Biochem. J. (1990) 265, 533–538 (Printed in Great Britain)

Conformational equilibria of the L-iduronate residue in non-sulphated di-, tetra- and hexa-saccharides and their alditols derived from dermatan sulphate

Yuko INOUE, Yoshinobu INOUYE* and Kinzo NAGASAWA†
School of Pharmaceutical Sciences, Kitasato University, 5–9–1, Shirokane, Minato-ku, Tokyo 108, Japan

The conformation of the L-iduronate residue in non-sulphated di-, tetra- and hexa-saccharides and their alditol derivatives derived from rooster comb dermatan sulphate was investigated by 400 MHz ^{1}H-n.m.r. spectroscopy. The ratio of conformational isomers is obtained by the average spin–spin coupling constants of a mixture of nearly isoenergetic conformers ($^1C_4$, $^4C_1$ and $^2S_0$). The non-reducing terminal L-iduronate residue in the tetrasaccharides (I–H–I–H and I–H–G–H) and their alditols (I–H–I–H-ol and I–H–G–H-ol) is in equilibrium with three conformers ($^1C_4$, 30 %; $^4C_1$, 40 %; $^2S_0$, 30 %) of nearly equal population. Whereas the internal L-iduronate residue in the tetrasaccharides (I–H–I–H and G–H–I–H) exists as an equilibrium mixture of $^1C_4$ (54 %) and $^2S_0$ (42–44 %) conformers, that of their alditols (I–H–I–H-ol and G–H–I–H-ol) is in equilibrium between $^2S_0$ conformer (66 %) and $^1C_4$ conformer (28 %). The conformational population for the internal L-iduronate residue $\overset{2}{\text{I}}$ in the hexasaccharide ($\overset{3}{\text{I}}$–H–$\overset{2}{\text{I}}$–H–$\overset{1}{\text{I}}$–H) is also calculated and compared with that for the L-iduronate residue in native dermatan sulphate, which was calculated on the basis of the spin–spin coupling constants reported by Gatti, Casu, Torri & Vercellotti [(1979) Carbohydr. Res. **68**, c3–c7].

INTRODUCTION

The L-iduronate residue is found in dermatan sulphate, heparan sulphate and heparin (Comper & Laurent, 1978). The ring conformation of the L-iduronate residue in the glycosaminoglycans, especially in heparin, with peculiar biological activities has attracted attention in recent years (Rees *et al.*, 1985; Torri *et al.*, 1985; Casu *et al.*, 1986; Ferro *et al.*, 1986).

A force-field calculation of the conformational properties of methyl 4-*O*-methyl-2-*O*-sulpho-L-idopyranosiduronate has demonstrated that its pyranoside ring structure adopts three nearly isoenergetic conformers, two chair forms $^1C_4$ and $^4C_1$ and one skew-boat form $^2S_0$ (Ragazzi *et al.*, 1986). Ferro *et al.* (1986) interpreted the ^{1}H-n.m.r. coupling constant data for 2-*O*-sulpho-L-iduronate residues in synthetic heparin mono- and oligosaccharides that exist in the specific pentasaccharide sequence responsible for binding to antithrombin III, and stated that the sulphated L-iduronate residues are in conformational equilibrium as $^1C_4$ and $^2S_0$ conformers, and that the $^2S_0$ conformer becomes predominant (64 %), when the sulphated L-iduronate residue is substituted at C-4 by the *N*,3,6-trisulphated D-glucosamine residue typical of the binding site to antithrombin III.

The $^2S_0$ conformer has been proposed to contribute to the conformational equilibrium of the terminal non-sulphated L-iduronate residue in heparan sulphate tetrasaccharides, from ^{1}H-n.m.r. studies (Sanderson *et al.*, 1985). The L-iduronate residue in dermatan sulphate has been shown by Casu *et al.* (1986) to exist as an equilibrium mixture of $^1C_4$ (58 %) and $^2S_0$ (42 %) conformers, on the basis of the proton–proton spin coupling constants of the L-iduronate ring in dermatan sulphate, as previously observed by Gatti *et al.* (1979).

Nagasawa *et al.* (1984) reported solvolytic desulphation and depolymerization of rooster comb dermatan sulphate, which resulted in the formation of four isomeric tetrasaccharides (I–H–I–H, I–H–G–H, G–H–I–H and G–H–G–H) and eight isomeric hexasaccharides. The L-iduronate residue in the tetrasaccharides containing L-iduronate unit(s) is located in two different environments; one is the internal L-iduronate residue and the other is the non-reducing terminal L-iduronate residue. The conformational population of the former is expected to be different from that of the latter. The internal L-iduronate residue, $\overset{2}{\text{I}}$ in the major hexasaccharide ($\overset{3}{\text{I}}$–H–$\overset{2}{\text{I}}$–H–$\overset{1}{\text{I}}$–H) derived from dermatan sulphate, is expected to possess conformational population similar to that of native dermatan sulphate, if one ignores the effect of sulphate substitution. Recently, Sanderson *et al.* (1989) reported assignments of the ^{1}H- and ^{13}C-n.m.r. spectra of dermatan sulphate and its oligosaccharides obtained by chondroitin ABC lyase digestion of the polysaccharide, but no information as to the proton–proton spin coupling constants for the L-iduronate residue has been given.

In the present work the proton–proton spin coupling

Abbreviations used: I, α-L-iduronate; G, β-D-glucuronate; H, *N*-acetyl-β-D-galactosamine; H-ol, *N*-acetyl-D-galactosaminitol; GlcNAc, *N*-acetyl-α-D-glucosamine; $GlcNAc_{6S}$, 6-sulphated *N*-acetyl-α-D-glucosamine; anManOH, 2,5-anhydro-D-mannitol; $anManOH_{6S}$, 6-sulphated 2,5-anhydro-D-mannitol.

* Present address: Department of Chemistry, University of Tsukuba, Tennodai, Tsukuba, Ibaraki 305, Japan.
† To whom correspondence should be addressed.

constants are determined for the L-iduronate residue in non-sulphated di-, tetra- and hexa-saccharides and their alditol derivatives obtained by solvolysis of dermatan sulphate, and the conformational populations are calculated on the basis of the observed coupling constants.

EXPERIMENTAL

Materials

Preparation of the purified fraction (RC-20) of rooster comb dermatan sulphate was as described previously (Nagasawa *et al.*, 1984). ^2H_2O (99.8 atom% ^2H) for ^2H_2O exchange was purchased from E. Merk. ^2H_2O (99.95 atom% ^2H_2O) for ^1H-n.m.r. studies was purchased from C.E.A. (Gif-sur-Yvette, France).

N.m.r. spectroscopy

Samples of oligosaccharides (5–10 mg) were exchanged several times with ^2H_2O and finally dissolved in 99.95 atom% ^2H_2O (0.4–0.5 ml). pH values of the solutions were 5.0–5.2. ^1H-n.m.r. spectra were recorded with a Varian XL-400 n.m.r. spectrometer at 20 or 80 °C. Chemical shifts (δ) were expressed relative to dioxan as internal standard (δ 3.70 p.p.m.). Proton resonances were assigned by means of spin-decoupling difference experiments and homonuclear COSY-45 and heteronuclear $^{13}C/H$ correlation experiments (Bax & Freeman, 1981; Bax, 1983).

Preparation of oligosaccharides containing L-iduronate residue

Oligosaccharide samples for measurement of ^1H-n.m.r. spectra were prepared as described previously (Nagasawa *et al.*, 1984). Briefly, the pyridinium salt (320 mg) of rooster comb dermatan sulphate (RC-20) was heated in aq. 90% (v/v) dimethyl sulphoxide (80 ml) for 14.5 h at 95 °C. Five cycles of this reaction were performed. The total reaction mixture obtained was evaporated *in vacuo*, and the residue was applied on to a column (3.0 cm × 86 cm) of Dowex AG-1 X4 (Cl^-form), which was eluted with linear gradients of 0.1–0.2 M-LiCl (2.4 litres), 0.2–0.25 M-LiCl (1.2 litres) and 0.5 M-LiCl (1 litre). The di-, tetra- and hexa-saccharide fractions obtained were separately subjected to rechromatography on the Dowex AG-1 X4 column, and each purified oligosaccharide fraction was applied on to a column (1.0 cm × 85 cm) of Dowex AG-1 X4 (formate form) kept at 37 °C, and eluted with linear gradients of 0–0.2 M-formic acid (1 litre) for the disaccharide fraction, 0.1–0.4 M-formic acid (2 litres) for the tetrasaccharide fraction and 0.3–0.8 M-formic acid (3 litres) for the hexasaccharide fraction. Each of the isomeric oligosaccharides was isolated as the lithium salt, after neutralization with pyridine followed by freeze-drying and passage through a column of Dowex 50 W X2 (Li^+ form).

Borohydride reduction of oligosaccharides

To each of di- or tetra-saccharides (20 mg) in water (1 ml) was added $NaBH_4$ (20 mg) dissolved in 1 M-NH_3 (1 ml). The mixture was left to stand for 1 h at room temperature, then excess borohydride was destroyed by the addition of acetic acid until evolution of H_2 stopped. The reaction mixture was passed through a column of Dowex 50 W X2 (H^+ form) and the eluent was freeze-dried. Boric acid was removed by repeated evaporation with methanol. The residue dissolved in water (5 ml) was neutralized with 1 M-LiOH, and concentrated, then applied to a column (1.0 cm × 55 cm) of Sephadex G-15 prepared in 10% (v/v) ethanol for desalting.

RESULTS AND DISCUSSION

The 400 MHz ^1H-n.m.r. spectrum of I–H–I–H, one of the tetrasaccharides obtained by solvolysis of rooster comb dermatan sulphate in ^2H_2O at 20 °C and pH 5.2, is shown in Fig. 1. Chemical shifts (δ) and proton–proton spin-coupling constants (J) for the L-iduronate residue in the disaccharide (I–H), three tetrasaccharides (I–H–I–H, I–H–G–H and G–H–I–H), and their alditols, and the hexasaccharide ($\overset{3}{\text{I}}$–$\overset{2}{\text{H}}$–$\overset{1}{\text{I}}$–H–I–H) were assigned by means of spin-decoupling difference experiments, homonuclear

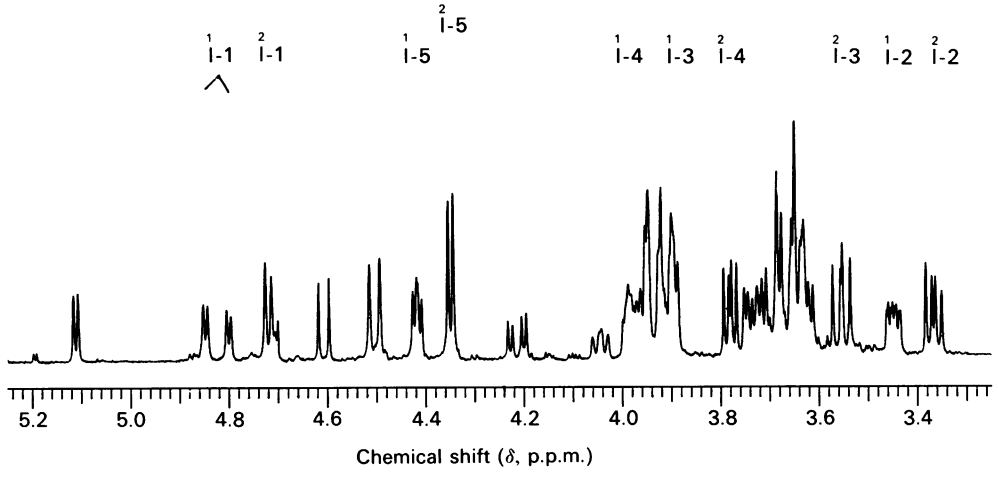

Fig. 1. 400 MHz ^1H-n.m.r. spectrum of the tetrasaccharide $\overset{2}{\text{I}}$–$\overset{1}{\text{H}}$–I–H (*N*-acetyldermosine dimer)

The resonances due to $\overset{1}{\text{I}}$-1–5 and $\overset{2}{\text{I}}$-1–5 protons are indicated.

Table 1. Observed and calculated chemical shifts and proton–proton spin-coupling constants for L-induronate residue in dermatan disaccharide (*N*-acetyldermosine) and its alditol

Values in parentheses and in square brackets are the observed and calculated coupling constants respectively.

Sample	Anomer	Temperature (°C)	Chemical shift (δ, p.p.m.) and coupling constant (Hz)				
			H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5
I–H	α	20	4.781 (4.9) [5.6]	3.384 (7.8) [7.7]	3.578 (6.3) [6.0]	3.796 (4.0) [3.8]	4.380
	β	20	4.732 (5.2) [5.7]	3.380 (7.8) [7.6]	3.560 (6.4) [6.2]	3.788 (4.0) [4.0]	4.367
	α	80	4.848 (5.5) [5.9]	3.402 (7.7) [7.6]	3.614 (7.0) [6.6]	3.816 (4.1) [4.3]	4.390
	β	80	4.807 (5.5) [5.9]	3.398 (7.7) [7.6]	3.607 (7.0) [6.6]	3.813 (4.1) [4.3]	4.378
I–H-ol		20	4.715 (6.4) [6.6]	3.445 (9.0) [8.9]	3.571 (7.2) [7.1]	3.813 (4.8) [4.8]	4.418
		80	4.771 (6.5) [6.6]	3.425 (8.5) [8.5]	3.591 (7.4) [7.4]	3.807 (5.0) [4.9]	4.395

COSY-45 and heteronuclear $^{13}C/H$ correlation experiments. Data for the disaccharide and for the tetra- and hexa-saccharides are listed in Tables 1 and 2 respectively. The L-iduronate residues in the tetrasaccharides are located in two different environments. Proton–proton spin-coupling constants for the internal L-iduronate residue, which is substituted at the C-1 and C-4 positions by two *N*-acetylgalactosamine residues, are clearly different from those for the non-reducing terminal L-iduronate residue, substituted only at the C-1 position by the same substituent. Proton–proton spin-coupling constants for the L-iduronate residue in the disaccharide are close to those for the L-iduronate at the non-reducing terminal position in the tetrasaccharides (I–H–I–H and I–H–G–H). H-2, H-3 and H-4 Protons of the internal L-iduronate residue in the tetrasaccharides (I–H–I–H and G–H–I–H) give complex multiplets, owing to small differences in chemical shifts between α- and β-anomers of the reducing *N*-acetylgalactosamine residue, but the chemical shifts for the non-reducing terminal L-iduronate protons in the tetrasaccharides (I–H–I–H and I–H–G–H) are similar for both α- and β-anomers of the distant reducing terminal residue.

There are two kinds of internal L-iduronate residues, $\overset{1}{\text{I}}$ and $\overset{2}{\text{I}}$, in $\overset{3}{\text{I}}$–H–$\overset{2}{\text{I}}$–H–$\overset{1}{\text{I}}$–H, which is a major hexasaccharide derived from desulphated dermatan sulphate. H-2, H-3 and H-4 protons of the L-iduronate residue $\overset{2}{\text{I}}$ of the hexasaccharide showed complex signals containing multiplets due to the protons of $\overset{1}{\text{I}}$ adjacent to α- and β-anomers of the reducing *N*-acetylgalactosamine residue. Under these circumstances, proton–proton spin-coupling constants for $\overset{2}{\text{I}}$ are satisfactorily determined by spin-decoupling difference experiments, but those for $\overset{1}{\text{I}}$ could not be determined. On the other hand proton–proton spin-coupling constants for the non-reducing terminal L-iduronate residue $\overset{3}{\text{I}}$ in the hexasaccharide are unequivocally determined.

The oligosaccharides were converted by borohydride reduction into the alditol derivatives, and their ^1H-n.m.r. spectra were examined. Although the proton–proton spin-coupling constants for the internal L-iduronate residue of the alditols (I–H–I–H-ol and G–H–I–H-ol) were significantly changed by the reduction, those for the non-reducing terminal L-iduronate residue remained nearly unchanged.

A force-field calculation (Ragazzi *et al.*, 1986) of the conformational properties of methyl 4-*O*-methyl-2-*O*-sulpho-L-idopyranosiduronate has demonstrated that the skew-boat conformer ($^2S_0$) is nearly isoenergetic with the two chair conformers ($^1C_4$ and $^4C_1$). The conformational populations of the internal or non-reducing terminal L-iduronate residues were obtained by fitting the average coupling constants $J_{1,2}$, $J_{2,3}$, $J_{3,4}$ and $J_{4,5}$, computed for a mixture of the $^1C_4$, $^4C_1$ and $^2S_0$ forms, to the experimental values by least-squares analysis. Predicted coupling constants for the three conformers used in these calculations were obtained from the values for a glycosidically linked L-iduronate residue that had been reported by Sanderson *et al.* (1987), except for the $J_{4,5}$ value for the $^4C_1$ conformer, for which the largest value experimentally observed (6.35 Hz) was employed in accordance with the discussion by Sanderson *et al.* (1987). The results of the calculations are summarized in Table 3, which reveals the following points. (1) The non-reducing terminal L-iduronate residue in the disaccharide (I–H), tetrasaccharides (I–H–I–H and I–H–G–H), their alditols (I–H–I–H-ol and I–H–G–H-ol) and the hexasaccharide ($\overset{3}{\text{I}}$–H–$\overset{2}{\text{I}}$–H–$\overset{1}{\text{I}}$–H) is in equilibrium with nearly equal populations of $^1C_4$ (30%), $^4C_1$ (40%) and $^2S_0$ (30%) conformers. This fact indicates that the three conformers are approximately isoenergetic with one another in the non-reducing terminal L-iduronate residue at 20 °C. (2) The internal L-iduronate residue in the tetrasaccharides (I–H–I–H and G–H–I–H) is in a

Table 2. Observed and calculated chemical shifts and proton–proton spin-coupling constants for L-iduronate residues in dermatan tetrasaccharides, their alditols and dermatan hexasaccharide (*N*-acetyldermosine trimer)

Values in parentheses and in square brackets are the observed and calculated coupling constants respectively.

Sample	Anomer	Temperature (°C)	Chemical shift (δ, p.p.m.) and coupling constant (Hz)									
			Internal L-iduronate					Non-reducing L-iduronate				
			H-1 ($J_{1,2}$)	H-2 ($J_{3,4}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{1,5}$)	H-5
I–H–I–H	α	20	4.875 (3.4) [4.1]	3.476 (6.4) [6.3]	N.D.* (4.1) [4.0]	4.018 (2.6) [2.2]	4.452	4.747 (5.0) [5.7]	3.395 (7.9) [7.8]	3.581 (6.2) [6.0]	3.808 (4.2) [3.9]	4.377
	β	20	4.828 (3.5) [4.1]	3.480 (6.5) [6.3]	N.D.* (4.1) [4.0]	4.015 (2.6) [2.2]	4.441					
	α	80	4.926 (4.0) [4.6]	3.492† (6.4) [6.3]	3.959† (5.0) [4.8]	4.076† (3.0) [2.8]	4.463	4.806 (5.3) [5.8]	3.402 (7.5) [7.5]	3.615 (6.5) [6.4]	3.818 (4.4) [4.1]	4.383
	β	80	4.885 (3.9) [4.6]	3.492† (6.4) [6.3]	3.959† (5.0) [4.8]	4.076† (3.0) [2.8]	4.453					
G–H–I–H	α	20	4.879 (3.4) [4.1]	3.472 (6.4) [6.3]	N.D.* (4.1) [3.9]	4.019 (2.5) [2.1]	4.444					
	β	20	4.831 (3.4) [4.1]	3.476 (6.4) [6.3]	N.D.* (4.1) [3.9]	4.015 (2.5) [2.1]	4.434					
I–H–G–H	$\alpha\beta$	20						4.761 (5.0) [5.7]	3.402 (7.8) [7.7]	3.593 (6.2) [6.1]	3.820 (4.2) [3.9]	4.387
I–H–I–H-ol		20	4.798 (5.2) [4.9]	3.606 (8.0) [8.1]	3.919 (4.2) [4.6]	4.035 (3.1) [2.7]	4.480	4.761 (5.0) [5.6]	3.408 (7.8) [7.7]	3.595 (6.2) [6.0]	3.820 (4.1) [3.8]	4.387
		80	4.845 (5.1) [5.0]	3.582 (7.5) [7.6]	3.942 (4.5) [4.9]	4.081 (3.4) [3.0]	4.459	4.804 (5.2) [5.8]	3.403 (7.7) [7.6]	N.D.* (6.4) [6.2]	3.819 (4.2) [4.0]	4.382
G–H–I–H-ol		20	4.815 (5.1) [4.9]	3.614 (8.0) [8.1]	3.933 (4.3) [4.6]	4.052 (3.0) [2.7]	4.488					
I–H–G–H-ol		20						4.783 (4.9) [5.6]	3.421 (7.8) [7.7]	3.611 (6.1) [5.9]	3.837 (4.1) [3.8]	4.406
^{3}I–H–^{2}I–H–^{1}I–H	$\alpha\beta$	20	4.817 (3.7)‡ [4.4]	3.471 (6.7)‡ [6.5]	3.924 (4.5)‡ [4.4]	4.005 (2.9)‡ [2.5]	4.430‡	4.746 (5.0) [5.7]	3.398 (7.9) [7.7]	3.582 (6.3) [6.1]	3.810 (4.1) [3.9]	4.377

* Not determined.
† Value at the central position of group of peaks.
‡ Data for ^{2}I in ^{3}I–H–^{2}I–H–^{1}I–H.

Table 3. Calculated proportions of the L-iduronate conformer ($^2S_0$, $^1C_4$ and $^4C_1$) populations for dermatan di- and tetra-saccharides, their alditols and dermatan hexasaccharide (*N*-acetyldermosine trimer)

Sample	Anomer	Temperature (°C)	Proportion in conformer (%)					
			Internal L-iduronate			Non-reducing L-iduronate		
			$^2S_0$	$^1C_4$	$^4C_1$	$^2S_0$	$^1C_4$	$^4C_1$
I–H	α	20				30	30	40
	β	20				26	30	44
	α	80				16	30	54
	β	80				16	30	54
I–H–I–H	α	20	42	54	4			
						32	28	40
	β	20	42	54	4			
	α	80	26	52	22			
						18	32	50
	β	80	28	52	20			
G–H–I–H	α	20	44	54	2			
	β	20	44	54	2			
I–H–G–H	αβ	20				28	30	42
I–H-ol		20				32	10	58
		80				18	16	66
I–H–I–H-ol		20	66	28	6	30	30	40
		80	50	34	16	24	30	46
G–H–I–H-ol		20	66	28	6			
I–H–G–H-ol		20				32	30	38
^{3}I–H–^{2}I–H–^{1}I–H	αβ	20	38*	50*	12*	28	30	42

* Data of ^{2}I in ^{3}I–H–^{2}I–H–^{1}I–H.

conformational equilibrium that is different from that of the non-reducing terminal L-iduronate; the $^1C_4$ (54%) and $^2S_0$ (42–44%) conformers are predominant in the former. This characteristic conformational population of the internal L-iduronate residue may be explained by hydrogen-bonding together with the steric effects of being flanked by two neighbours. Since the carboxy group of the L-iduronate $^1C_4$ and $^2S_0$ conformers adopts an equatorial or quasi-equatorial orientation, it is close to the amido NH group of the *N*-acetylgalactosamine substituted at the 4-OH of the L-iduronate residue. Moreover, the axial 3-OH group of the L-iduronate $^1C_4$ conformer may be located closely to the amido $>C=O$ group of the other adjacent *N*-acetylgalactosamine at the reducing end, and the quasi-equatorial 2-OH group of the L-iduronate $^2S_0$ conformer may be close to the same amido $>C=O$ group. (3) The small difference in the conformational population between ^{2}I in the hexasaccharide and the internal L-iduronate residue in the tetrasaccharides seems to be attributable to the difference in the distance from the reducing terminal residue. (4) The conformational population of the internal L-iduronate residue varies significantly with borohydride reduction of the reducing terminal residue. Whereas the internal L-iduronate residue in the tetrasaccharide alditols (I–H–I–H-ol and G–H–I–H-ol) is predominantly in the $^2S_0$ conformation (66%), the contribution of the $^1C_4$ conformation decreases to 28%. As to the L-iduronate residue in the disaccharide alditols (I–H-ol), the contribution of the $^4C_1$ conformation increases to 58% and that of the $^1C_4$ conformation decreases to 10%. The results indicate that the conformation of the L-iduronate residue in these oligosaccharides is affected by the substituent at the C-1 position of the L-iduronate, and that the L-iduronate residue substituted with an acyclic alditol, *N*-acetylgalactosaminitol, at its C-1 position favours the $^2S_0$ and $^4C_1$ conformations where the substituent is oriented equatorially. On the other hand, the conformational population of the non-reducing terminal L-iduronate residue in the tetrasaccharides (I–H–I–H and I–H–G–H) is the same before and after the borohydride reduction. (5) At an elevated temperature (80 °C) the proportion of the $^2S_0$ conformer decreases and that of the $^4C_1$ conformer increases in both internal and non-reducing terminal L-iduronate residues of all the oligosaccharides. However, the population of the $^1C_4$ conformer is almost unchanged except for a slight increase in its proportion of the L-iduronate residue in I–H-ol and the internal L-iduronate residue in I–H–I–H-ol.

The conformational population for the L-iduronate residue in native dermatan sulphate was calculated by least-squares analysis based on the published proton–proton spin-coupling constants (Gatti *et al.*, 1979) and

compared with that for ^{2}I in the hexasaccharide (^{3}I–^{2}H–^{1}I–H–^{2}I–H). The conformational population for ^{2}I in the hexasaccharide is expected to be similar to that of the L-iduronate residue in a dermatan polysaccharide chain without sulphate substitution. Therefore a small difference in the conformational population between ^{2}I in the hexasaccharide ($^{2}S_0$, 38%; $^{1}C_4$, 50%; $^{4}C_1$, 12%) and the L-iduronate residue in native dermatan sulphate ($^{2}S_0$, 36%; $^{1}C_4$, 60%; $^{4}C_1$, 4%) appears to be associated with the presence of the sulphate groups in the polysaccharide. A similar difference, due to the presence of sulphate groups, is observed in the conformational populations for the non-reducing L-iduronate residues in the non-sulphated and sulphated tetrasaccharides derived from bovine lung heparan sulphate (for I–GlcNAc–G–anManOH: $^{2}S_0$, 14%; $^{1}C_4$, 24%; $^{4}C_1$, 62%; for I–GlcNAc$_{6S}$–G–anManOH$_{6S}$: $^{2}S_0$, 16%; $^{1}C_4$, 40%; $^{4}C_1$, 44%), which were calculated by us on the basis of the proton–proton spin-coupling constants reported by Sanderson *et al.* (1985). Tables 1 and 2 show that the observed proton–proton spin-coupling constants are in good agreement with the calculated values, except for a few cases of deviation (max. 0.7 Hz) for the $J_{1,2}$ values.

The above results show that the $^{2}S_0$ conformer makes an important contribution to the conformational equilibrium of the non-sulphated L-iduronate residues, and that the position of the conformational equilibrium among the three conformers is sensitive to the local structural environment in the oligo- and poly-saccharides.

Received 31 May 1989/27 July 1989; accepted 10 August 1989

We thank Mrs. A. Hatano and Mrs. N. Sato of our School for the recording of the ^{1}H-n.m.r. spectra.

REFERENCES

Bax, A. (1983) J. Magn. Reson. **53**, 517–520

Bax, A. & Freeman, R. (1981) J. Magn. Reson. **44**, 542–561

Casu, B., Choay, J., Ferro, D. R., Gatti, G., Jacquinet, J.-C., Petitou, M., Provasoli, A., Ragazzi, M., Sinaÿ, P. & Torri, G. (1986) Nature (London) **322**, 215–216

Comper, W. D. & Laurent, T. C. (1978) Physiol. Rev. **58**, 255–315

Ferro, D. R., Provasoli, A., Ragazzi, M., Torri, G., Casu, B., Gatti, G., Jacquinet, J.-C., Sinaÿ, P., Petitou, M. & Choay, J. (1986) J. Am. Chem. Soc. **108**, 6773–6778

Gatti, G., Casu, B., Torri, G. & Vercellotti, J. R. (1979) Carbohydr. Res. **68**, c3–c7

Nagasawa, K., Ogamo, A., Ichihara, H. & Yoshida, K. (1984) Carbohydr. Res. **131**, 301–314

Ragazzi, M., Ferro, D. R. & Provasoli, A. (1986) J. Comput. Chem. **7**, 105–112

Rees, D. A., Morris, E. R., Stoddart, J. F. & Stevens, E. S. (1985) Nature (London) **317**, 480

Sanderson, P. N., Huckerby, T. N. & Nieduszynski, I. A. (1985) Glycoconjugate J. **2**, 109–120

Sanderson, P. N., Huckerby, T. N. & Nieduszynski, I. A. (1987) Biochem. J. **243**, 175–181

Sanderson, P. N., Huckerby, T. N. & Nieduszynski, I. A. (1989) Biochem. J. **257**, 347–354

Torri, G., Casu, B., Gatti, G., Petitou, M., Choay, J., Jacquinet, J.-C. & Sinaÿ, P. (1985) Biochem. Biophys. Res. Commun. **128**, 134–140