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# CONFORMER POPULATIONS OF L-IDURONIC ACID RESIDUES IN GLYCOSAMINOGLYCAN SEQUENCES*

DINO R. FERRO†, AUGUSTO PROVASOLI, MASSIMO RAGAZZI,
*Istituto di Chimica delle Macromolecole del C.N.R., via E. Bassini 15, I-20133 Milano (Italy)*

BENITO CASU, GIANGIACOMO TORRI,
*Istituto di Chimica e Biochimica "G. Ronzoni", via G. Colombo 81, I-20133 Milano (Italy)*

VERONIQUE BOSSENNEC, BRUNO PERLY,
*C.E.A. IRDI/DPC, F-91191 Gif-sur-Yvette (France)*

PIERRE SINAŸ,
*Ecole Normale Supérieure, Laboratorie de Chimie, UA 1110, 24 rue Lhomond, F-75231 Paris (France)*

MAURICE PETITOU, AND JEAN CHOAY
*Institut Choay, 46 avenue Th. Gautier, F-75016 Paris (France)*
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## ABSTRACT

The $^1$H-n.m.r. $^3J$ values for the L-iduronic acid (IdoA) residues for solutions in $D_2O$ of natural and synthetic oligosaccharides that represent the biologically important sequences of dermatan sulfate, heparan sulfate, and heparin have been rationalized by force-field calculations. The relative proportions of the low-energy conformers $^1C_4$, $^2S_0$, and $^4C_1$ vary widely as a function of sequence and of pattern of sulfation. When IdoA or IdoA-2-sulfate units are present *inside* saccharide sequences, only $^1C_4$ and $^2S_0$ conformations contribute significantly to the equilibrium. This equilibrium is displaced towards the $^2S_0$ form when IdoA-2-sulfate is preceded by a 3-*O*-sulfated amino sugar residue, and towards the $^1C_4$ form when it is a non-reducing terminal. For terminal non-sulfated IdoA, the $^4C_1$ form also contributes to the equilibrium. N.O.e. data confirm these conclusions. Possible biological implications of the conformational flexibility and the counter-ion induced changes in conformer populations are discussed.

## INTRODUCTION

L-Iduronic acid (IdoA) is the major uronic acid of the glycosaminoglycans dermatan sulfate (DeS) and heparin (HEP), and is also present, in minor amounts

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†Author for correspondence.

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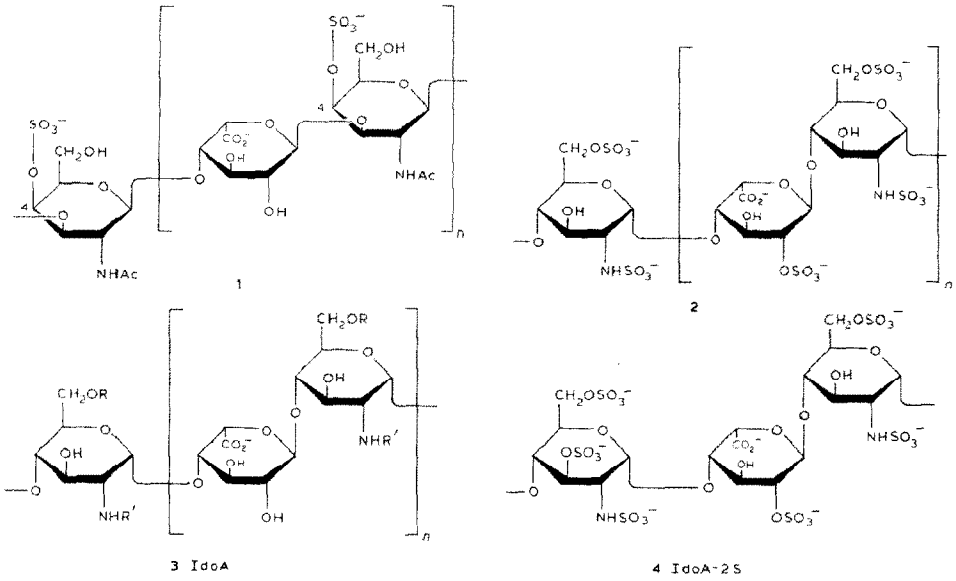


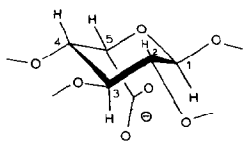

Fig. 1. Sequences **1** and **2** account for a major part of mammalian dermatan sulfate and heparin, respectively; sequences **3** are minor components of heparan sulfate, and very minor components of heparin; and sequences **4**, which are part of the pentasaccharide representing the active site for antithrombin III, are contained in only about one-third of the polysaccharide chains of mammalian heparins (R = H or $SO_3^-$, R' = Ac or $SO_3^-$).

relative to D-glucuronic acid, in heparan sulfate (HS). IdoA residues are mostly non-sulfated in the "regular regions" (*i.e.*, in the prevalent products of biosynthesis) of DES, and mostly 2-sulfated (IdoA-2S) in those of HEP. In HS, IdoA and IdoA-2S occur, in various relative amounts, in minor, "heparin-like" sequences[2]. Typical environments of IdoA and IdoA-2S are shown in **1–4** (Fig. 1).

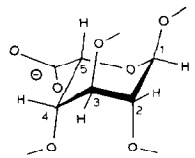
IdoA-containing glycosaminoglycans bind to several tissue and plasma proteins, and have various biological properties, some of which, especially the antithrombotic activity of heparin, have current or potential applications in therapy[3–5]. The binding and associated biological properties of glycosaminoglycans that contain D-glucuronic acid as the major uronic acid are much weaker than those of glycosaminoglycans that contain mainly IdoA. Thus, DeS binds to several plasma proteins[3–5] and is antithrombotic[6], whereas the binding properties and biological activities of its formal isomer, chondroitin 4-sulfate, are poor[7]. The main reason for these differences in binding properties has been suggested to involve the orientation of the carboxylate groups of the IdoA residues[8,9].

Whereas the conformation of hexopyranose residues of most common oligo- and poly-saccharides is well-established as one of the two chair forms $^4C_1$ or $^1C_4$, the conformation of IdoA residues has long been a matter of controversy[4,5,10,11]. The X-ray diffraction patterns of DeS fibres were reported[12] to be compatible only with IdoA residues in the $^4C_1$ chair, as was suggested also by kinetic studies of periodate oxidation[9]. However, this conformation was ruled out by n.m.r. data. In

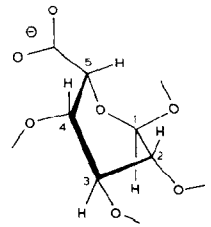
fact, the $^3J_{HH}$ values for the IdoA residues in DeS[13] and the IdoA-2S residues in HEP[14] suggested a "$^1C_4$-like" form, possibly a slightly distorted $^1C_4$ chair, as also proposed for methyl α-D-idopyranuronate[15]. Unusual $^3J$ values were observed subsequently for the IdoA-2S residues in a synthetic pentasaccharide closely related to the active site for antithrombin[16]. The $^3J$ values reported[17] for IdoA in various fragments of HS also indicated the conformation to be variable. Force-field studies[18] indicated that the skew-boat form $^2S_0$ is equi-energetic with the $^1C_4$ and $^4C_1$ forms and showed that the energy barrier between the three forms is quite high (as large as 9 kcal/mol). These studies strongly indicated that the "non-standard" $^3J$ values for IdoA should be interpreted in terms of an equilibrium between two or three low-energy conformers (**5–7**) rather than a distorted chair form. The population of conformers was calculated from the $^3J$ values for heparin, methyl α-L-idopyranuronate, several synthetic oligosaccharides related to heparin[19,20], and disaccharide fragments of heparin[21]. Calculations also showed that interconversion of th conformations of an internal IdoA (or IdoA-2S) unit may occur without affecting the end-to-end distance in an oligo- or poly-saccharide chain[19].



5 $^4C_1$



6 $^1C_4$



7 $^2S_0$

We now present and discuss a more complete set of $^3J_{HH}$ values for IdoA and IdoA-2S residues in natural and synthetic oligosaccharides that simulate sequences in DeS, HS, and HEP, and also n.O.e. data.

## EXPERIMENTAL

*Materials.* — Natural and synthetic compounds studied were obtained as indicated by the references in Table I.

*N.m.r. spectroscopy.* — $^1$H-N.m.r. (300 and 500 MHz) spectra for compounds *1, 2, 4, 9–11, 13, 20–23,* and *25–27* were obtained with Bruker CXP-300 and WM-500 spectrometers for ~5% solutions in $D_2O$ (99.96%), at 23° unless otherwise stated. Coupling constants were calculated from spectra simulated with the PANIC84 version of the LAOCOON computer program. Signal assignments were made by homonuclear spin-decoupling and confirmed by 2D (COSY) experiments. Assignment of all protons of the pentasaccharide **27** in the presence of excess of NaCl and $CaCl_2$ was achieved using multiple-relay homonuclear correlation experiments[22]. Semi-quantitative n.O.e.'s were derived from a 2D NOESY map[23] obtained with a mixing time of 300 ms. Quantitative values were then ob-

TABLE I

$^3J_{HH}$ VALUES (Hz) AND CALCULATED PERCENT OF MAJOR CONFORMERS OF IDOA RESIDUES IN DIFFERENT ENVIRONMENTS[a]

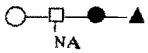
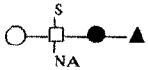
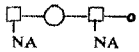
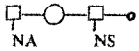
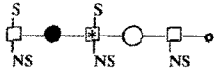
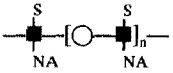
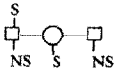
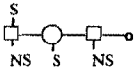
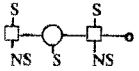
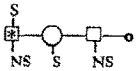
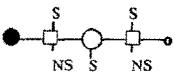
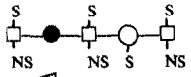
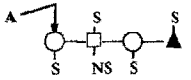
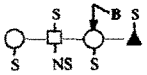
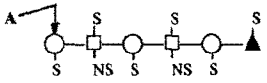
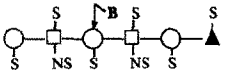
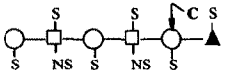
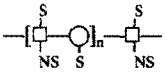
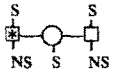
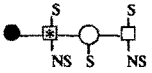
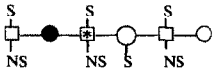
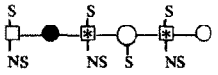
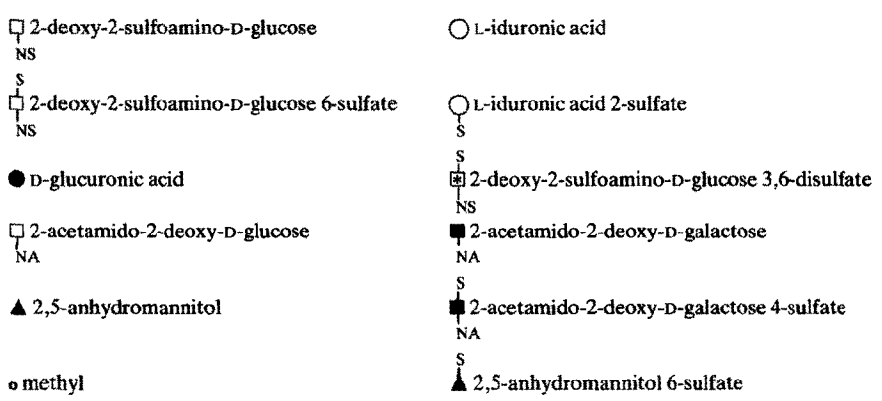
| | *Non-sulfated IdoA*[b] | *Ref.* | $J_{1,2}$ | $J_{2,3}$ | $J_{3,4}$ | $J_{4,5}$ | $^1C_4$ | $^4C_1$ | $^2S_0$ |
|---|---|---|---|---|---|---|---|---|---|
| *1* |  | 19 | 4.9 | 6.6 | 6.0 | 4.0 | 41 | 59 | 0 |
| *2* |  | 19 | 3.0 | 4.6 | 4.6 | 3.2 | 71 | 29 | 0 |
| *3* |  | 20 | 4.0 | 6.6 | 5.2 | 3.7 | 45 | 29 | 26 |
| *4* |  | 25 | 4.8 | 7.2 | 6.0 | 3.8 | 34 | 41 | 25 |
| *5* |  | 21 | 5.9 | 8.05 | 7.3 | 4.7 | 19 | 67 | 14 |
| *6* |  | 21 | 4.92 | 7.14 | 6.20 | 3.87 | 35 | 46 | 19 |
| *7* |  | 20 | 3.9 | 6.6 | 3.6 | 3.2 | 47 | — | 53 |
| *8* |  | 20 | 3.3 | 5.4 | 3.6 | 2.4 | 64 | — | 36 |
| *9* |  | 26 | 2.6 | 4.6 | 3.6 | 2.4 | 74 | 7 | 19 |
| *10* |  | 27 | 5.0 | 7.8 | 6.5 | 4.3 | 26 | 48 | 26 |
| *11* |  | 27 | 4.6 | 7.5 | 5.8 | 3.9 | 31 | 34 | 35 |
| *12* |  | 13 | 3.0 | 6.0 | 3.5 | 3.3 | 57 | — | 43 |
| | *Sulfated IdoA* | | | | | | | | |
| *13* |  | 19 | 1.76 | 3.34 | 3.44 | 2.22 | 90 | — | 10 |
| *14* |  | 28 | 2.62 | 4.42 | 4.19 | 2.67 | 78 | 22 | 0 |
| *15* |  | 20 | 1.9 | 3.7 | 3.7 | 2.2 | 87 | 13 | 0 |
| *16* |  | 20 | 1.4 | 3.2 | 2.8 | 2.1 | 96 | 4 | 0 |
| *17* |  | 20 | 2.8 | 5.4 | 3.1 | 2.5 | 65 | 0 | 35 |
| *18* |  | 20 | 2.8 | 5.4 | 3.1 | 2.5 | 65 | 0 | 35 |
| *19* |  | 20 | 3.9 | 7.3 | 3.9 | 3.3 | 39 | 7 | 54 |
| *20* |  | 26 | 3.0 | 6.14 | 3.28 | 2.5 | 56 | 0 | 44 |

*Table 1 (continued)*

| | *Sulfated IdoA*[b] | *Ref.* | $J_{1,2}$ | $J_{2,3}$ | $J_{3,4}$ | $J_{4,5}$ | $^1C_4$ | $^4C_1$ | $^2S_0$ |
|---|---|---|---|---|---|---|---|---|---|
| *21* |  | 19 | 2.8 | 6.1 | 3.3 | 3.3 | 57 | 0 | 43 |
| *22* |  | 26 | 1.0 | 3.6 | 3.6 | 2.3 | 93 | 7 | 0 |
| *22'* |  | 26 | 3.3 | 6.3 | 3.9 | 2.8 | 54 | 9 | 37 |
| *23* |  | 26 | 1.0 | 3.0 | 3.7 | 2.3 | 95 | 5 | 0 |
| *23'* |  | 26 | 2.8 | 5.7 | 3.7 | 2.7 | 63 | 7 | 30 |
| *23''* |  | 26 | 3.3 | 6.35 | 3.75 | 2.8 | 52 | — | 48 |
| *24* |  | 14 | 2.64 | 5.90 | 3.44 | 3.09 | 59 | 0 | 41 |
| *25* |  | 19 | 3.5 | 8.3 | 4.2 | 3.0 | 31 | 0 | 69 |
| *26* |  | 19 | 3.75 | 8.1 | | 3.0 | 32 | 0 | 68 |
| *27* |  | 19 | 3.95 | 7.54 | 3.56 | 3.13 | 36 | 0 | 64 |
| *28* |  | 29 | 5.3 | 9.4 | 4.4 | 4.4 | 10 | 12 | 78 |

[a]Data obtained at 23°, except for *24* (37°); *5*, *6*, *13*, and *14* (60°); and *12* (70°). [b]Symbols:




Iduronic acid residues are $\alpha$-(1→4)-linked in heparin and heparan sulfate, and $\beta$-(1→3)-linked in dermatan sulfate; amino sugar residues (either *N*-sulfated or *N*-acetylated) are all (1→4)-linked, $\alpha$ in heparin and heparan sulfate, and $\beta$ in dermatan sulfate; glucuronic acid residues are $\beta$-(1→4)-linked in heparin and heparan sulfate, and $\beta$-(1→3)-linked in dermatan sulfate.

TABLE II

N.O.e. EFFECTS (%) FOR IdoA-2S AS A MONOMER *13* AND IN THE HEXASACCHARIDE SEQUENCE *23*

| | *13*[a] | *23*[b] | | |
|---|---|---|---|---|
| | | *Residue A* | *Residue B* | *Residue C* |
| H-1→H-2 | 6 | −6 | −2 | −2 |
| H-4→H-5 | 5.5 | −11 | −10 | −10 |
| H-2→H-5 | 0.8 | 0 | −10 | −11 |

[a]At 39°. [b]At 10°.

tained from difference spectra after semi-quantitative inversion of a given mutliplet using the decoupler channel. Data for other compounds were taken from the literature (refs. in Table I).

*Methods of calculation.* — The populations of the $^1C_4$, $^2S_0$ and $^4C_1$ conformations for each compound in Table I were obtained by least-squares fitting of the $^3J$ values, computed for a mixture of the three forms, to the observed values. The contribution of a given form was set to zero when it was <5% and its removal did not increase the r.m.s. deviation by >0.02 Hz. The $^3J$ values for each conformer were computed following a reported procedure[19] which utilizes the equation given by Haasnoot *et al.*[24] on the basis of geometrical models obtained from force-field computations. Slightly different sets of $^3J$ values were found, depending on whether the IdoA residue was sulfated and/or flanked by other residues, as shown in Table II of ref. 19.

## RESULTS AND DISCUSSION

The $^3J$ values listed in Table I for IdoA and IdoA-2S in monosaccharides, disaccharides, higher saccharides, dermatan sulfate, and heparin vary significantly. Some differences are large. Thus, the $J_{1,2}$ values for *13* and *27* are 1.76 and 3.95 Hz, respectively. As also illustrated in the partial spectra of Fig. 2, large differences are observed for $J_{2,3}$ between the monosaccharide *13* (3.34 Hz) and the tetrasaccharide *26* (8.1 Hz)*. A $J_{2,3}$ value as large as 9.4 Hz was observed[29] for pentasaccharide *28*.

The variation in $^3J$ values is not homogeneous. Whereas the increasing values for $J_{2,3}$ of IdoA in *1–6*, *10*, and *11* are paralleled by increasing values of the other $^3J$ values, $J_{2,3}$ of IdoA in *7* and *8*, and of IdoA-2S in *19–21*, *22'*, and *23–28* increase much more than the other $^3J$ values.

Table I also reports the calculated population of major conformers ($^1C_4$, $^2S_0$, and $^4C_1$) of IdoA and IdoA-2S for the various compounds. For most compounds, the $^1C_4$ form is the major contributor to the conformational equilibrium. As ex-

*Perhaps because of concentration effects, chemical shifts and long-range coupling patterns of *13* (Fig. 2a) are somewhat different from those reported[19].

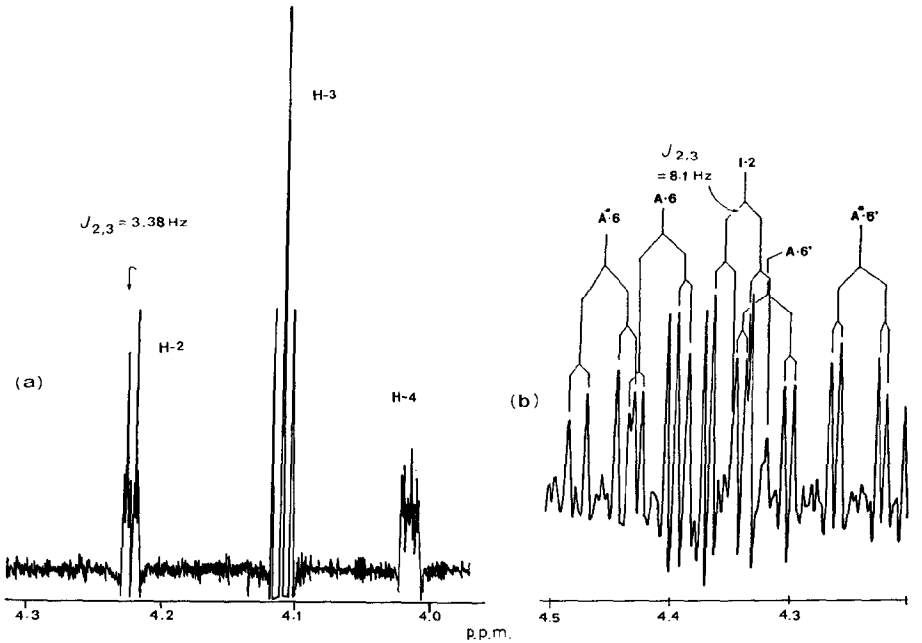



Fig. 2. Partial $^1$H-n.m.r. spectra (300 MHz) of (a) the monomer *13* and (b) the tetrasaccharide *26*, showing the large difference in the $J_{2,3}$ values of IdoA in the two compounds.

pected from the *gauche* protons of the $^1C_4$ form (**6**), the prevalence of this form is associated with small $^3J$ values (1–3 Hz), as observed for the sulfated monomer *13*. In contrast, the all-*trans* orientation (except for H-4,5) of protons in the $^4C_1$ chair (**5**) is expected to result in large $^3J$ values (7–10 Hz)[10]. Calculations show that the homogeneously large $^3J$ values are indeed associated with a substantial contribution of the $^4C_1$ form to the conformational equilibrium. The contribution of the $^2S_0$ form (**7**) is reflected mainly by large $J_{2,3}$ values and intermediate values for the other constants[19]. The $^2S_0$ conformer contributes up to 40% to the conformational equilibrium of "internal" IdoA and IdoA-2S residues of regular sequences of DeS (*12*) and HEP (*24*), becomes preponderant (64–69%) in HEP sequences where the IdoA-2S residue is preceded by a 3-sulfated 2-deoxy-2-sulfoamino residue (as in *25–27*), and (as observed previously[29]) is the major conformer (78%) when the IdoA-2S residue is flanked by two 3-*O*-sulfated amino sugars (as in *28*).

Substantial contributions of the $^4C_1$ and $^2S_0$ forms are associated with smoothing-out of long-range ($^4J$) couplings usually related to a planar "W" arrangement of the protons. A clear pattern of long-range couplings is observable for methyl α-L-idopyranuronate (Fig. 2a; see also ref. 15 for methyl α-D-idopyranuronate).

A sensitive probe for the contribution of the unusual $^2S_0$ form is a substantial intra-residue n.O.e. effect between H-2 and H-5, which are in close proximity (2.3 Å) only in this conformer (Fig. 3). As shown in Table II, the only significant n.O.e. observed for IdoA-2S in the monomer *13* and in position A of hexasaccharide *23*

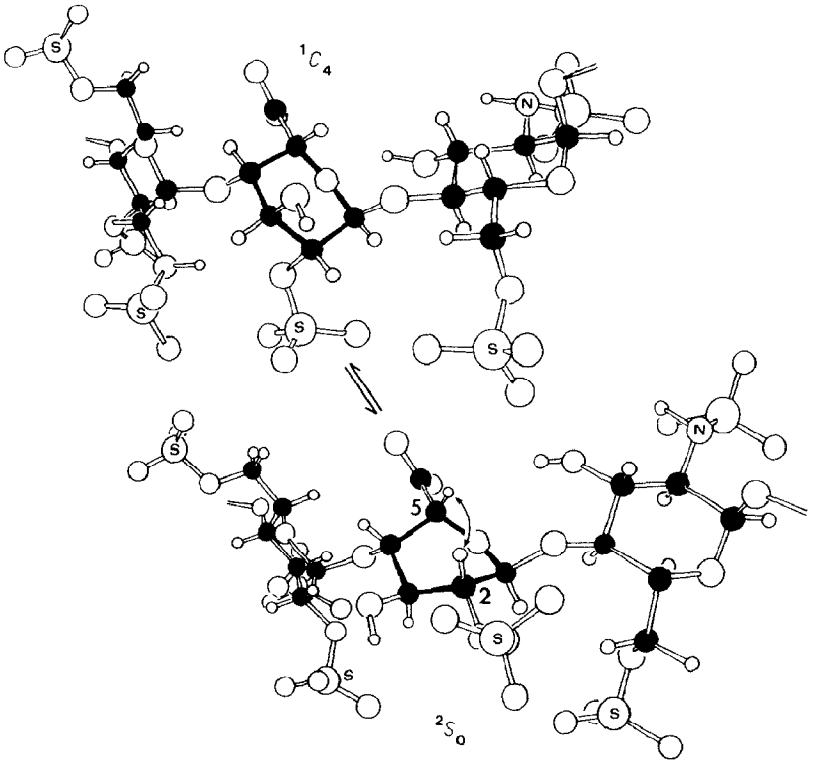



Fig. 3. Low-energy conformations of a trisaccharide segment of the pentasaccharide *27*, with the Ido-2S residue in the $^1C_4$ and $^2S_0$ conformations. The arrow indicates a pair of protons of the iduronate residue with an expected strong n.O.e. effect.

are those between vicinal protons. In contrast, a significant H-2/H-5 n.O.e. was oberved for the same residue in positions B and C of *23*, in accordance with a substantial contribution of the $^2S_0$ conformer as calculated from the coupling pattern[30].

There is ample evidence that the $^3J$ values of iduronate are significantly affected by temperature[14,15,20,21]. Thus, the $^3J$ values of IdoA-2S in regular sequences of heparin (*24*) increase with increase of temperature[14]. Calculations show that such an increase is mainly associated with an increasing proportion of the $^2S_0$ conformer. On the other hand, the more general increase of the $^3J$ values for methyl $\alpha$-idopyranuronates (ref. 15 and the present work), as well as for terminal non-reducing IdoA residues of HS tetrasaccharides[17], also reflects increasing proportions of the $^4C_1$ form.

Although not strictly homogeneous because of the different temperatures, comparison of data in Table I indicates the following trends.

(*a*) *Non-sulfated IdoA* in monomers (*1* and *2*) is present as an equilibrium of the two chair forms, which is displaced towards the $^1C_4$ form on methylation at position 4. When at the non-reducing terminal of disaccharides and higher

TABLE III

INFLUENCE OF SALTS ON THE $^3J_{H,H}$ VALUES FOR IdoA-2S IN THE MONOMER *13* AND IN THE SEQUENCES *24* AND *27*[a]

| *Compound* | | *Saltless solution* | *+NaCl* | *+$CaCl_2$* |
|---|---|---|---|---|
| *13* | $J_{1,2}$ | 1.015 | 1.3 | 1.3 |
| | $J_{2,3}$ | 3.277 | 3.0 | 3.0 |
| | $J_{3,4}$ | 3.383 | 3.3 | 3.2 |
| | $J_{4,5}$ | 1.994 | 2.0 | 1.92 |
| *24* | $J_{1,2}$ | 2.64[b] | | 1.8 |
| | $J_{2,3}$ | 5.90[b] | n.d. | 4.6 |
| | $J_{3,4}$ | 3.44[b] | | n.d. |
| | $J_{4,5}$ | 3.09[b] | | 1.9 |
| *27* | $J_{1,2}$ | 3.95 | 3.37 | 3.4 |
| | $J_{2,3}$ | 7.54 | 6.62 | 6.18 |
| | $J_{3,4}$ | 3.56 | 3.70 | 3.13 |
| | $J_{4,5}$ | 3.13 | 3.10 | 3.13 |

[a]Data obtained at 23° for solutions in $D_2O$, and with added 0.5M NaCl or $CaCl_2$ for *13* and *27*, and a 1:1 molar ratio of heparin (average disaccharide unit) and $CaCl_2$ for *24*. [b]At 37°, from ref. 19.

saccharides (*3–6, 10,* and *11*), the $^2S_0$ form also contributes to the equilibrium, the $^1C_4$ and $^4C_1$ forms being favoured when the amino sugar is *N*-sulfated and *N*-acetylated, respectively. When IdoA is flanked by two amino sugar residues, the $^1C_4$ and $^2S_0$ forms preponderate and there is <10% of the $^4C_1$ form. As for the terminal position, an adjacent *N*-sulfated amino sugar displaces the equilibrium towards the $^1C_4$ form.

(*b*) *2-Sulfated IdoA* is mainly in the $^1C_4$ form in the monomer *13*, and 2-sulfation displaces the equilibrium towards the $^1C_4$ form in all the sequences studied. This effect is superimposed on sequence (next-neighbour) effects similar to those observed for non-sulfated IdoA, *i.e.*, a further displacement towards the $^1C_4$ form when IdoA-2S is at the non-reducing terminal (as for residue A of *22* and *23*) and a substantial contribution of the $^2S_0$ form when it is internal (sequences *17–28*). As observed previously, when the amino sugar preceding the IdoA-2S residue bears an extra sulfate group (at O-3), the major conformer becomes $^2S_0$. Such a displacement towards the $^2S_0$ conformation is even more marked when IdoA-2S is flanked by two 3-*O*-sulfated amino sugars.

The relative proportions of conformers may also be a function of ionic strength[20] and the type of counter-ion. The data in Table III, and conformer populations calculated therefrom, indicate that, whereas the conformational equilibrium of IdoA-2S in the monomer *13* is barely affected by NaCl or $CaCl_2$, it is displaced towards the $^1C_4$ form when IdoA-2S is internal in an oligo- or poly-saccharide chain. For heparin (*24*), the ratio $^1C_4$:$^2S_0$ changes from 59:41 to 79:21 in the presence of $CaCl_2$, and for the pentasaccharide *27*, from 36:64 to 48:52 and 53:47 in the presence of NaCl and $CaCl_2$, respectively.

The electrostatic repulsion between anionic charges on neighbouring residues may be the driving force that determines the conformational equilibrium of IdoA-2S residues in heparin oligosaccharides[19,20]. The screening of these charges by an excess of counter-ions could relieve the inter-residue interactions, thus modulating the conformational equilibrium.

Whereas the flexibility of the backbones of polysaccharides is usually associated[31] only with rotation of the monosaccharide residues around the glycosidic bonds (variation of angles $\varphi$ and $\psi$), the extra-flexibility induced by the presence of an equilibrium of two or more conformations of monosaccharide residues is a peculiar characteristic of IdoA-containing glycosaminoglycans which could contribute to their binding properties and biological "versatility". These features contrast with the poor binding and biological properties of other glycosaminoglycans having approximately the same degree of sulfation and molecular weight, but the more rigid glucuronic acid as the major uronic acid.

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