Biochem. J. (1989) 257, 347–354 (Printed in Great Britain)

Chondroitinase ABC digestion of dermatan sulphate

N.m.r. spectroscopic characterization of the oligo- and poly-saccharides

Paul N. SANDERSON,*‡ Thomas N. HUCKERBY† and Ian A. NIEDUSZYNSKI*§
*Department of Biological Sciences, University of Lancaster, Bailrigg, Lancaster LA1 4YQ, U.K., and †Department of Chemistry, University of Lancaster, Bailrigg, Lancaster LA1 4YA, U.K.

Dermatan sulphates, in which iduronate was the predominant uronate constituent, were partially digested by chondroitinase ABC to produce oligosaccharides of the following structure:

$$\Delta UA\text{-}[GalNAc(4SO_3)\text{-}IdoA]_m GalNAc(4SO_3)$$

[where $m = 0$–5, ΔUA represents β-D-gluco-4-enepyranosyluronate, IdoA represents α-L-iduronate and GalNAc($4SO_3$) represents 2-acetamido-2-deoxy-β-D-galactose 4-*O*-sulphate], which were fractionated by gel-permeation chromatography and examined by 100 MHz ^{13}C-n.m.r. and 400/500 MHz ^1H-n.m.r. spectroscopy. Experimental conditions were established for the removal of non-reducing terminal unsaturated uronate residues by treatment with $HgCl_2$, and reducing terminal *N*-acetylgalactosamine residues of the oligosaccharides were reduced with alkaline borohydride. These modifications were shown by ^{13}C-n.m.r. spectroscopy to have proceeded to completion. Assignments of both ^{13}C-n.m.r. and ^1H-n.m.r. resonances are reported for the GalNAc($4SO_3$)-IdoA repeat sequence in the oligosaccharides as well as for the terminal residues resulting from enzyme digestion and subsequent modifications. A full analysis of a trisaccharide derived from dermatan sulphate led to the amendment of published ^{13}C-n.m.r. chemical-shift assignments for the polymer.

INTRODUCTION

Dermatan sulphate proteoglycans are components of the extracellular matrices of many tissues, including skin (Cöster *et al.*, 1975) and articular cartilage (Rosenberg *et al.*, 1985). Within extracellular matrices dermatan sulphate proteoglycans interact specifically with other macromolecules, including collagen (Scott & Haigh, 1985) and fibronectin (Schmidt *et al.*, 1987; Lewandowska *et al.*, 1987). The precise nature of these interactions remains to be determined; however, a specific association between dermatan sulphates and collagen has been described (Gallagher *et al.*, 1983). Dermatan sulphates also interact with plasma heparin cofactor II, resulting in a dramatic increase in the rate of inhibition of thrombin by heparin cofactor II (Tollefsen *et al.*, 1983). Specific dermatan sulphate fragments of dodecasaccharide size or larger have been shown to be responsible for this binding (Tollefsen *et al.*, 1986).

Dermatan sulphates are composed of alternating galactosamine 4-(or 6-)sulphate and uronate residues. The uronate component may be either glucuronate or iduronate, and the latter may be ester-sulphated at C-2 (Lindahl & Höök, 1978). Considerable scope therefore exists for sequence heterogeneity, with the possibility that particular structural features within dermatan sulphate chains may be required for specific biological interactions. This heterogeneity complicates structural analysis of dermatan sulphate polymers, and consequently it is necessary when investigating biological interactions to study oligosaccharide fragments produced by chemical [e.g. periodate oxidation/borohydride reduction (Fransson & Carlstedt, 1974)] or enzymic methods. Digestion of dermatan sulphates by the bacterial eliminase chondroitinase ABC (chondroitin ABC lyase) (Yamagata *et al.*, 1968) results in oligosaccharide products with unsaturated uronate non-reducing terminal residues. It has been reported (Ludwigs *et al.*, 1985, 1987) that such terminal unsaturated uronate residues can be removed from hyaluronate disaccharides by mild treatment with mercuric salts. In the present study this technique was used to remove unsaturated uronate residues from oligosaccharides obtained by chondroitinase ABC digestion of a dermatan sulphate preparation of high iduronate content. These oligosaccharides were characterized by n.m.r. spectroscopy and the major ^1H-n.m.r. and ^{13}C-n.m.r. resonances were assigned as a prelude to further interaction studies.

EXPERIMENTAL

Materials

By-products from heparin manufacture were supplied by Dr. W. E. Lewis, formerly of Glaxo Operations, Runcorn, Cheshire, U.K. Chondroitinase ABC and hyaluronidase (type I-S, from bovine testes) were

Abbreviations used: ΔUA, β-D-gluco-4-enepyranosyluronate; GalNAc, 2-acetamido-2-deoxy-β-D-galactose; GalNAc-ol, 2-acetamido-2-deoxy-D-galactosaminitol; IdoA, α-L-iduronate; SO_3, sulphate ester; DS(*x*), dermatan sulphate oligosaccharide with *x* residues (unreduced); R (reduced).

‡ Present address: Department of Food Research and Technology, Cranfield Institute of Technology, Silsoe College, Silsoe, Bedfordshire MK45 4DT, U.K.

§ To whom correspondence and requests for reprints should be addressed.

obtained from Sigma Chemical Co., Poole, Dorset, U.K. All other materials were obtained from sources described previously (Sanderson *et al.*, 1987).

Preparation of dermatan sulphates

Dermatan sulphates were prepared from pig mucosal heparin by-products by precipitation with cetylpyridinium chloride followed by fractionation of the calcium salt with ethanol (Rodén *et al.*, 1972). The polysaccharides, which were precipitated in 18% (v/v) ethanol, had a galactosamine/glucosamine ratio of 22:3 as determined by hexosamine analysis on an LKB 4101 amino acid analyser (LKB Biochrom, Cambridge, U.K.). The major contaminant, heparin, was removed by precipitation with 3 M-potassium acetate (Scott *et al.*, 1968). The product, in which galactosamine accounted for more than 98% of the hexosamine, was not significantly degraded by testicular hyaluronidase and was therefore a dermatan sulphate preparation of high iduronate content. The ^{13}C-n.m.r. spectrum (Fig. 1) confirms the purity of the preparation, the 12 distinct resonances representing the 12 carbon atoms of the disaccharide repeat unit. Any glucuronate residues present would give rise to an anomeric resonance at 106.4 p.p.m. [cf. chondroitin 4-sulphate (Bociek *et al.*, 1980)]. No signals are observed in this region.

Chondroitinase ABC digestion of dermatan sulphate

For a preliminary analytical-scale digestion, dermatan sulphate (1 mg) was dissolved in 10 ml of digest buffer (50 mM-Tris/HCl buffer, pH 8.0, containing 60 mM-sodium acetate and 0.01% bovine serum albumin). A reference solution was prepared by adding 0.0015 unit of chondroitinase ABC to 3 ml of this solution and heating the mixture at 100 °C for 10 min. The absorbance at 232 nm of this blank solution did not increase significantly over the reaction period. The digest was started by the addition of 0.001 unit of enzyme to 2 ml of the dermatan sulphate solution at 37 °C, and the increase in absorbance at 232 nm was monitored with time. To determine the size distribution of oligosaccharide intermediates produced during the digestion, 20 mg of dermatan sulphate was dissolved in 20 ml of digest buffer and incubated at 37 °C with 0.1 unit of chondroitinase ABC. At specified times 2 ml portions were withdrawn and heated at 100 °C for 15 min. These samples were freeze-dried before being dissolved in 200 μl of 0.2 M-NH_4HCO_3 (containing 0.1% bovine serum albumin and 2 M-NaCl as V_0 and V_t markers respectively) and were then applied to an analytical Sephadex G-50 (superfine grade) column (size, 140 cm × 0.9 cm; eluent, 0.2 M-NH_4HCO_3; flow rate, 6.6 ml/h). Fractions were assayed for uronic acid by an automated carbazole procedure (Heinegård, 1973). For the preparative digest, 5 units of chondroitinase ABC were added to 1 g of dermatan sulphate in 100 ml of digest buffer at 37 °C and the digestion was stopped after 25 min by raising the temperature to 100 °C for 15 min. The freeze-dried sample was then dissolved in approx. 10 ml of 0.2 M-NH_4HCO_3 and applied to a Sephadex G-50 (superfine grade) column (size, 169 cm × 2.6 cm; eluent, 0.2 M-NH_4HCO_3; flow rate, 12.6 ml/h). The fractions in the peaks were pooled and then run on the analytical Sephadex G-50 column to remove any contaminating oligosaccharides of different sizes.

Borohydride reduction of oligosaccharides

Reduction of the reducing terminal galactosamine residues was achieved by addition of 120 mg of $NaBH_4$ in 5 ml of 100 mM-NaOH to approx. 120 mg of dermatan sulphate di-, tetra- or hexa-saccharide in 5 ml of water and leaving the mixture to stand overnight at room temperature after initial stirring. A further 100 mg of solid $NaBH_4$ was then added and the mixture left for 4–5 h. Excess borohydride was then destroyed by careful addition of HCl to lower the pH to 2–3 from the final reaction mixture pH of 10.3. The sample was neutralized with NaOH, and the mixture was concentrated by rotary evaporation and applied directly to a Sephadex G-25 desalting column (size, 156 cm × 2 cm; eluent 0.2 M-NH_4HCO_3; flow rate, 28 ml/h).

Cleavage of unsaturated uronic acid with $HgCl_2$

Reduced dermatan sulphate tetra- and hexa-saccharides (40–70 mg) were dissolved at a concentration of 1 mg/ml in 50 mM-Tris/HCl buffer, pH 6.8, containing 5 mM-$HgCl_2$ and incubated at 65 °C for 3 h. After cooling, approx. 10 ml of Dowex 50W-X8 (H^+ form) resin that had been pre-washed with water was added and the mixture was gently stirred for 15 min. The resin was then discarded and the solution titrated to pH 8 with a few drops of aq. NH_3. During this titration a precipitate formed. The entire mixture was rotary-evaporated to dryness and taken up in a minimum volume of 0.2 M-NH_4HCO_3. Any precipitated salt remaining was removed by centrifugation and the mixture was then applied directly to a Sephadex G-25 (superfine grade) desalting column (size, 158 cm × 2 cm; eluent, 0.2 M-NH_4HCO_3; flow rate, 28 ml/h). The column effluent was assayed for absorbance at 232 nm and uronic acid. Material pooled as the tri- and penta-saccharide had no significant absorbance at 232 nm.

N.m.r. spectroscopy

Oligosaccharide samples were converted into the Na^+ form and prepared for n.m.r. studies as previously described (Sanderson *et al.*, 1987) with sodium 3-trimethylsilyl[$^2H_4$]propionate as internal standard for both ^{1}H and ^{13}C n.m.r. measurements. ^{1}H-n.m.r. spectra were determined at 400 or at 500 MHz with Bruker WH400 and AM500 spectrometers respectively with 5 mm V.T. probes at ambient temperature or at 60 °C as indicated. ^{13}C-n.m.r. spectra were obtained at 25 MHz on a JEOL FX-100 system equipped with dual ^{1}H/^{13}C 5 mm V.T. probe and at 100 MHz on a Bruker WH400 instrument with a 5 mm or 10 mm V.T. probe as shown. Homonuclear COSY-45 and heteronuclear C/H correlations were performed as previously described (Huckerby *et al.*, 1986). Some of the data handling was performed by using NMR1 (Lab One NMR1 Spectroscopic Data Analysis System, Release 3.8; New Methods Research, Syracuse, NY, U.S.A.).

RESULTS AND DISCUSSION

The dermatan sulphate parent material was composed essentially of *N*-acetyldermosine repeat units in which the GalNAc residues were ester-sulphated at C-4. The composition of this material was confirmed by a 100 MHz ^{13}C-n.m.r. spectrum (Fig. 1). The two major

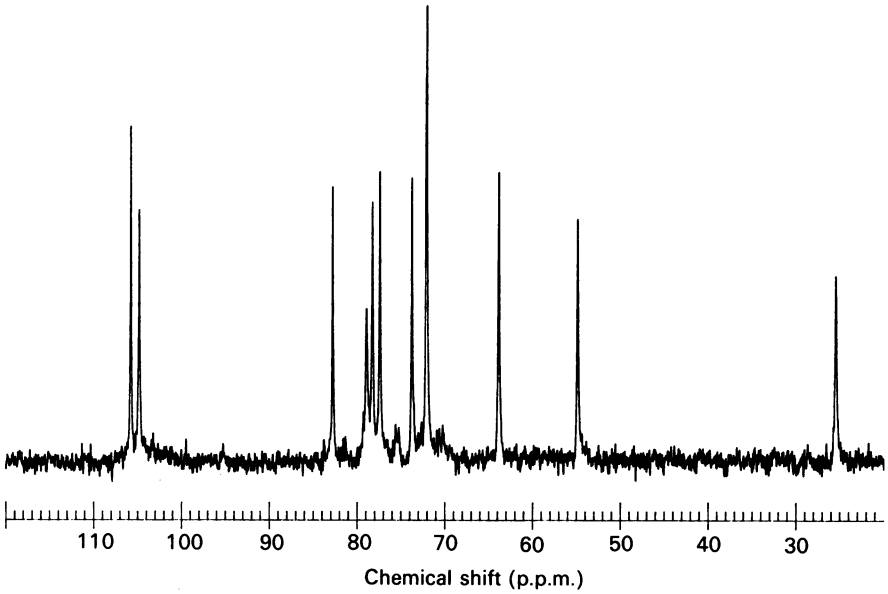

Fig. 1. 100 MHz ^{13}C-n.m.r. spectrum of the 'parent' dermatan sulphate (Na^+ form) at 60 °C

Carbonyl resonances at 176.57 and 177.76 p.p.m. have been omitted.

anomeric resonances at 105.73 and 104.74 p.p.m. represent C-1 of the IdoA and the GalNAc(4SO_3) residues respectively. Any glucuronate residues in the polymer would be expected to give rise to an anomeric resonance at 106.4 p.p.m (Bociek *et al.*, 1980). The absence of any significant peaks in this region confirms that the uronate component of this dermatan sulphate preparation is exclusively iduronate. Traces of minor structural components are also observed.

These dermatan sulphates give rise, upon chondroitinase ABC digestion, to oligosaccharide fragments consisting of GalNAc(4SO_3)-IdoA repeat sequences with unsaturated uronate residues at their non-reducing terminal and with reducing terminal GalNAc(4SO_3) residues. In order to produce oligosaccharide fragments that are representative of the dermatan sulphate parent structure it is necessary to remove the unsaturated uronate residues. A further modification of the oligosaccharide structure involves the reduction of the reducing terminal galactosamine residue. A summary of the digestion and modification procedures used to generate oligosaccharides from the dermatan sulphate is given in Scheme 1.

The rate of chondroitinase ABC digestion of the dermatan sulphates was determined, initially, by monitoring the increase in absorbance at 232 nm due to the unsaturated uronate residues of the oligosaccharide products. The digestion was 50% complete after 80 min, as determined by this method. Taking this value as a guideline, dermatan sulphate (20 mg) was incubated with enzyme, and at specified times portions were withdrawn and heated at 100 °C to stop the digestion. The size distribution of oligosaccharides generated at each stage of the digestion was determined by gel-filtration chromatography (Fig. 2). This showed that the optimum digestion period for production of a wide range of differently sized oligosaccharides was 25 min. For subsequent studies oligosaccharides were prepared from a 25 min chondroitinase ABC digestion of 1 g of dermatan

Scheme 1. Summary of digestion and modification procedures used to produce oligosaccharides from dermatan sulphate

$m > 0$; $n = 1$ or 2.

sulphate and were fractionated according to size by gel-permeation chromatography.

A 100 MHz ^{13}C-n.m.r. spectrum was obtained for each of the oligosaccharide fractions. The larger oligosaccharides [e.g. DS(10) and DS(12)] have spectra similar to that of the parent polymer (Fig. 1), but the spectra of smaller oligosaccharides are significantly complicated by resonances from the terminal residues. Reducing terminal galactosamine residues are in a state

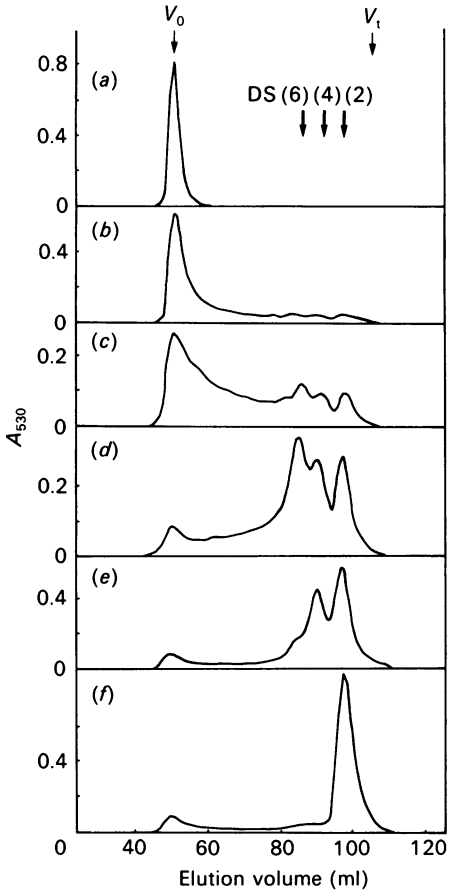

Fig. 2. Gel-permeation chromatography on Sephadex G-50 (superfine grade) of dermatan sulphate oligosaccharides produced by chondroitinase ABC digestion for (*a*) 0 min, (*b*) 10 min, (*c*) 25 min, (*d*) 55 min, (*e*) 120 min and (*f*) 31 h

of mutarotation; hence signals from both α- and β-forms are observed. Such forms also cause perturbations for chemical shifts of sites in adjacent residues. Simplification of the spectra is achieved by borohydride reduction of these galactosamine residues to produce galactosaminitol residues. The effect of this reduction upon the ^{13}C-n.m.r. spectrum of the tetrasaccharide is shown in Fig. 3. The signals from C-1 of the α- and β-galactosamine residues (at approx. 94 and 97.4 p.p.m. respectively; see Fig. 3*a*) disappear completely upon reduction (see Fig. 3*b*), to be replaced by the methylene resonance from C-1 of galactosaminitol.

The non-reducing unsaturated uronate residue gives rise to six resonances in the ^{13}C-n.m.r. spectra of the smaller oligosaccharides. This residue was completely removed from the tetrasaccharide by mild treatment with $HgCl_2$. The effectiveness of this removal is demonstrated by comparing the ^{13}C-n.m.r. spectrum of the reduced tetrasaccharide (Fig. 3*b*) with that of the trisaccharide remaining after removal of the unsaturated uronate (Fig. 3*c*). Note the complete absence of signals from C-1, C-4 and C-5 of the unsaturated uronate (at approx. 103, 109 and 147 p.p.m. respectively) in the trisaccharide spectrum.

Detailed assignments of the oligosaccharide spectra may be divided into two categories: first, the *N*-acetyldermosine repeat unit which is of various lengths, and, secondly, the terminal residues whose contribution to the total spectrum diminishes with molecular size. In even-numbered oligomers the non-reducing terminal residue is ΔUA, but in odd-numbered oligomers this becomes *N*-acetylgalactosamine.

The end-residue resonances may be assigned by detailed examination of data from DS(2R) and DS(3R). A two-dimensional COSY-45 proton spectrum for DS(2R) (not shown) permitted a total assignment of chemical shifts for ΔUA ^{1}H-n.m.r. signals and an extensive identification of signals from the strongly second-order galactosaminitol residue. It should be noted that this oligosaccharide is not representative since the two terminal residues are attached to one another. These assignment data are summarized in Table 1. Extensive assignment of proton resonances for DS(3R) was made via COSY and Relayed-COSY experiments combined with accurate determinations of spin–spin couplings obtained through MEFSD resolution enhancement of segments of one-dimensional 400 MHz and 500 MHz spectra by using the computer program suite NMR1. In this system total assignments for the non-reducing galactosamine were precluded because of second-order characteristics. Assignment of H-2 in the galactosaminitol came from a C–H correlation experiment (not shown) in which the C-2–N carbon atom could be unambiguously assigned. By using this correlation map, assignments for the other carbon atoms could then be achieved. ^{1}H-n.m.r. data are summarized in Table 1, and ^{13}C-n.m.r. shifts are given in Table 1.

There is considerable confidence in both ^{1}H-n.m.r. and ^{13}C-n.m.r. shift assignments for the iduronate residue in DS(3R) because these data derive from extensive homo- and hetero-nuclear correlation experiments. However, attention must now be given to the main repeat unit assignments for higher oligosaccharides and for the parent polymer, dermatan sulphate. Previous n.m.r. investigations of dermatan sulphate (Hamer & Perlin, 1976; Gatti *et al.*, 1979) have produced iduronate and partial galactosamine assignments for ^{13}C-n.m.r. and ^{1}H-n.m.r. spectra respectively. The earlier iduronate ^{1}H-n.m.r. assignments (Gatti *et al.*, 1979) were confirmed here via a series of spin–spin decoupling experiments. The second-order nature of the proton resonances from the *N*-acetylgalactosamine residues again precluded totally accurate shift determinations; nevertheless ^{1}H-n.m.r. assignments for these residues in dermatan sulphate were made and are reported here for the first time.

A comparison of the ^{13}C-n.m.r. data for DS(3R) with that from the polymer reveals an inconsistency. In earlier work on dermatan sulphate (Hamer & Perlin, 1976) the resonance at 82.74 p.p.m. (in our referencing system) was claimed to arise from GalNAc(4SO_3) C-3. In spectra of DS(3R) this resonance occurs at 72.82 p.p.m. for the unsubstituted non-reducing terminal galactosamine residue, indicating an unacceptably high downfield-shift perturbation of 10 p.p.m. upon substitution at C-3. Also, in DS(3R) the resonance at 81.81 p.p.m. was unequivocably assigned to C-4 of iduronate. In order to resolve this anomaly, a C–H correlation experiment was, with difficulty, performed on a sample of the dermatan sulphate polymer. The result is shown in Fig. 4. The lowfield carbon signal at 82.74 p.p.m. is clearly connected to the resonance at 4.11 p.p.m., which arises from

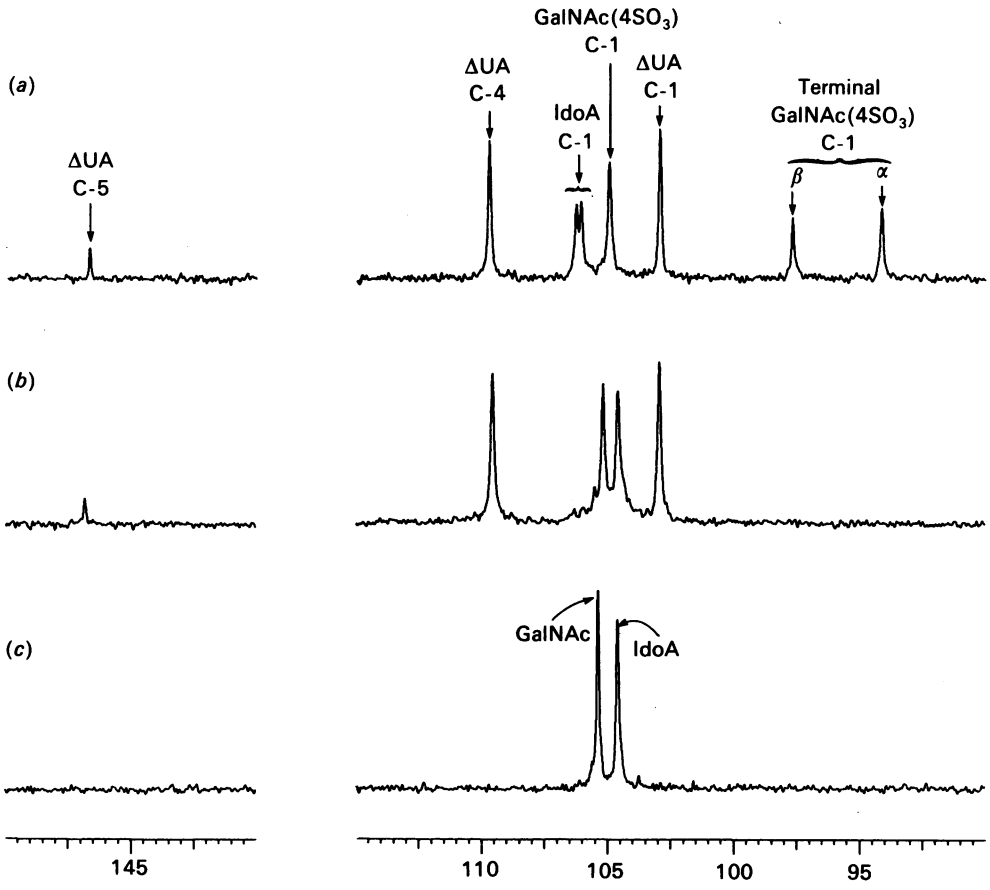

Fig. 3. Anomeric region of 25 MHz ^{13}C-n.m.r. spectrum for (*a*) DS(4), (*b*) the borohydride-reduced DS(4R) and (*c*) the trisaccharide, DS(3R), produced by the removal of the unsaturated uronate from DS(4R)

The identity of major resonances is indicated. Notes: (i) the IdoA C-1 signal in DS (4) has a doublet character because it 'senses' both α- and β-forms of the adjacent reducing terminal residue. This doublet character disappears upon reduction of the terminal GalNAc(4SO$_3$); (ii) upon reduction, the reducing terminal anomeric resonances also disappear, to be replaced by an additional methylene resonance (not shown); (iii) ΔUA resonances are no longer observed in the spectrum of DS(3R).

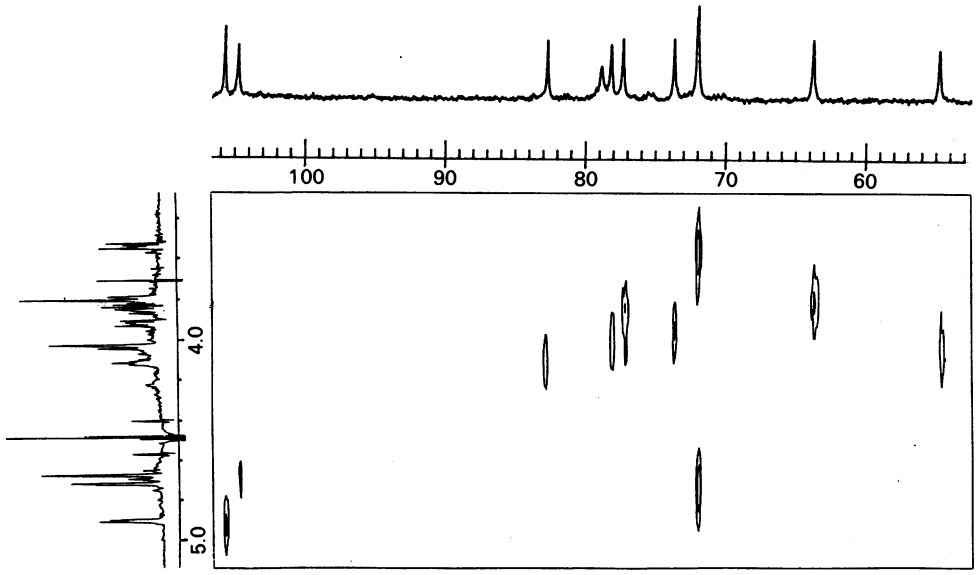

Fig. 4. ^{13}C/^{1}H correlation plot for the 'parent' dermatan sulphate

Table 1. ^{1}H chemical-shift values for dermatan sulphate and derived oligosaccharides

^{1}H signal positions determined at 400 MHz are given in p.p.m. downfield from internal sodium 3-trimethylsilyl[$^2H_4$]propionate. Residues -X- are flanked; X- and -X are at non-reducing and reducing termini respectively.

Residue		DS 70 °C	DS(2R) 20 °C	DS(3R) 20 °C	DS(2) 60 °C	DS(4) 60 °C	DS(6) 60 °C	DS(8) 60 °C	DS(10) 60 °C
ΔUA-									
	1		5.263		5.13				
					5.17				
	2		3.938						
	3		4.173						
	4		5.943		5.82	5.967			
					5.83				
GalNAc(4SO_3)-									
	1			4.58					
	2			3.83–3.91					
	3			3.83–3.91					
	4			4.694					
	5			3.83–3.91					
	6			3.83–3.91					
	6′			3.83–3.91					
	Me								
-GalNAc(4SO_3)-ol									
	1		3.65–3.7						
	1′		3.65–3.7						
	2		4.322	4.251					
	3		4.369	4.293					
	4		4.431	4.442					
	5		4.075	4.036					
	6		~ 3.65						
	6′		~ 3.65						
-GalNAc(4SO_3)-									
	1	4.7							
	2	4.05							
	3	3.95							
	4	4.65–4.7							
	5	3.8							
	6	3.75–3.8							
	6′	3.75–3.8							
	Me	2.081 (60 °C)							
-IdoA-									
	1	4.904		4.978			4.909	4.901	4.899
	2	3.536		3.647			3.537	3.53	3.528
	3	3.918		4.059			3.92	3.921	3.919
	4	4.113		4.151			4.124		
	5	4.717		4.647			4.721	4.715	4.714

iduronate H-4, confirming that this carbon resonance in the dermatan sulphate spectrum does, indeed, represent iduronate C-4. One signal is missing from the correlation map because of problems of broad resonances; this is the signal corresponding to the galactosamine C-4 resonance at 78.88 p.p.m. (see Fig. 1), which would be expected to connect to a corresponding ^{1}H signal at 4.65–4.7 p.p.m. as assigned by analogy with GalNAc(4SO_3) in DS(3R). This revised assignment leads to a much more reasonable downfield-shift movement of 5.3 p.p.m. for GalNAc-(4SO_3) C-3 in the polymer relative to the unsubstituted C-3 of GalNAc(4SO_3) in DS(3R). Hence assignments of the ^{1}H-n.m.r. and ^{13}C-n.m.r. resonances for the GalNAc(4SO_3)-IdoA repeat unit may be made as shown in Tables 1 and 2 respectively.

In conclusion, the assignments reported here provide a sound basis for the n.m.r. characterization of dermatan sulphates and, especially, of biologically active oligosaccharide derivatives of dermatan sulphates. Also, these assignments will facilitate determination of proton–proton coupling constants for iduronate in dermatan sulphates and, consequently, allow further examination of the conformational 'versatility' of iduronate residues already demonstrated in oligosaccharides derived from heparan sulphate and heparin (Sanderson *et al.*, 1985, 1987), in synthetic oligosaccharides (Ferro *et al.*, 1986) and in heparin itself (Casu *et al.*, 1986).

We thank the Science and Engineering Research Council (S.E.R.C.) and the Wellcome Trust for financial support, the S.E.R.C. for use of their 400 MHz and 500 MHz n.m.r. facilities, Dr. O. W. Howarth and Dr. A. T. Harrison (Warwick University) and Dr. L. Y. Lian (Leicester University) for assistance and valuable discussions, and Mr. H. Morris for technical assistance.

Table 2. ^{13}C chemical-shift values for dermatan sulphate and derived oligosaccharides

^{13}C signal positions determined at 100 MHz are given in p.p.m. downfield from internal sodium 3-trimethylsilyl[$^2H_4$]propionate. Anomeric carbon ^{13}C shifts are sensitive to temperature; e.g. DS(3R) here shows two coincident signals, which are clearly separated under the conditions of Fig. 3 and also of the $^{13}C/^1H$ correlation experiment. Residues -X- are flanked; X- and -X are at non-reducing and reducing termini respectively.

Residue		DS 60 °C	DS(3R) 60 °C	DS(2) 60 °C	DS(4) 60 °C	DS(6) 60 °C	DS(8) 60 °C	DS(10) 60 °C	DS(12) 60 °C
ΔUA-									
	1			102.73	102.73	102.81	102.82	102.83	102.9
	2			71.53	71.26	71.46	71.43	71.43	71.57
	3			67.51	67.36	67.44	67.41	67.41	67.53
	4			109.24	109.14	109.25	109.25	109.27	109.42
	5			146.97	146.86	146.98	146.97	146.97	146.97
	6			177.1	177.1	177.2	177.2	177.1	177.1
-GalNAc(4SO_3)									
	1α			94.07	93.95				
	1β			97.65	97.44				
	2α			52.49	52.27				
	2β			56.11	55.89				
	6α			63.76	63.69				
	6β			63.89					
	C=O			176.71					
	Meα			24.91	24.97				
	Meβ			25.07	25.12				
GalNAc(4SO_3)-									
	1		104.83						
	2		55.51						
	3		72.82						
	4		78.51						
	5		77.13						
	6		63.66						
	C=O		177.95						
	Me		{24.91 / 25.31}						
-GalNAc(4SO_3)-ol									
	1		{63.3 / 65.39}						
	2		54.71						
	3		78.88						
	4		81.1						
	5		73						
	6		{63.3 / 65.39}						
	C=O								
	Me		{24.91 / 25.31}						
-GalNAc(4SO_3)-									
	1	104.74			104.65	104.72	104.77	104.78	104.78
	2	54.79			54.63	54.79	54.75	54.76	54.84
	3	78.14			78.31	78.27	78.21	78.22	78.33
	4	78.88			78.79	78.91	78.91	78.92	78.93
	5	77.33				77.33	77.31	77.32	77.36
	6	63.74			63.68	63.71	63.72	63.74	63.77
	C=O	177.77			177.67	177.76	177.75	177.77	177.73
	Me	25.42			25.43	25.42	25.4	25.4	25.44
-IdoA-									
	1	105.73	104.83			105.79	105.81	105.83	105.83
	2	~ 72.05	72.13			72.08	72.13	72.14	72.11
							72.16	72.17	72.17
	3	73.72	73.31			73.81	73.86	73.89	73.83
	4	82.74	81.81		82.75	82.86	83.04	83.05	82.93
	5	~ 72.05	72.13			72.08	72.13	72.14	72.11
							72.16	72.17	72.17
	C=O	176.56	176.5		176.5	176.58	176.59	176.59	176.4

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Received 25 May 1988/13 July 1988; accepted 26 July 1988