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A ^{13}C-N.M.R. SPECTRAL STUDY OF CHONDROITIN SULFATES A, B, AND C: EVIDENCE OF HETEROGENEITY*

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ABSTRACT

Chondroitin sulfates A, B, and C produce well-resolved ^{13}C-n.m.r. spectra which allow for a more complete characterization than that available from their p.m.r. spectra. The ^{13}C data fully support earlier evidence as to the main structural features of these glycosaminoglycans, but they also show that many chondroitin preparations are substantially heterogeneous in composition. Thus, spectra of chondroitin A and C have the appearance of composites representative of both types of polymer: specimens of A may contain 25% of the C-type of structural sequence, and C, 30% of the A-type of sequence; 10–20% of unidentified constituents, including a residue bearing a 6-sulfate group, are present in the specimens of chondroitin B. Chemical-shift and $^{1}J_{C-H}$ values found for the L-iduronic acid residues of chondroitin B, as well as the effect of gadolinium nitrate on the relaxation properties of its ^{13}C nuclei, indicate that this moiety possesses the α configuration and favors the $^{1}C_{4}$(L) conformation. Corresponding data for the acetamidodeoxy-D-galactose and D-glucuronic acid residues of the chondroitins are consistent with the β-anomeric configuration and $^{4}C_{1}$(D) conformation in all instances.

INTRODUCTION

A variety of chemical and enzymic data from many sources[1–3] favor formulas **1**, **2**, and **3** as the major types of structural arrangement found in chondroitins A, B, and C, respectively. Support for these structures is also provided by p.m.r. spectroscopy[4]. However, because solutions of the polymers, particularly of chondroitin C, give rise to broad proton resonances, signal overlap (even at 220 MHz) has seriously interfered with attempts at detailed analyses of these spectra. ^{13}C-Signal line-widths in polymer spectra tend to be relatively much narrower[5], which should favor a better resolution of the ^{13}C spectra of chondroitin sulfates as compared with their ^{1}H spectra. Indeed, preliminary reports[6–8] show this to be the case, and we now present a fuller interpretation of these spectra and discuss their bearing on the chemical structure of the chondroitins.

*Dedicated to the memory of Professor Edward J. Bourne.

RESULTS AND DISCUSSION

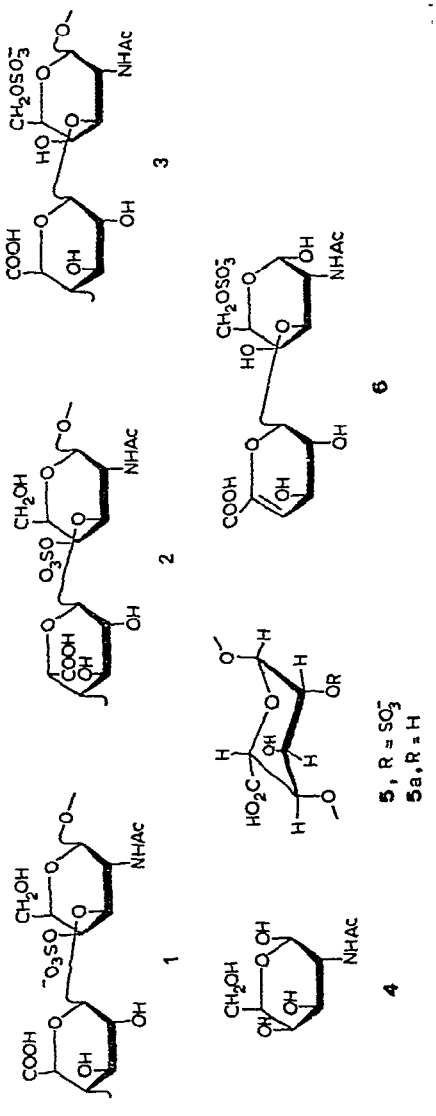
Analysis of the ^{13}C spectra. — Chondroitin sulfate B (dermatan sulfate) affords a well-resolved spectrum (Fig. 1B). A total of 12 major signals are clearly distinguishable, two of which represent two carbons each. From the shapes of these signals and their relative intensities, it is concluded that this spectrum reasonably accounts for a basic repeating sequence containing 14 non-equivalent carbons, as required by formula **2**. Because of their distinctive chemical shifts, signals for the two carbonyl groups (A-C and U-6), the anomeric carbons (A-1 and U-1), the carbon (A-2) bearing the acetamido group, and the acetamido methyl (CH_3) are most readily designated. Specific assignments of the carboxyl carbon (U-6) and U-1 signals—and hence, by inference, of A-C* and A-1—were facilitated by the finding that the intensities of these signals were selectively and strongly reduced in the presence of a trace proportion of gadolinium nitrate [$Gd(NO_3)_3$]. Since Gd^{3+} has a marked effect on T_1 of C-1 and C-6 of α-anomers of uronic acids[9], this observation served not only as an aid to signal identification but also to provide stereochemical information, as discussed below.

Assignments of other signals were made by reference to spectral data for appropriate model compounds. Thus, ^{13}C chemical-shifts were calculated for the acetamidodeoxyhexose residue of **2** from values reported[10] for 2-acetamido-2-deoxy-β-D-galactopyranose (**4**), allowance being made for the strong, deshielding change expected of A-1 and A-3 due to glycosidic bond formation[11] and of A-4 due to the sulfate group[12,13], and for small increases in shielding at adjacent carbons. As can be seen (Table I), these calculated values coincide quite well with the chemical shifts of the designated signals in Fig. 1B. Similarly, data for the α-L-idopyranosyluronic acid residue (**5**) of heparin[12] and methyl α-D-idopyranosiduronic acid[13] gave the calculated values of Table I, on which the signal assignments of uronic acid in Fig. 1B are based. Again, the agreement between the results observed and expected is good.

Hence, formula **2** is consistent with the major features of the ^{13}C spectrum of chondroitin sulfate B. However, several relatively weak signals are visible (arrows) in Fig. 1B, and the significance of these is discussed below.

Since 2-acetamido-2-deoxy-β-D-galactopyranose 4-sulfate is a constituent not only of chondroitin B but also of A, it is noteworthy that eight signals ascribed to this type of residue in Fig. 1B find close counterparts in the ^{13}C spectrum of chondroitin A (Fig. 1A). Hence, the assignments presented in the latter Figure are based in part on this analogy; they are favored also by the fact that treatment of the polymer with sodium periodate had virtually no effect on these signals, whereas most of the others were strongly shifted. Since only the uronosyl moiety of sequence **1** possesses a vicinal (2,3) diol, the oxidative cleavage should appreciably affect only signals of that residue.

*By way of confirmation, this signal appears in the ^{1}H-coupled spectrum (see below) as a narrow multiplet, attributable to small, two-bond coupling with the methyl protons.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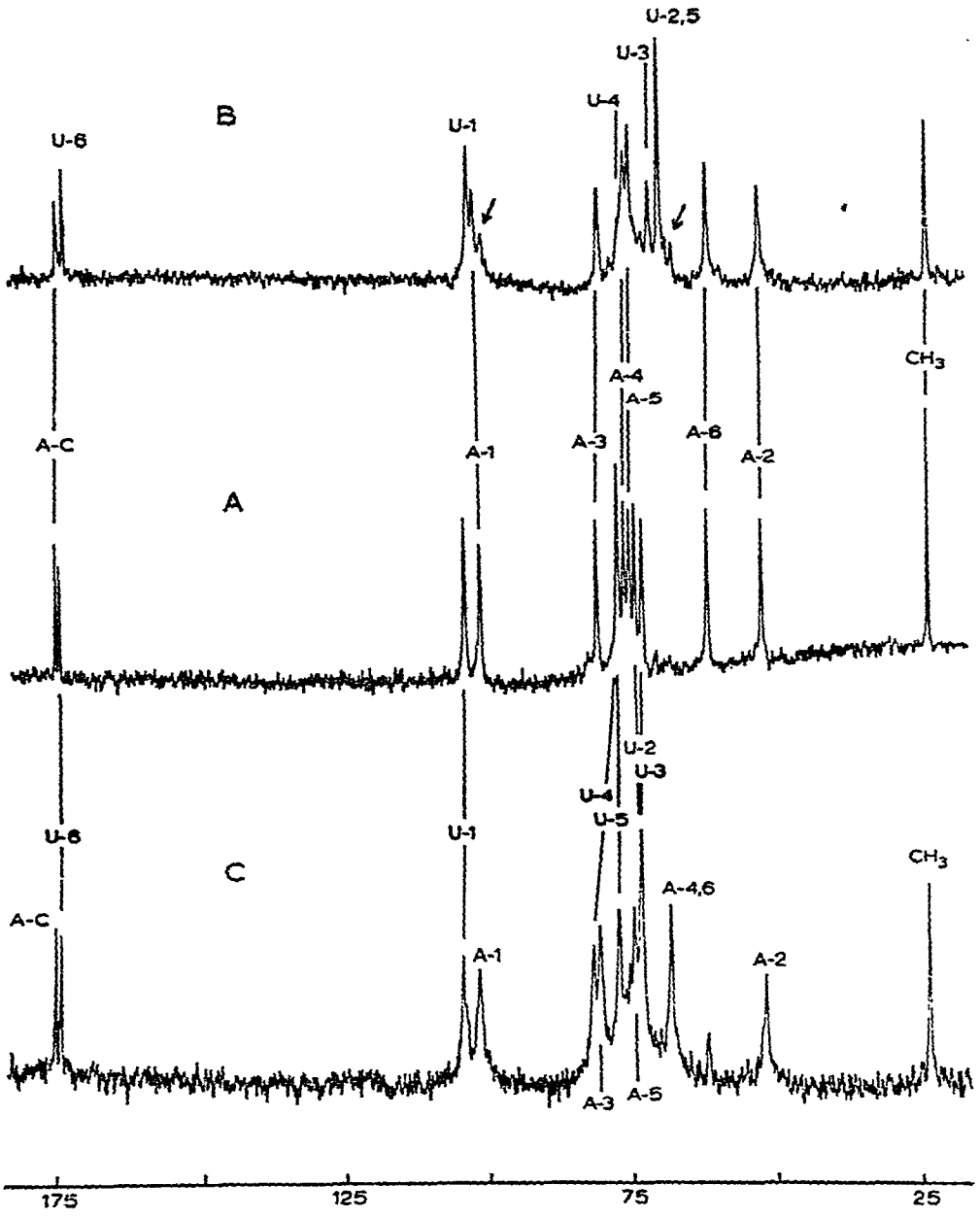

Fig. 1. ^{13}C-N.m.r. spectra (^{1}H-decoupled; 22.6 MHz) of chondroitins A, B, and C (spectra A, B, and C, respectively) as sodium salts in deuterium oxide. Temp.: 45° for chondroitins A and B, 60° for chondroitin C. Signals designated by "A" refer to those produced by acetamidodeoxyhexose residues, whereas those of uronosyl residues are labelled "U". The vertical lines relate resonances for analogous ^{13}C-nuclei in different polymers. Two minor signals are identified by arrows (see text below).

TABLE I

OBSERVED AND CALCULATED ^{13}C-CHEMICAL-SHIFTS OF MAJOR SIGNALS IN THE SPECTRA OF CHONDROITINS A, B, AND C

Carbon	*2-Acetamido-2-deoxy-β-D-galacto-pyranose Obs.*[a]	*Acetamidodeoxyhexose in chondroitins A and B*				*Acetamidodeoxyhexose in chondroitin C*		
		Increment[b]	*Calc.*	*Obs.*		*Increment*[b]	*Calc.*	*Obs.*
				A	*B*			
A-1	96.5	+7	103.5	102.3	102.6	+7	103.5	102.7
A-2	54.9	−1	53.9	53.0	53.4	−1	53.9	52.4
A-3	72.3	−1, +9	80.3	81.9	81.5	+9	81.3	81.3[c]
A-4	69.0	−1, +7	75.0	77.0	76.7	−1	68.0	69.0
A-5	76.3	−1	75.3	76.0	75.8	−1	75.3	75.4
A-6	62.2	0	62.2	62.5	62.5	+7	69.2	69.0
CH_3	23.4	0	23.4	24.0	24.0	0	23.4	24.0
Ac	175.8	0	175.8	176.4	176.3	0	175.8	176.3

	β-D-glucuronic acid[d] *Obs.*	*D-Glucosyluronic acid in chondroitins A and C*[d]				*α-L-Idosyluronic acid 2-sulfate in heparin*[d] *Obs.*	*L-Idosyluronic acid in chondroitin B*[d]		
		Increment[b]	*Calc.*	*Obs.*			*Increment*[b]	*Calc.*[e]	*Obs.*
				A	*C*				
U-1	97.4	+7	104.4	105.0	105.4	101.5	+1	102.5	103.5
U-2	75.5	−1	74.5	73.8	74.1	77.5	−7	70.5	70.4
U-3	77.1	−1	76.1	75.1	74.1	71.5	+1	72.5	72.0
U-4	73.3	+9	82.3	78.1	82.5[c]	77.5	0	77.5	76.7
U-5	77.5	−1	76.5	78.1	78.0	71.5	0	71.5	70.4
U-6	177.5	0	177.5	175.7	175.4	175.5	0	175.5 (177.2)[e]	174.6

[a]Ref. 10. [b]Approximate change in chemical shift expected relative to the corresponding carbon of the model compound: on formation of glycosidic bond at anomeric (+7 p.p.m.) or at a secondary C (+9 p.p.m.)[11,15]; on introduction of sulfate group (+7 p.p.m.), or its removal (−7 p.p.m.)[12,13]; due to bond or sulfate group introduced at adjacent position (−1 p.p.m.), or removed (+1 p.p.m.)[11–13,15]. [c]Assignments for A-3 and U-4 may be reversed. [d]Sodium salt. [e]Chemical shifts for sodium (methyl α-D-idopyranosid)uronate[14] are closely similar: U-1, 102.0; U-2 to U-5 (not individually assigned), 73.5, 72.0, 71.0, and 71.0 p.p.m. One of these (U-4) should be displaced downfield by glycosidic bond formation. The chemical shift of U-6 for β-L-idopyranuronic acid is 177.2 p.p.m.

Reference to the ^{13}C chemical-shifts of β-D-glucopyranuronic acid (Table I) provided a basis for assigning the individual signals attributed in Fig. 1A to the uronic acid residues of chondroitin A. The most notable discrepancy concerns U-4, although it is possible that the allotted increment of +9 p.p.m. is too large in this instance; *i.e.*, glycosidic bonding at a secondary carbon in oligosaccharides occasionally involves a downfield shift of only 5–6 p.p.m.[15]. A confirmation of the U-5 and U-6 assignments was obtained by acidifying the solution (pH ~1). This promoted an upfield displacement of one of the signals (U-4 and U-5) at 78.1 p.p.m. and of signal U-6 by −3.2 and −3.3 p.p.m., respectively, whereas all other signals were affected by <0.6 p.p.m. Analogously, under similar conditions, only C-5 and C-6 of β-D-glucuronic acid exhibited strong upfield shifts*. The periodate-oxidation experiment mentioned above helped to confirm the assignments for U-1, U-2, and U-3.

Many signals in the spectra of chondroitins A and C should have closely similar shifts, because the structural sequences (**1** and **3**) in these polymers differ only in the location of the sulfate group. Accordingly, there are five resonances in Fig. 1C that have almost the same chemical shifts as those assigned in Fig. 1A to the uronic acid carbons. The sixth carbon, U-4, differs by several p.p.m. although, in this instance, there is close agreement between the calculated and observed values. As with chondroitin A, the signals assigned to U-5 and U-6 were strongly shifted upfield at pH 1 (by −3.1 and −3.2 p.p.m., respectively), substantiating these assignments. Spectral changes observed on periodate oxidation were more complex with this polymer, although they readily served to confirm the assignments of U-1 and several of those for the acetamidodeoxyhexose residue.

Signals ascribed to the latter residue, and designated A-1, A-2, A-5, A-C, and CH_3 in Fig. 1C, all correspond closely to signals found in Fig. 1A, and also to the calculated values of Table I. As expected, however, signal A-4 of chondroitin C is far upfield of that of chondroitin A, in keeping with the absence of a sulfate group at this position in **3**, whereas, consistent with the 6-sulfate in **3**, A-6 for chondroitin C is much further downfield than for chondroitin A. Hence, the relatively strong resonance at 69 p.p.m. in Fig. 1C is treated as a composite of A-4 and A-6. In agreement with this is the fact that C-4 of the monosaccharide model has a chemical shift of 69.0 p.p.m., and C-6 a shift of 62.2 p.p.m., which is expected to be displaced downfield in the polymer by ~7 p.p.m. due to the appended 6-sulfate group.

Further evidence that these two resonances are coincident is furnished by the spectrum of the unsaturated disaccharide (**6**) formed by the action of chondroitinase AC[16] on the polymer: in the range 69.0–69.5 p.p.m. are several signals which may be attributed to C-4 and C-6 of the acetamidodeoxyhexose residues of the mixed α and β anomers of **6**. Accordingly, these signals appear in the proton-coupled ^{13}C-spectrum as a mixture of doublets (C-4) and triplets (C-6). An earlier examination of the p.m.r. spectra of unsaturated disaccharides analogous to **6** has indicated[17], on the basis of

*It is noteworthy, by contrast, that signal H-5 of uronosyl residues is shifted strongly *downfield* when the salt is converted into the acid[4].

observed, long-range ^{1}H–^{1}H-coupling, that the α,β-unsaturated uronosyl moiety adopts a conformation in which O-1 is *quasi*-axial*. It is noteworthy, therefore, that coupling between C-1 and H-1 of this residue is 170.2 Hz, which coincides with the value associated with an axial C–O bond [in contrast to ~160 Hz for equatorial anomers (see below)]. Other ^{13}C chemical-shift data for **6** helped to confirm the assignments for A-3 and A-5 in Fig. 1C.

In summary, there is a generally good correspondence between the observed ^{13}C chemical-shifts for the chondroitins and those expected from the data for compounds of low molecular weight chosen as models. Such agreement is consistent with ^{13}C-n.m.r. observations relating various types of macromolecule and small molecules chemically akin to them. Hence, it appears that the influence of macromolecular architecture *per se* on chemical shifts is usually rather minor. Among the present data, the most striking differences between observed and expected chemical shifts (Table I) involve uronosyl residues: the calculated value for U-4 of chondroitin A differs by +4 p.p.m., U-3 of chondroitin C by +2 p.p.m., and U-6 of all three polymers by +2 p.p.m. Such disparities may arise, for example, from electrostatic changes introduced when uronic acids are incorporated into a polymer. This question invites further examination.

Structural heterogeneity of the chondroitin sulfates. — The spectrum of chondroitin B (Fig. 1B) contains a group of relatively weak signals, at 102.1 (arrow), 77.8, 73.8, 69.6, and 68.6 (arrow) p.p.m. Their presence indicates that the sample contains ~20% of material in addition to that constituted as in **2**. These minor signals were slightly less prominent in the spectrum of a different preparation of chondroitin sulfate B but, otherwise, the spectrum of the latter was essentially superimposable on Fig. 1B. There is evidence[18–21] that chondroitin B contains a small proportion of D-glucosyluronic acid residues. Both the p.m.r. spectrum[4] and the current ^{13}C-spectrum are not inconsistent with this information: for example, according to Table I, the signals at 77.8 and 73.8 p.p.m. could be associated with a β-D-glucuronsyl constituent. However, yet other types of sugar residues must be present in minor proportion to account for the signal at 102.1 p.p.m. and the two at ~69 p.p.m. From ^{13}C data on the other chondroitins (Table I) and heparin, the most likely source of these signals are α-aldohexopyranosides that bear a 6-sulfate group.

In all, four samples of chondroitin A and chondroitin C from different origins have been examined. That represented by Fig. 1A is unique among the group in exhibiting evidence of very high homogeneity: a tiny peak is visible at 69 p.p.m., suggesting that a few percent of aminodeoxyhexose residues bear a sulfate group at position-6. By contrast, relatively strong signals are found at 69 p.p.m. in the spectra of the other samples of chondroitin A (*e.g.*, Fig. 2A), corresponding to a range of 10–25% of sulfation at C-6 (and the absence of a sulfate at C-4). All four of the chondroitin-C samples produce a prominent signal at 62.5 p.p.m. (as in Fig. 1C),

*This applies equally well for **6**, as its olefinic H-4 exhibits long-range coupling (^{4}J) of 0.8 Hz with H-2.

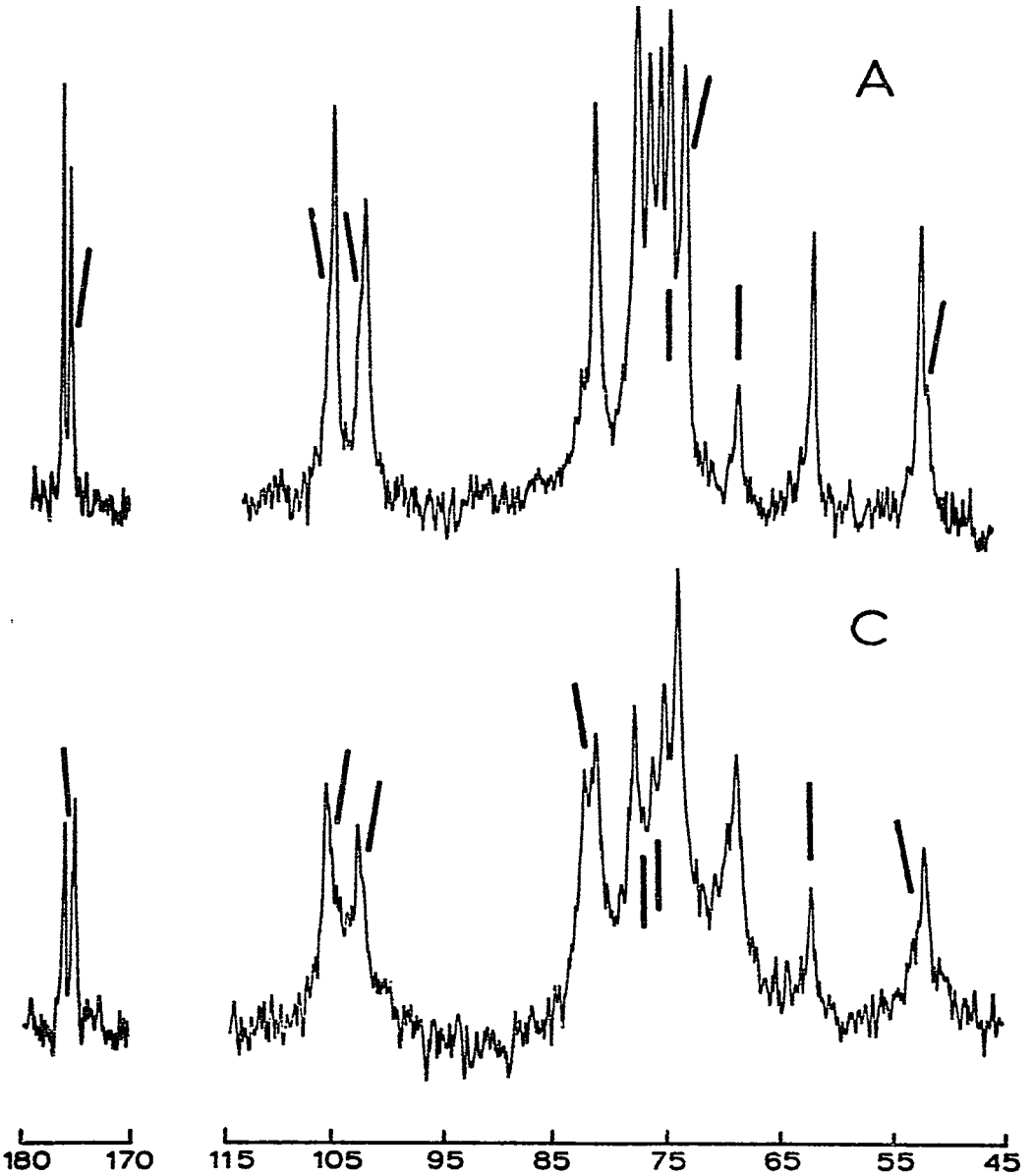

Fig. 2. Partial ^{13}C-n.m.r. spectra (^{1}H-decoupled; 22.6 MHz) of samples of chondroitins A and C (spectra A and C, respectively) that exhibit a lower degree of homogeneity than those represented in Fig. 1; as sodium salts in deuterium oxide at 45° (A) and 60° (C). The heavy lines signify the locations of minor signals attributed to contamination of chondroitin A with a polymer structurally akin to chondroitin C (spectrum A), and *vice versa* (spectrum C).

indicating that 15–30% of the aminodeoxyhexose residues in these preparations are not sulfated at the 6 position. There are also many other indications of inhomogeneity. In Fig. 2A, several minor signals, coincident with signals of the chondroitin-C spectrum (vertical lines), are detected as shoulders, or as a broadening of the major signals having the same chemical shifts as signals of chondroitin A. The same is apparent in Fig. 2C, which contains a relatively stronger signal at 62 p.p.m. than does Fig. 1C, as well as stronger peaks at 76.9 and 76.0 p.p.m., and more-prominent shoulders on several of the major signals; as indicated by the vertical lines in Fig. 2C, all of these minor features are characteristic of chondroitin A.

Therefore, it appears that the samples of chondroitin A and C consist of both structures **1** and **3** in various proportions: members of one group (A) contain a small (to a trace) proportion of **3**, whereas those of the other (C) incorporate a minor proportion of **1**. Whether **1** and **3** occur together as a physical mixture or in a co-polymer relationship (or both) is not apparent from these spectral data. According to Fig. 1A, it is clear that sequence **1** may occur essentially independently of **3**. Enzymic studies are reported[22] to show, however, that some preparations of both chondroitins A and C contain copolymerized sequences. In any event, ^{13}C-n.m.r. spectroscopy affords a highly useful technique for detecting structural heterogeneity in these polymers, and for providing information about the kinds and proportions of the different types of structures present.

TABLE II

DIRECT BOND–COUPLING ($^1J_{C-H}$) BETWEEN ^{13}C-1 AND ^{1}H-1, AND ANOMERIC CONFIGURATION

	$^1J_{C-H}$ (*Hz*)	
	α	β
D-Glucopyranose	169	160
Methyl D-glucopyranoside	169	160
2-Acetamido-2-deoxy-D-glucopyranose	172.8	160
D-Glucopyranuronic acid[a]	170.4	161.6
(Chondroitin A residues)		
2-Acetamido-2-deoxy-D-glucopyranosyl		161
D-Glucopyranosyluronic acid[a]		162
(Chondroitin B residues)		
2-Acetamido-2-deoxy-D-glucopyranosyl		162
L-Idopyranosyluronic acid[a]	168	
(Chondroitin C residues)		
2-Acetamido-2-deoxy-D-glucopyranosyl		161
D-Glucopyranosyluronic acid[a]		160
(Heparin residues)		
2-Deoxy-2-sulfamino-D-glucopyranosyl	170	
L-Idopyranosyluronic acid	172	

[a]Sodium salt.

Conformational aspects. — Conformational aspects of formulas **1–3** may be considered in terms of the proton-coupled spectra of the chondroitins. Coupling between each anomeric carbon and its appended proton (*i.e.*, $^1J_{C-H,H-1}$) is either around 160 Hz or 170 Hz (Table II). According to data[23–25] on carbohydrates of low molecular weight (see also, Table II), these values are characteristic of an equatorial and axial anomeric configuration, respectively. Hence, the results are consistent with the expectation that each of the constituent sugars possessing the D-*gluco* configuration is a β anomer having the $^4C_1$(D) conformation and an equatorial C-1–O-1 bond.

By contrast, the larger ^1J-value for the L-idosyluronic acid moiety of chondroitin B indicates that the anomeric bond is axial in this instance, which accords with earlier evidence, from p.m.r. studies[4], that this residue is an α-L anomer and favors the $^1C_4$(L) conformation, as shown in **5a**. The fact, as noted above, that C-1 and the carboxyl carbon interact with Gd^{3+} (as found also with heparin[9]) is a further indication that the C-1–O-1 bond is axial and that the carboxyl group is equatorial. It has been proposed, on the basis of X-ray data[26,27], that the L-idosyluronic acid residues in oriented fibres of chondroitin B favor the $^4C_1$(L) conformation; also, the rate of periodate oxidation of the polymer is thought[21] to be consistent with this latter conformation. Nevertheless, it appears wholly reasonable to interpret the current ^{13}C-n.m.r. results, comprising chemical-shift, coupling, and relaxation characteristics, and supplemented with the earlier p.m.r.-data, in terms of the $^1C_4$(L) conformation—or perhaps a closely related, skew conformation—when the chondroitin B is in aqueous solution.

EXPERIMENTAL

Materials. — Some specimens of chondroitin sulfates A, B, and C were kindly furnished by Dr. J. A. Cifonelli; the p.m.r. spectra of these, or closely related, preparations have been described earlier[4]. Other samples of the three polymers were purchased from Miles Laboratories, Nutritional Biochemicals, or Sigma Chemicals. All preparations were in the form of sodium salts.

^{13}C-n.m.r. spectra. — Spectra were recorded at 22.63 MHz with a Bruker WH-90 spectrometer. Proton-decoupled FT spectra were measured using a repetition time of 0.6 sec, pulse width of 18 μsec (70°), 4 K real data points, sweep width of 4000 Hz, and, typically, 80–100,000 scans. For ^{1}H-coupled spectra, the repetition time was 1.2 sec, decouple time 0.5 sec, and pulse width 24 μsec (90°).

The samples of chondroitin sulfate were examined as solutions in D_2O (without deuterium-exchange) at concentrations of 0.1–0.4 g ml; over this range, there was no significant change in chemical shift, although the relative intensities of signals varied slightly. Solutions of the sodium salts had a pH (pD) of ~6. In some experiments, hydrochloric acid was introduced to give pH ~1.

A probe temperature of 60° was used for the solutions of chondroitin C in order to reduce viscosity and, thereby, line widths. For chondroitins A and B, which gave

less-viscous solutions, a probe temperature of 45° was used; ^{13}C chemical-shifts measured for one of the chondroitin A samples run at 60° differed from those at 45° by <0.2 p.p.m.

Chemical shifts are given with respect to external tetramethylsilane, using methanol as an internal reference; at 45–60°, the chemical shift of methanol in deuterium oxide relative to that of tetramethylsilane contained in a coaxial capillary was 50.7 ±0.1 p.p.m.

Direct-bond coupling ($^1J_{C-H}$) was measured as the frequency difference between the mid-points of the two peaks of the C-1 "doublet"; with the chondroitin sulfates, half-line widths were 10–13 Hz, and the estimated error in ^1J values was ±2 Hz.

Periodate oxidation. — To a solution of chondroitin A or C (350 mg) in deuterium oxide (2 ml), sodium metaperiodate (250 mg) was added, and the ^{1}H-decoupled FT spectrum was then recorded over a period of 12 h at 30° (viscosities decreased markedly as the oxidation proceeded).

Enzymic preparation of **6** *from chondroitin C.* — A solution of chondroitinase AC (Sigma, 10 units) in water (0.5 ml) was added to chondroitin C (350 mg) in deuterium oxide (1.5 ml), and, after incubation at 37° for 5 h (a marked drop in viscosity was then evident), the ^{1}H-decoupled FT spectrum was recorded at 37° over a period of 12 h. From the intensities of signals of the product (**6**) relative to those of polymer remaining, the degree of degradation was estimated to be >70%. Methanol (6 vol.) was added, the polymeric precipitate was removed by centrifugation, and the supernatant liquid was concentrated. A solution of the residue in deuterium oxide gave a ^{1}H-decoupled spectrum consisting of a series of sharp lines, attributable to disaccharide **6**, interspersed with minor, broader signals corresponding closely to those

TABLE III

^{13}C-CHEMICAL SHIFTS (P.P.M.) AND $^1J_{C-H}$ (Hz) FOR DISACCHARIDE **6**

Acetamidodeoxyhexosyl residue				*Unsaturated uronosyl residue*	
A-1(β)	96.7 (160, d)	A-5(β)	74.5 (147, d)	U-1	102.8 (170.2, d)
A-1(α)	93.0 (172.8, d)	A-5(α)	70.2 —	U-2 or U-3	71.2 (149, d) or 67.5 (149, d)
A-2(β)	54.0 (147, d)	A-6(α,β)	69.4 (148, t) 69.1		
A-2(α)	50.5 (142, d)		176.7	U-4	109.0 (169.2, d)
A-3(β)	81.4 (143, d)	A-C	176.3 (s) 176.0[a]	U-5	146.3 (s)
A-3(α)	78.6 (141, d)		24.3[a]	U-6	171.0 (s)
A-4(α,β)	69.8 (142, d) 69.7	CH_3	24.0 (141, q) 23.8		

[a]This relatively minor signal may be due to residual, partially degraded, polymer.

of the original polymer. In the proton spectrum, the olefinic proton signal (H-4, δ 5.9 p.p.m.) appeared as a quartet ($^3J_{3,4} = 4.1$ Hz, $^4J_{2,4} = 0.8$ Hz).

^{13}C Chemical-shifts and $^1J_{C-H}$ values for **6** are listed in Table III; ^1J is given in parenthesis, along with the multiplicity of the signal: *e.g.*, a triplet (t) when there are two large spacings due to directly bonded protons.

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REFERENCES

1 R. W. Jeanloz, *Mucopolysaccharides*, in M. Florkin and E. H. Stotz (Eds.), *Comprehensive Biochemistry*, Vol. 5, Elsevier, Amsterdam, 1963, pp. 262–278.
2 J. S. Brimacombe and J. M. Webber, *Mucopolysaccharides*, Elsevier, Amsterdam, 1964.
3 G. O. Aspinall, *Polysaccharides*, Pergamon, New York, 1970, p. 166–170.
4 A. S. Perlin, B. Casu, G. R. Sanderson, and L. F. Johnson, *Can. J. Chem.*, 43 (1970) 2260–2268.
5 J. Schaefer, in G. C. Levy (Ed.), *Topics in Carbon-13 NMR Spectroscopy*, Wiley, New York, 1974, pp. 150–208.
6 S. E. Lasker and M. L. Chiu, *Ann. N. Y. Acad. Sci.*, 222 (1973) 971–977.
7 A. S. Perlin, *Proc. Intern. Symp. Macromolecules, IUPAC*, Rio de Janeiro (July 26–31, 1974), Elsevier, Amsterdam, 1975, pp. 337–348.
8 A. S. Perlin, *Abstr. Pap. Amer. Chem. Soc. Meeting*, Chicago, Aug. 24–29, 1975.
9 B. Casu, G. Gatti, N. Cyr, and A. S. Perlin, *Carbohyd. Res.*, 41 (1975) C6–C8.
10 D. R. Bundle, H. J. Jennings, and I. C. P. Smith, *Can. J. Chem.*, 51 (1973) 3812–3819.
11 D. E. Dorman and J. D. Roberts, *J. Amer. Chem. Soc.*, 93 (1971) 4463–4472.
12 A. S. Perlin, N. M. K. Ng Ying Kin, S. S. Bhattacharjee, and L. F. Johnson, *Can. J. Chem.*, 50 (1972) 2437–2441.
13 S. Honda, H. Yuki, and K. Takiura, *Carbohyd. Res.*, 28 (1973) 150–153.
14 A. S. Perlin, B. Casu, G. R. Sanderson, and J. Tse, *Carbohyd. Res.*, 21 (1972) 123–132.
15 T. Usui, N. Yamoska, K. Matsuda, K. Tuzimura, H. Sugiyawa, and S. Seto, *J. Chem. Soc. Perkin I*, (1973) 2425–2432.
16 S. Suzuki, H. Saito, T. Yamagata, K. Ammo, N. Seno, Y. Kalwai, and T. Furukashi, *J. Biol. Chem.*, 243 (1968) 1543–1550.
17 A. S. Perlin, D. M. Mackie, and C. P. Dietrich, *Carbohyd. Res.*, 18 (1971) 185–194.
18 P. Hoffman, A. Linker, and K. Meyer, *Science*, 124 (1956) 1252.
19 J. A. Cifonelli, J. Ludowieg, and A. Dorfman, *J. Biol. Chem.*, 233 (1958) 541–545.
20 L. A. Fransson and L. Roden, *J. Biol. Chem.*, 242 (1967) 4161–4169.
21 L. A. Fransson, *Carbohyd. Res.*, 36 (1974) 339–348.
22 C. P. Dietrich, personal communication.
23 A. S. Perlin and B. Casu, *Tetrahedron Lett.*, (1969) 2921–2924.
24 J. A. Schwarcz and A. S. Perlin, *Can. J. Chem.*, 50 (1972) 773–775.
25 K. Bock, J. Lundt, and C. Pederson, *Tetrahedron Lett.*, (1972) 1037–1040.
26 E. D. T. Atkins and T. C. Laurent, *Biochem. J.*, 133 (1973) 605–606.
27 S. Arnott, J. M. Guss, D. W. L. Hukins, and M. B. Mathews, *Biochem. Biophys. Res. Commun.*, 54 (1973) 1377–1383.