.4
	5	63.63	59.93	66.1	61.9

[a]Measured relative to internal 1,4-dioxane, and converted to the Me_4Si scale by applying the relationship δ_{Me_4Si} (dioxane) = 67.3.

for A1 compared with C-1 in α-D-glucuronic acid (downfield shift of 5.3 p.p.m.). Such downfield shifts are characteristically experienced on glycosylation by both the α carbon in the aglycon and the anomeric carbon in the glycosyl residue[8,9], thus proving again that the position of the glycosidic linkage is between A1 and B2. Furthermore, strong downfield-shifts are observed for C4 ($\Delta\delta$ = 7 p.p.m.) and A4 ($\Delta\delta$ = 10 p.p.m.), which reflect the position of the β-(1→4) linkage in the xylobiose residue and of the methoxyl group in the glucuronic acid residue, respectively.

Therefore, it is concluded that the main component of the mixture is 2′-*O*-(4-*O*-methyl-α-D-glucosyluronic acid)xylobiose (**2**), and that the primary structure of this trisaccharide can be safely determined by direct analysis of the mutarotated aqueous solution, using n.m.r. techniques only.

EXPERIMENTAL

Pure (4-*O*-methyl-D-glucurono)-D-xylan was obtained from birchwood. The wood was first delignified with chlorite, and the resulting holocellulose was extracted with alkali. The hemicelluloses [in the case of hardwoods, mainly (4-*O*-methyl-D-glucurono)-D-xylan] were precipitated from the extract after acidifica-

tion. This material was subjected to partial hydrolysis with aqueous 45% formic acid and yielded a mixture of neutral and acidic xylo-oligomers. Separation into two fractions was obtained with Dowex 1-X4 (acetate form). The neutral xylo-oligosaccharides were eluted with water, and the acidic sugars were obtained after elution with aqueous 30% acetic acid. The aldo-oligouronic acids were fractionated by gel-permeation chromatography on Sephadex G-15. The main fraction, consisting of a mixture (~97:3) of two aldotriouronic acids, was then subjected to n.m.r. analysis.

Spectra were recorded with a Bruker WM 400 spectrometer for 2% solutions in D_2O (0.5 mL). The data were recorded at 313 K in order to optimise resolution, with the exception of the insert at 4.5 p.p.m. in Fig. 1 and the n.O.e. measurements in Fig. 3. Proton chemical shifts were measured relative to that of internal acetone ($\delta_{Me_4Si} = 2.12$); carbon chemical shifts were measured relative to internal dioxane ($\delta_{Me_4Si} = 67.3$) and then converted to the Me_4Si scale.

The COSY experiments were performed by applying the $90°–t_1–45°–t_2$ pulse sequence. Incrementation of t_1 yielded the first, and acquisition of the FID's during t_2 yielded the second, time domaine; 256 time-averaged FID's of 1 k data points and 64 scans each were acquired for 256 different t_1 values, which were incremented by 800 μs between successive experiments. Before Fourier transformation, a "zero-filling" in the t_1 dimension and a multiplication of the data with a sinus function in both dimensions were performed.

The heteronuclear shift-correlated spectra were measured by using the pulse sequence described in ref. 5; 256 time-averaged FID's of 4 k data points and 480 scans each were recorded for the 256 different values of $t_1/2$, which were incremented by 400 μs between the successive experiments; this amounts to a proton spectral width of 1,250 Hz. Prior to the Fourier transformation, the data were again subjected to zero filling (t_1-dimension) and multiplication with a sinus function (both dimensions).

Before the n.O.e. measurements, the solution was subjected to three freeze–thaw cycles, and then the tube was sealed under vacuum. A careful selection of the presaturation time (1.2 s) and of the decoupler power (44 dB below 0.2 W) was crucial in this experiment.

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