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Preliminary communication

Evidence for a boat–chair equilibrium in the glucuronate residue of chondrosine

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Chondrosine [**1**, 2-amino-2-deoxy-3-*O*-(β-D-glucopyranosyluronic acid)-D-galactose] is obtained by degradation of the chondroitin sulphates. Previous n.m.r. studies were performed on solutions of the sodium salt of *N*-acetylchondrosinate in methyl sulphoxide[1]. The ^{1}H-n.m.r. spectrum (400.13 MHz) of a solution of **1** in D_2O (3 mg/mL) at 24° (internal acetone, 2.225 p.p.m.) indicated an α,β-ratio of 65:35. The ^{1}H-n.m.r. spectrum was analysed completely using ^{1}H–^{1}H LRCOSY (long range correlated spectroscopy) and by simulation with a modified version of the LAOCN3 program[2] (Table I).

TABLE I

^{1}H-N.m.r. data (δ in p.p.m. and J in Hz) for chondrosine (**1**) from spectral analysis and relative assignments (r.m.s. <0.1 Hz)

Residue	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6A*	*H-6B*
D-GalN, α anomer	5.504	3.671	4.262	4.360	3.795	3.561	3.535
β anomer	4.900	3.340	4.058	4.302	3.802	3.561	3.535
D-GlcA, α anomer	4.705	3.464	3.770	4.172	3.768		
β anomer	4.694	3.478	3.770	4.172	3.768		
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6A}$	$J_{5,6B}$	$J_{6A,6B}$
D-GalN, α anomer	3.66	10.76	2.93	0.00	4.77	4.12	−9.50
β anomer	8.55	10.99	3.18	0.00	5.17	3.97	−9.44
D-GlcA, α anomer	7.60	9.49	5.38	7.08			
β anomer	7.84	9.51	5.38	7.08			

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The values of $J_{6A,6B}$ for the α and β anomers of the GalN moiety of **1** (−9.50 and −9.44 Hz, respectively) deviated much from reported data[3], but the ^3J values accorded with a $^4C_1$ chair conformation. However, the values for the GlcA moiety ($J_{3,4}$ 5.4, and $J_{4,5}$ 7.1 Hz) correspond[4] to dihedral angles for H-3,4 of 147° or 51° and for H-4,5 of −155° or −24°, respectively, and are not consistent with a single conformation.

These observations were rationalised in a molecular mechanics study using the REFINE program[5], in which the zwitterionic character of **1** was simulated by conferring a formal charge (*e*) on each of the carboxylic and amino groups. As in a study of the conformation of L-iduronate[6], the effect of various partial charges was tested. Whereas the conformation of the GalN moiety was not affected, that of the GlcA moiety was dependent on the values of partial charges. For charges up to 0.8*e*, the $^4C_1$ chair conformation was the most stable by far, but values of 1.0*e* favoured boat conformations with calculated energies of ≤2 kcal/mol more than that of the chair conformation. These boat conformations are stabilised by an interaction between the ionic groups $O^- \cdots H\text{–}N^+$ (2.4 Å).

The calculated low-energy conformations of **1** have inter-glycosidic dihedral angles that are quite different from those observed in the crystal[7].

The relative coupling constants derived from the computed geometries (Table II), on the assumption of an equilibrium between the chair (**A**) and boat (**B**) forms, yield a best-fit ratio of 60:40 with an r.m.s. value of 0.5 Hz.

A drawing of the chondrosine molecule with the GlcA ring in the boat conformation is shown in **2**. The results are in good agreement with the above-mentioned energy differences and show that conformations other than $^4C_1$ and $^{2,5}B$ do not produce a satisfactory fit.

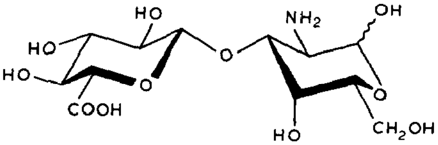
To our knowledge, this is the first experimental and computational evidence for a boat-like conformation of the glucuronate ring in GlcA. The effect of ionic strength, n.O.e. effects, and computations on the crystal structure are being studied.

TABLE II

Main geometrical features of the minimum-energy structures for the GlcA moiety in chondrosine[a–c]

Conformation	θ (°)	φ_2 (°)	Q (Å)	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	*Glycosidic linkage*		
								π (°)	ψ (°)	E[d]
(A) $^4C_1$	177	285	0.57	7.67	9.13	9.06	10.04	165	20	−39.00
(B) $^{2,5}B$	93	236	0.70	7.84	8.06	1.06	1.47	−172	15	−36.93
(C) ^2S_o	97	261	0.70	7.17	9.80	5.02	0.74	−172	4	−38.17
Experimental (α)				7.60	9.49	5.38	7.08			
Experimental (β)				7.84	9.51	5.38	7.08			

[a] Best fit of the $^3J_{H,H}$ values for an **A**:**B** equilibrium ratio of 61:39 gave an r.m.s. value of 0.53 Hz; an **A**:**C** equilibrium ratio of 59:42 gave a value of 1.12 Hz. [b] Ring-puckering parameters θ, φ_2 [phase angles (°)] and Q [total puckering amplitude (Å)] are defined as in ref. 8. [c] Dihedral angles of the glycosidic linkage are defined as φ = H-1′–C-1′–O-3–C-3 and ψ = C-1′–O-3–C-3–H-3. [d] The energy *E* is given in kcal/mol.



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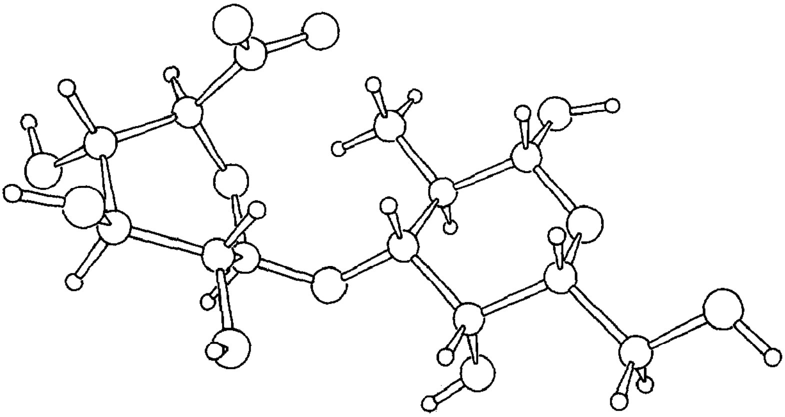

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