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Base-catalyzed conversion of the α-L-iduronic acid 2-sulfate unit of heparin into a unit of α-L-galacturonic acid, and related reactions

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ABSTRACT

Residues of α-L-idopyranosyluronic acid 2-sulfate in heparin undergo a loss of the sulfate group, by intramolecular displacement in sodium carbonate solution at 100°, leading to the formation of a heparin analog containing residues of α-L-galactopyranosyluronic acid (**4**). The configuration of these latter residues, which was determined by chemical evidence in combination with n.m.r. and c.d. spectroscopic data, is accounted for by selective hydrolysis of the oxirane ring of 2,3-anhydro-α-L-guluronic acid (**3**), a transient intermediate formed during the desulfation step. Regio- and stereo-selective aspects of ring-opening reactions of **3** are described. Several observations suggest that the reaction temperature is critical in determining whether the nucleophilic attack on the anhydro ring occurs at C-2 or C-3. Among these is the finding that, in contrast to the conditions used in the formation of the α-L-*galacto* diastereomer (**4**), ammonolysis of **3** at room temperature gives rise to a 2-amino-2-deoxyaldohexuronic acid having the α-L-*ido* configuration.

INTRODUCTION

The sulfate group of the α-L-iduronic 2-sulfate residue (**1**) of heparin is readily displaced in alkali giving rise[1,2] to a 2,3-anhydride residue. As the sulfate groups of 2-deoxy-2-sulfamino-α-D-glucose 6-sulfate residues (**2**) remain intact, and only moderate depolymerization occurs, this facile loss of the 2-sulfate group is attributed to intramolecular displacement by O-3 of **1** within the favorable 2,3-antiperiplanar orientation shown. Hence, the anhydride present in the modified polymer is taken to be the α-L-*gulo* diastereomer (**3**). Hydrolysis of its oxirane ring in water or dilute sodium hydroxide at 65–70° gives a polymer containing, according to n.m.r. evidence[2], residues of α-L-galacturonic acid (**4**). Chemical evidence is presented herein which confirms the configuration proposed for **4**. In addition, a simple procedure is given for the high-yield conversion of residues of **1** into **4**, and stereochemical aspects of the opening of the 2,3-anhydro ring of **3** are described.

RESULTS AND DISCUSSION

Ring-opening of the anhydro ring of **3** under basic conditions stronger than those required for the formation of **4** has been shown[1,2] to furnish another modified heparin in

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which the uronic acid constituent is almost exclusively nonsulfated *α*-L-iduronic acid (**5**). That is, there is a strikingly high degree of stereoselectivity in each of the two reactions found for the formation of either **4** or **5**. Other experimental conditions have now been tested on the assumption that an intermediate stage containing *both* diastereomers would be observed. One such experiment, in which beef lung heparin was heated under reflux in 0.1M sodium carbonate for 18 h, promoted conversion of the 2-sulfate directly into **4** in 80–85% yield, as shown by a comparison of the ^{1}H-n.m.r. spectra in Figs. 1A and 1C for heparin and the modified polymer, respectively. Shorter reaction times gave rise to spectra (*e.g.*, Fig. 1B) that corresponded to the presence of residues of **1** and **4**, but not of the intermediary anhydride **3**, which is in accord with the heat-lability of the latter. It is also worth noting that residues of **4** were formed more rapidly from hog mucosal heparin than from the beef lung sample, *i.e.*, 60 *vs.* 40% conversion within 5 h. This result is consistent with the higher rates of transformations **1**→**3** and **3**→**4** observed[2] for fractions of hog mucosal heparin as compared with beef lung heparin, a

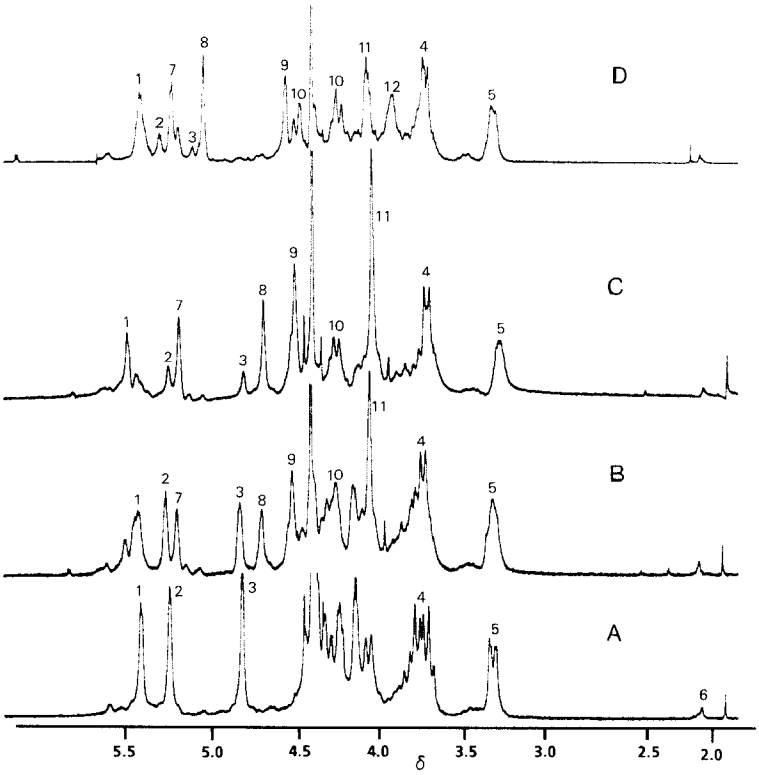


Fig. 1. The ^{1}H-n.m.r. spectra (300 MHz) for a solution in D_2O at 65° of: (A) Beef lung heparin (sodium salt); (B) modified heparin containing residues of **4**, at 5 h; (C) corresponding to B, at 18 h; (D) sample from C adjusted to pD 3.0. Signal designations: 1, A-1; 2, I-1; 3, I-5; 4, A-3, -4; 5, A-2; 6, acetamido CH_3; 7, G-1; 8, G-5; 9, G-4; 10, A-6; 11, G-2,-3; and 12, A-5 (I = **1**, A = **2**, G = **4**).

difference that appears to be favored by the greater constitutional heterogeneity in the mucosal polymer. That the nature of the cation may also be significant is suggested by the observation that, although 0.1M lithium carbonate induced the formation of **4** at about the same rate as sodium carbonate, a slower (by $\sim 1/3$) rate of interconversion was observed with the potassium salt.

The α-L-galacto *configuration of the uronic acid residue in polymer* **4**. — As already noted, two different products have been obtained from the epoxide ring-opening reactions of the 2,3-anhydride **3** under appropriate conditions. According to n.m.r. spectroscopic evidence, these products are the α-L-*galacto* (**4**) and α-L-*ido* (**5**) diastereomers, which reinforced the initial expectation of an α-L-*gulo* configuration for **3**. A proof for the correctness of this stereochemical relationship between **3**, **4**, and **5** is given here by evidence that **4** is, indeed, an α-L-*galacto* isomer.

That the configuration at C-5 of the original α-L-iduronic acid 2-sulfate residue (**1**) had not undergone a base-catalyzed epimerization was shown by the c.d. spectra of polymers **3**, **4**, and **5**, each of which exhibited a *negative* n→π* band of λ_{max} close to 210 nm, analogous to that[3] of heparin. Consequently, each of the modified heparins contains pyranosuronic acid residues of the α-L-enantiomeric form (conformationally, the pyranose rings are taken to be analogous on the basis of n.m.r. evidence). The overall shape of the c.d. curve for **4**, specifically (Fig. 2) differs only slightly from that of heparin, and also bears close to a mirror-image relationship to the curve reported[4] for methyl α-D-galactopyranosiduronic acid.

Verification of the L-*galacto* configuration for **4** was provided by chemical evidence. A sequence of well-known procedures was applied to the polymer, whereby residue **4** was ultimately converted into a free aldose, identified as L-galactose. Deaminative degradation with nitrous acid, was followed by borohydride reduction of the 2,5-anhydromannose units of the oligosaccharides formed. The uronic acid units of the

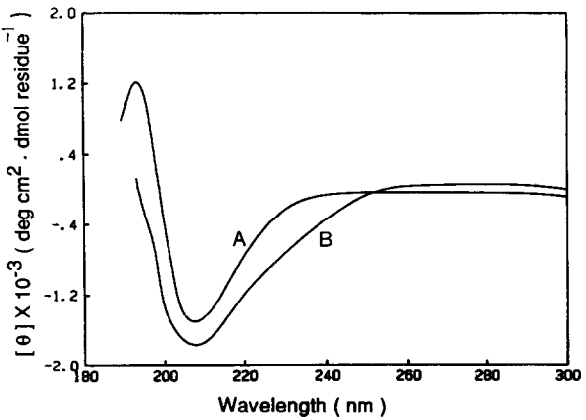
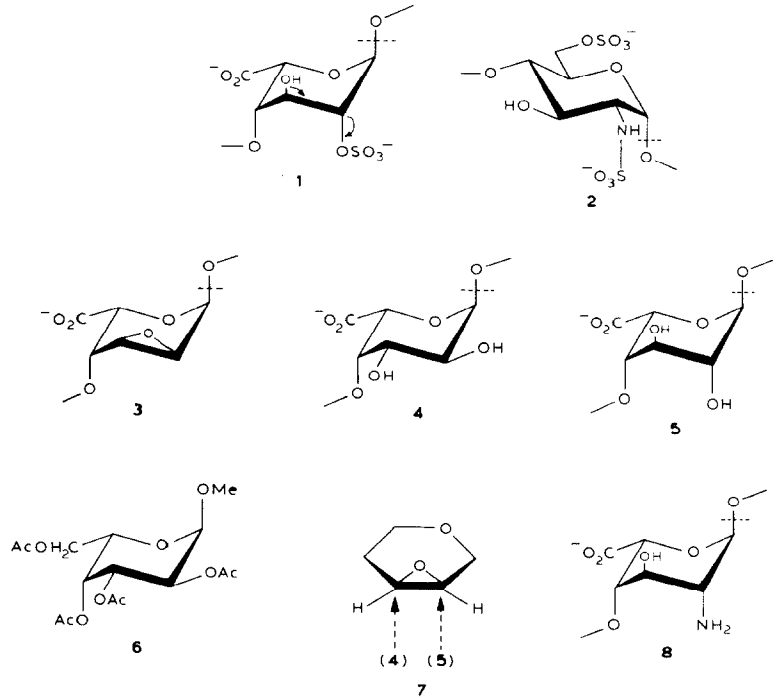


Fig. 2. C.d. spectra of: (A) Beef lung heparin (sodium salt), and (B) modified heparin containing residues of **4** (sodium salt), in water at 25° (4 mg·10 mL^{-1}; mol. wt. **1** + **2** = 665, **2** + **4** = 563).

latter fragments were converted into methyl esters with methanol containing chlorotrimethylsilane, and the esters were reduced with sodium borohydride. Acid-catalyzed methanolysis of the reduction product was then carried out, to induce further fragmentation of the oligosaccharides and the simultaneous formation of monomeric methyl glycosides from the aldose residues that they contained. Following acetylation, the organic-soluble material was chromatographed on silica gel giving, as a major product, methyl 2,3,4,6,-tetra-*O*-acetyl-*α*-L-galactopyranoside (**6**). The latter was deacetylated, and the glycoside hydrolyzed in aqueous trifluoroacetic acid to afford L-galactose, which was characterized as its distinctive[5], crystalline, methylphenylhydrazone.

Stereochemistry of the ring-opening reactions of anhydride **3**. — Two polymers, each containing a different diastereomeric uronic acid residue, have been obtained by hydrolysis of the oxirane ring of **3**. In ring-opening reactions of low-molecular-weight 2,3-anhydro derivatives, both of the possible diastereomers are often observed[6,7], although one of them may be formed preponderantly. The corresponding reactions of **3** are of unusually high stereoselectivity in that either one or the other feasible product, *i.e.*, **4** or **5**, is produced exclusively by the nucleophilic attack on C-3 or C-2, respectively (indicated by the arrows in structure **7**), depending on the experimental conditions. As shown in the present study, the treatment of heparin with hot sodium carbonate solution affords the *α*-L-*galacto* diastereomer (**4**). Undoubtedly, **3** is an intermediate in



its formation, because the hydrolysis of isolated **3** at 65° gave rise[2] to **4** as the sole product. By contrast, residues of the α-L-*ido* isomer (**5**) have been generated[1,2], either directly from heparin or from **3**, by lyophilization of an appropriately strongly alkaline solution of either polymer, although without the application of heat. Hence, the procedures used for the preparation of **4** and **5** have differed in terms of reaction temperature, as well as pH.

Some experiments were carried out to test the steric outcome of other ring-opening reactions of **3**. By treating the polymer under reflux with 20% aqueous diisopropylamine, residues of **4** were produced exclusively. Similarly, although phosphate anion has been used[8] successfully with an epoxide to introduce a phosphate substituent, in the reaction of **3** with dipotassium phosphate under reflux, only **4** was obtained, *i.e.*, hydrolysis again occurred specifically. By contrast, no reaction was observed when the solution of **3** and 10M dipotassium phosphate was lyophilized, which is analogous to conditions used for the formation of **5**. However, a successful ring-opening reaction was effected by the ammonolysis of **3** in 10M ammonium hydroxide at room temperature. That a second amino substituent had been introduced in high yield was evident from the observation that the ^{1}H-n.m.r. spectrum of the product (Fig. 3A), exhibited a new, prominent, upfield resonance (δ ~3.1; 19 in Fig. 3A) in the region characteristic of a proton in 1,2-position to an amino group. (This was accompanied by the disappearance of the H-2 and H-3 epoxide resonances of **3**). Analogously, the ^{13}C-n.m.r. spectrum contained an additional upfield signal attributable to the presence of an amino as well as the sulfamino group. In addition, selective *N*-acetylation was found to take place between the product and acetic anhydride in aqueous sodium hydroxide, as shown by the appearance of an acetamidomethyl signal at δ 2.0.

A ^{1}H–COSY spectrum (Fig. 3A) of the ammonolysis product showed conclusively that the newly-introduced amino group was located at C-2 of the uronic acid residues, because of the cross-correlation between the appropriate H-2 signal (19 in Fig. 3A) and a glycosyluronic acid H-1 signal (17 in Fig. 3A). On steric grounds, this indicated that the 2-amino-2-deoxyhexopyranosyluronic acid residue has the α-L-*ido* configuration (**8**). Consistent with that expectation is the observation that many features of the spectrum in Fig. 3A closely resemble those of *ido* isomer **5** (Fig. 3B), the O-2 analog of **8**, especially with regard to the chemical shifts of H-1 and H-5. Evidently, residues of **5** accompany those of **8**.

This finding provided one more indication that, in a strongly basic medium at room temperature, a nucleophilic attack on C-2 of the oxirane ring is far more highly favored than is an attack on C-3. As the latter reaction has been observed only at elevated temperatures, it is possible that conformational changes are involved, allowing for easier access of the nucleophile to one carbon atom or the other, or for a less crowded transition-state (or both). Nevertheless, as to the possibility of detectable changes in the solution conformation of **3** due to variations in the temperature or the concentration of base, n.m.r. spectra of the polymer were not substantially altered when the temperature was raised from 25 to 70°, or the pH raised from 6.5 to 13.5.

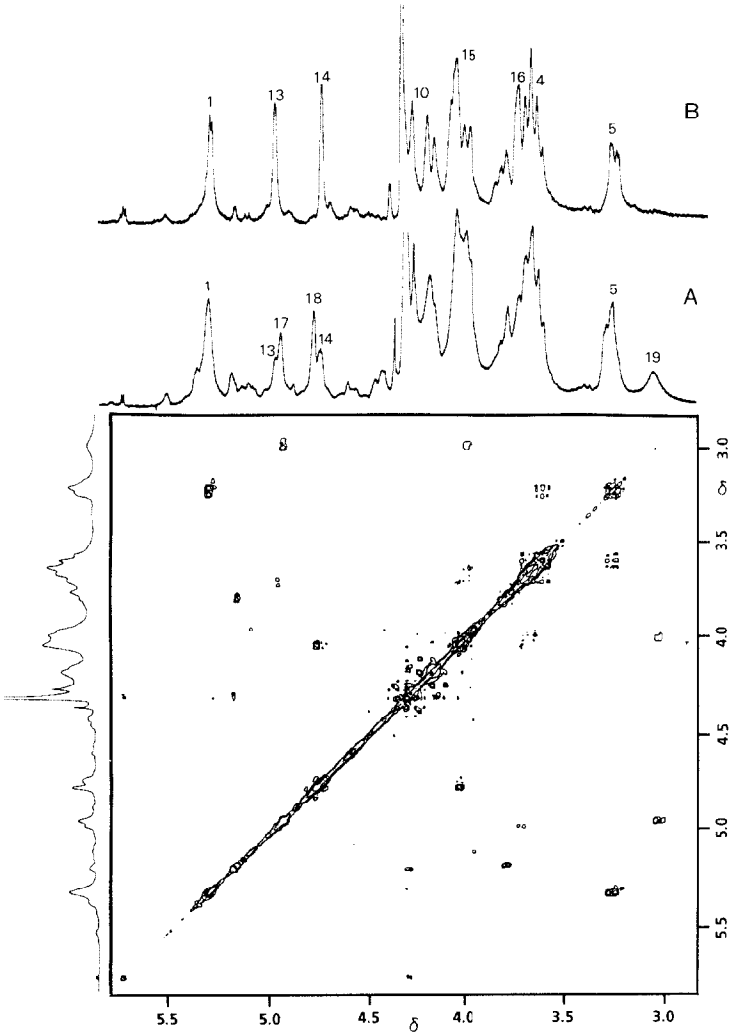
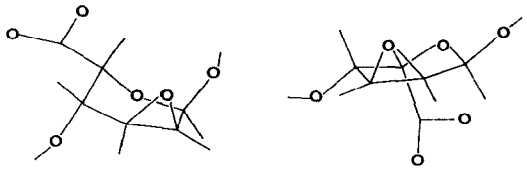


Fig. 3. (A) The ^{1}H-n.m.r. 2D correlation (COSY) spectrum (300 MHz) for a solution in D_2O, at 65°, of modified heparin containing residues of amino derivative **8**; and (B) the ^{1}H-n.m.r. spectrum (300 MHz) for a solution in D_2O, at 65°, of modified heparin[1,2] containing residues of α-L-iduronic acid (**5**). Signal designations: 1, A-1; 4, A-3,-4; 5, A-2; 10, A-6; 13, I′-1; 14, I′-5; 15, I′-3,-4; 16, I′-2; 17, I″-1; 18, I″-5; and 19, I″-2 (A = **2**; I′ = **5**, I″ = **8**).

Based on extensive spin-spin coupling data (*e.g.*, see ref. 9) for 2,3-anhydroaldohexopyranosides, residues of **3** are likely to exist in a half-chair conformation. Corresponding data are not available for **3** from the present spectra (nor from resolution-enhanced spectra) and, consequently, molecular-mechanics modelling was examined as a possible guide to the geometry of **3** and its reactivity. The information obtained indicated that each of the two half-chair conformations of this bicyclic structure,



9 10

represented by **9** (^5H_O, L) and **10** ($^OH_5$, L), is a minimum energy form and, not unexpectedly, that the former is more stable by $\sim 16 kJ \cdot mol^{-1}$. (In carrying out the molecular-mechanics calculations, methyl groups were located at O-1 and O-4 of structures **9** and **10**.)

Intuitively, conformation **10** appears to be equally susceptible to nucleophilic attack by HO^- at either C-2 or C-3. Also, approximately the same steric constraints are envisaged for both of the transition states, because the *quasi*-axial carboxylate group is positioned about equidistant from C-2 and C-3. (*Quasi*-equatorial O-1 and O-4 seem unlikely to be important factors, although the neighboring residues of **2** attached to them have yet to be factored into the modelling treatment.) With conformation **9**, a more selective attack of the nucleophile on C-2 would be expected not only owing to easier access to that position, but also because the alternative of a bond being formed between C-3 and the anion could introduce an eclipsing interaction with the *quasi*-axial C-4–O-4 bond. Such high selectivity is, indeed, seen in the production of the α-L-*ido* diastereomers, **5** and **8**, at *room temperature*. Moreover, they are the products favored according to the Fürst–Plattner rule[10], because an axial approach of the hydroxyl anion towards C-2 would directly give the 2,3-diaxial *ido* isomer in its stable[11], $^1C_4$(L), conformation (see **5**). This appears to be consistent with the possibility of an early transition-state more closely resembling the starting material than the products. However, neither conventional considerations such as these, nor the molecular-modelling information, appear able to account for the formation of the L-*galacto* diastereomer (**4**). As the latter requires an elevated temperature, the entropy of activation ($T\Delta S$) may be a major factor, or the involvement of a late, product-like, transition state (or both) leading to **4**, a feasible alternative.

Further observations on the n.m.r. spectra of the modified polymers containing **3** *and* **4**. — Although the chemical shifts of the resonances of residue **4** are consistent with its α-L-*galacto* configuration, the convergence of the H-2 and H-3 signals gave rise to a far more narrow signal (at δ 4.1) than should be formed for such antiperiplanar *vic* protons. However, on acidification of the solution, the chemical shift was altered sufficiently to show (see Fig. 1D) that the H-2 and H-3 signals, in fact, are now broader than their deceptively-narrow counterparts in the spectrum (Fig. 1C) of the neutral polymer. Regrettably, attempts to resolve the H-2–H-3 couplings by means of resolution enhancement or convolution difference were unsuccessful. Also evident from this spectrum (Fig. 1D) is the marked downfield location of the H-5 resonance, characteristic of the pH-induced shift of protons in 1,2-position to the carboxyl group. (It is worth noting

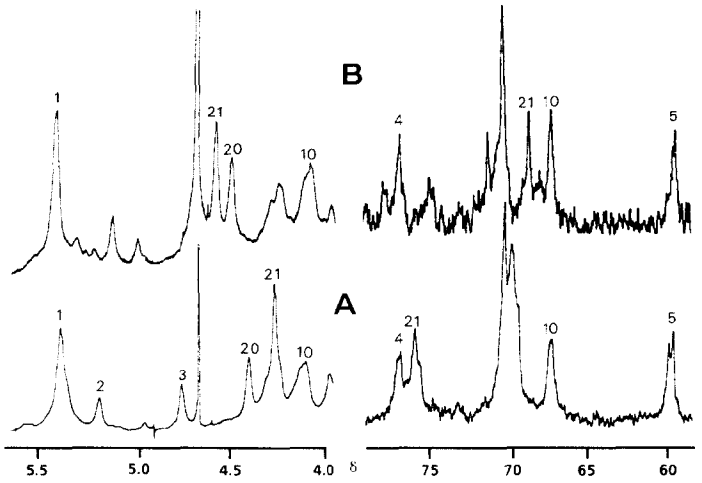


Fig. 4. Partial n.m.r. spectra of the modified heparin containing residues of **3** (~65%): (A, left) The ^{1}H-n.m.r. spectrum (300 MHz) for a solution in D_2O, at 65°; (B, left) the same sample in a solution adjusted to pD 3.0; (A, right) the ^{13}C-n.m.r. spectrum (75 MHz) for a solution in D_2O at 25°; and (B, right) the same sample in a solution adjusted to pD 3.0. Signal designations: 1, A-1 and B-1; 2, I-1; 3, I-5; 4, A-4; 5, A-2; 10, A-6; 20, B-4; and 21, B-5 (A = **2**; I = **1**; B = **3**).

the minor signal at δ 5.8 in Figs. 1B and 1C, probably due[12] to H-4 of a 4-enuronic acid formed by elimination, which is also strongly pH-dependent.)

The aforementioned[11] deshielding effect on the proton in 1,2-position at low pH was helpful in interpreting some unusual chemical shifts observed for the 2,3-anhydro residue **3**. According to the ^{1}H–^{13}C heterocorrelation (HETCOR) spectrum obtained for that polymer, one of the two ^{13}C signals (that at δ 78 in the partial spectrum in Fig. 4) found in the region associated with the linkage position showed connectivity with H-5, whereas it was expected to show a correlation with H-4. Hence, atypically, C-5 of **3** is more strongly deshielded than C-5 of **1**, **4**, or **5** (δ 71.7, 73.5, and 72.5, respectively), whereas H-5 (δ 4.3 Fig. 4) is more strongly shielded than H-5 of the other three species (δ 4.8, 4.6, and 4.8, respectively). The signal for H-5 for a solution at pD ~ 3.0 having become appropriately deshielded (see Fig. 4) is now correlated with a ^{13}C signal at $\delta \sim 70$, which amounts to an upfield shift of ~8 p.p.m. By contrast, corresponding data for pH effects on C-5 of **1**, showed[13] that only minor changes occur. Consequently, although this experiment verified the assignments for C-5 and H-5 of **3**, the source of the exceptionally large variations in the shielding of C-5 remains obscure.

Resistance of the modified heparins to enzymic degradation by heparinase. — The USP and anti-Xa activities of the modified heparins containing residues of **5**, as well as **3** and **4**[2], are markedly reduced relative to the potency of heparin[1,2]. The susceptibility of these heparins to the depolymerizing action of the enzyme, heparinase, is also grossly different. Hence, all three were found to be virtually unaffected, according to u.v. and n.m.r. spectroscopy, when incubated with a heparinase from *Flavobacterium hepari-*

num. A very weak response observed with **4** might be attributable to the presence, in the polymer, of the few intact residues of α-L-idopyranosyluronic 2-sulfate (**1**), which appears to be a required structural element of the substrate of this enzyme, whereby **1** is converted[12] into a hex-4-enopyranosyluronic acid end group of the oligosaccharides released.

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were determined at room temperature for solutions in 1-dm tubes, with a Jasco DIP 140 digital polarimeter. Circular dichroism spectra were recorded with a Jasco J-500 C spectropolarimeter. N.m.r. spectra were recorded with a Varian XL300 spectrometer operating at 300 MHz for ^1H and 75.4 MHz for ^{13}C, equipped with a 5-mm proton probe and a 5-mm broad-band probe, respectively, and are referenced to the signals of internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (δ 0.0). The 2D (^1H,H) COSY and (^{13}C,^1H) HETCOR experiments were performed with the Varian-supplied pulse sequences, and utilized for verifying most of the spectral assignments given. Molecular modelling was performed with PCMODEL (Serena Software), a molecular mechanics program as modified by K. Steliou. Solutions were evaporated below 50° under diminished pressure. Silica Gel Merck (230–400 mesh ASTM) was used for flash column chromatography.

Modification of heparin by reaction with aqueous sodium carbonate. Formation of residues of **4**. — A solution of beef lung heparin (0.33 g) and Na_2CO_3 (0.15 g) in water (16 mL) was heated for 20 h at 105–110°. The pH of the cooled solution was adjusted to 4.7 with Amberlite IR-120 (H^+) cation-exchange resin, and then to pH 6.5 with NaOH. After deuterium exchange with D_2O, the product was found by ^1H-n.m.r. spectroscopy to give the spectrum shown in Fig. 1C. This spectrum was virtually indistinguishable from that of the polymer, obtained alternatively[2] from heparin in a two-step procedure. When, instead of being acidified with ion-exchange resin as described above, the solution was dialyzed to neutrality against distilled water for 72 h, the yield of product recovered was 60% (0.17 g). The ^1H-n.m.r. spectrum of this preparation was indistinguishable from that shown in Fig. 1C, an indication that the constitution of both short- and long-chain molecules of the polymer was essentially the same.

Deaminative degradation of the modified heparin. — To a stirred solution of the polymer powder (0.40 g) in 1.1M acetic acid (3.8 mL) $NaNO_2$ (0.20 g) was added, and stirring was continued for 40 h. The solution was evaporated and toluene (10 mL) was introduced and then distilled off, this being repeated twice. The ^1H-n.m.r. spectrum of the residue (in D_2O) indicated that, on the basis of the relative intensity of the residual H-1 signal at δ 5.4, 75–80% of the aminodeoxyhexose residues had undergone reaction.

Borohydride reduction. — A solution of the degradation product in ice–water (2 mL) was added dropwise to a stirred suspension of $NaBH_4$ (0.12 g) in ice–water (3 mL). After 18 h at room temperature, the pH of the mixture was adjusted to 4.9 with Amberlite IR-120 (H^+) cation-exchange resin, the resin was filtered off, and the solution was concentrated to dryness. Methanol was added and distilled off, and this treatment was repeated twice to remove methyl borate, affording a white powder.

Esterification and borohydride reduction. — The crude product was suspended in methanol (20 mL), chlorotrimethylsilane (0.5 mL) was introduced dropwise, the suspension was stirred for 18 h, and the solvent was evaporated by codistillation with toluene (2 × 8 mL). The ^{1}H-n.m.r. spectrum of the residual solid (in D_2O) showed the presence of a methoxyl signal (δ 3.6).

To a stirred solution of the solid in ice–water (4 mL), adjusted to pH 6.5 with NaOH, $NaBH_4$ (0.16 g) in ice–water (6 mL) was added over a period of 1 h. After 18 h at room temperature, excess Amberlite IR-120 (H^+) cation-exchange resin was added, the solution was concentrated to dryness, and methyl borate was removed by treatment with methanol, followed by the introduction of toluene to facilitate the distillation of residual methanol. The ^{1}H-n.m.r. spectrum of the product (in D_2O) showed that the methoxyl resonance had been eliminated.

*Methanolysis. Isolation of methyl 2,3,4,6-tetra-*O*-acetyl-α-*L*-galactopyranoside.* — A suspension of the product in 5% methanolic HCl (20 mL) was heated under reflux for 18 h, the mixture was then rendered neutral with NaOH solution, and concentrated to dryness. Pyridine (10 mL), acetic anhydride (5 mL), and 4-dimethylaminopyridine (15 mg) were introduced, following which the suspension was stirred for 48 h. Ice–water was added, the product was extracted with dichloromethane, (3 ×), and the extract was washed with M HCl (2 ×), 5% $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated to dryness. The syrupy residue (0.30 g) was transferred onto a column (7 cm × 22 mm) of silica gel, and the column was eluted successively with petrol (100 mL), 1:10 ethyl acetate–petroleum ether (100 mL), and 1:5 ethyl acetate–petroleum ether (300 mL). A fraction of the latter eluate that, according to t.l.c. (solvent, 1:1 ethyl acetate–petroleum ether), contained mainly the methyl 2,3,4,6-tetra-O-acetylgalactoside was selected for a second chromatographic purification on silica gel, affording a yellow syrup (32 mg), $[\alpha]_D$ −60° (*c* 1.5, chloroform). According to its ^{1}H-n.m.r. spectrum (in $CDCl_3$), this product contained methyl 2,3,4,6-tetra-O-acetyl-α-L-galactopyranoside as the preponderant constituent, by comparison with the spectrum of the authentic D-enantiomer.

L-Galactose methylphenylhydrazone. — The product was deacetylated, at −5°, with methanol (3 mL) containing a catalytic proportion of barium methoxide (after 1.5 h, t.l.c. showed that the reaction was complete), and the solution was rendered neutral with Amberlite IR-120 (H^+) cation-exchange resin. Removal of the methanol afforded a syrup (18 mg) which was dissolved in 6M trifluoroacetic acid (4.5 mL) and heated under reflux for 4 h. The solution was concentrated to dryness, affording a yellow syrup, $[\alpha]_D$ −50° (*c* ~ 1, D_2O). The ^{1}H-n.m.r. spectrum (for a solution in D_2O) of the preponderant constituent was indistinguishable from that of an equilibrated solution of α,β-D-galactose (in D_2O). The solution of the hydrolysis product was mixed with a reagent[5] consisting of an ethanolic solution (1 mL) of methylphenylhydrazine (0.25 g) and acetic acid (30 μL). After storage for 18 h at 35°, and then for 18 h at 3°, a crystalline product, admixed with a fine, brown powder, was recovered by filtration and recrystallized from aqueous ethanol, m.p. 178–180° (dec.), undepressed by admixture with L-galactose methylphenylhydrazone (m.p. 180–184°).

Preparation of modified heparin containing residues of **3**. — The pH of a solution of

beef lung heparin (40 mg) in water (10 mL) was adjusted to 11.5–11.8 with 0.1M NaOH, and the solution was lyophilized. The residue was fibrous and faintly yellow. The ^{1}H-n.m.r. spectrum of its solution in D_2O (see Fig. 4) indicated that ~65% of the residues of **1** had been converted into residues of **3**.

Ammonolysis of the polymer anhydride. — To a solution of the polymer (0.11 g) in water (2 mL) was added conc. NH_3 solution (6 mL). After storage at room temperature for 48 h, NH_3 was gently evaporated off *in vacuo* as the temperature was slowly raised to 35°, and the residue was then subjected to a high vacuum for 18 h to yield a pale-yellow powder (0.11 g). The ^{1}H-n.m.r. spectrum in D_2O solution is shown in Fig. 3A. The product, when subjected to selective *N*-acetylation by treatment[14] at pH 10 and 0° with acetic anhydride, exhibited a strong ^{1}H-singlet at δ 2.05, corresponding to the presence of an acetamidomethyl group.

Heparinase treatment of the base-modified heparins. — To a solution of heparin or modified heparin (1.2 mg) in 5mM phosphate buffer (pH 7.0; 1.2 mL) was added a solution of *Flavobacterium heparinum* heparinase (0.1 mL, 25 units; Sigma) prepared with the same buffer. The mixture was stored at 30°, and assayed spectrophotometrically (λ 200–350 nm) at intervals. Whereas the u.v. spectrum of the heparin control exhibited a typical, marked change, and the concomittant emergence of a strong band at λ_{max} 232 nm, little change was observed in the u.v. spectra of the modified heparins over a period of 48 h. At that time, the relative absorbances at λ 232 nm for heparin and the polymers containing residues of **3**, **4** and **5**, were 0.171, 0.022, 0.032, and 0.013, respectively.

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REFERENCES

1 R. Rej, M. Jaseja, and A. S. Perlin, *Thromb. Haemostasis*, 61 (1989) 540.
2 M. Jaseja, R. Rej, F. Sauriol, and A. S. Perlin, *Can. J. Chem.*, 67 (1989) 1449–1456.
3 A. L. Stone, *Biopolymers*, 2 (1964) 315–325; 3 (1965) 617–624.
4 E. R. Morris, D. A. Ress, G. R. Sanderson, and D. Thom, *J. Chem. Soc., Perkin Trans. 2*, (1975) 1418–1425.
5 E. L. Hirst, J. K. N. Jones, and E. A. Woods, *J. Chem. Soc.*, (1947) 1048–1051.
6 S. Peat, *Adv. Carbohydr. Chem.*, 2 (1946) 37–77.
7 R. D. Guthrie, in W. Pigman and D. Horton (Eds.) *The Carbohydrates*, Vol. IA, Academic Press, 1972, pp. 423–478.
8 W. E. Harvey, J. J. Michalski, and A. R. Todd, *J. Chem. Soc.*, (1951) 2271–2278.
9 M. M. Abdel-Malik, Q. J. Peng, and A. S. Perlin, *Carbohydr. Res.*, 159 (1987) 11–23.
10 A. Fürst and P. A. Plattner, *Helv. Chim. Acta*, 32 (1949) 275–283.
11 A. S. Perlin, B. Casu, G. R. Sanderson, and J. Tse, *Carbohydr. Res.*, 21 (1972) 123–132.
12 A. S. Perlin, D. M. Mackie, and C. P. Dietrich, *Carbohydr. Res.*, 18 (1971) 185–194.
13 G. Gatti, B. Casu, G. K. Hamer, and A. S. Perlin, *Macromolecules*, 12 (1979) 1001–1007.
14 S. Roseman and J. L. Ludowieg, *J. Am. Chem. Soc.*, 78 (1954) 301–302.