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Spectroscopic analysis of mutarotated methyl(β-d-glucopyranosyluronate) -(1→3)-2-amino-2-deoxy-D-galactopyranose in aqueous solution

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ABSTRACT

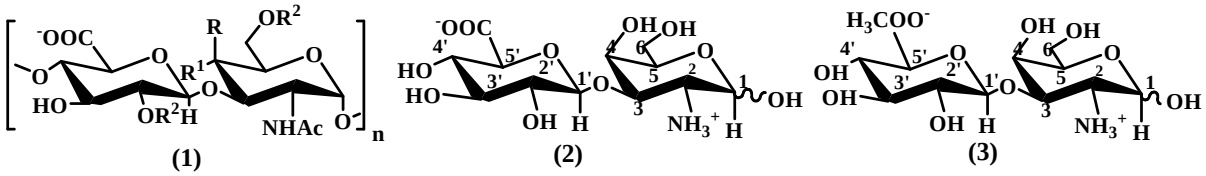
Chondrosine [β-D-Glc*p*A-(1→3)-α,β-D-GalNH_2] is a natural disaccharide accessible from Chondroitin sulfate (CS). We report in this communication the ^1H and ^{13}C NMR spectral data of the chondrosine methyl ester **(3)** in D_2O. Data revealed that two isomers of **(3)** namely methyl [β-D-GlcpA-(1→3)-α,β-D-GalNH_2] **(3a)** and methyl [α-D-GlcpA-(1→3)-α,β-D-GalNH_2] **(3b)** are present in solution and undergoing mutarotation on the GalNH_2 reducing end. Since the glycosidic linkage of the GlcpA residue can not undergo interconversion, it is rational to conclude that the αβ anomers of this residue are either generated in an early step during the preparation of chondrosine itself or both are natural products. This is the first experimental evidence for the presence of two combinations of mutarotated condrosine methyl ester in aqueous solution and thus the αβ designation can be made for the GlcpA residue in **(3)**.

KEYWORDS

Chondrosine;
Mutarotation;
Pyranose;
Hemiacetal;
NMR.

INTRODUCTION

Chondroitin sulfate (CS) **(1)** (SCHEME 1) is a class of glycosaminoglycans (GAGs) among others such as heparin, heparan sulfate and dermatan sulfate[1]. Biosynthesis of CS in animals begins with the synthesis of the linkage tetrasaccharide D-glucuronic acid (GlcA)-Gal-Gal-Xyl on the serine residues of core proteins. The chain then elongates from the linkage by the alternate addition of N-acetyl-D-galactosamine (GalNAc) and GlcA residues catalyze by CS synthases in the Golgi apparatus[2]. During the polymerization step the chain



(1) **(2)** **(3)**

R = OH, OSO_3^-; R^1 = H; R^2 = H, SO_3^-

SCHEME 1

undergoes sulfation reaction catalyze by a variety of sulfotransferases at various positions[3]. Structural studies showed that **(1)** consists of a dimeric unit of D-glucuronic acid (GlcA) and 2-acetamido-2-deoxy-D-galactose (GalNAc), namely [4)-β-D-GlcpA-(1→3)-β-D-GalNAc-(1→]$_n$ [4]. Chondrosine **(2)** is obtained from **(1)** either by acid-catalyzed hydrolysis[5] or enzymatic degradation processes[6]. Various chemical structures for compound **(2)** have been proposed[7] and to date the formula [β-D-Glc*p*A-(1→3)-α,β-D-GalNH$_2$] with a β-configuration of the glucuronidic linkage[8] is established.

Despite considerable interest in chemistry of chondroitin sulfate and hyaluronic acid[9], the use of **(2)** as starting material for glycoside synthesis is limited[10]. Additionally, there are no literature data on spectroscopic[11] or crystallographic studies for any chondrosine derivative to unequivocally establish the conformation of the disaccharide and the configuration of the interglycosidic (1→3)-linkage. As a continuation of our work in the field of natural carbohydrate oligosaccharides[12,13] and their mimetic analogues[14], we report in this communication a study of configuration analysis of chondrosine methyl ester **(3)** in aqueous solution based on ^{1}H and ^{13}C NMR spectral data.

Methyl esterification step was carried out by slow addition of acetyl chloride(1 equiv.) to a cold (0°C) suspension of **(2)** in MeOH and the mixture was cooled at -5°C for 4 d[10] to give the corresponding ester **(3)** in 40% yield. Mass spectral analysis recorded for $C_{13}H_{24}ClNO_{11}$ (Calc; M$^+$ 405.5) revealed a molecular ion peak at m/z 370.1 (+MS) assigned for (M-Cl)$^+$(100%) and a molecular ion peak at m/z 404.5 (-MS) assigned for (M-1)$^+$(100%). Signals of ^{1}H NMR spectrum (D_2O, 500 MHz, rt), assigned with the aid of ^{1}H-^{1}H-COSY, TABLE 1, for ester **(3)** indicated a number of anomers with variable populations. For instance, the two strong doublets displayed at δ 5.33 (d, $J_{1,2}$ 3.5 Hz), 5.32 (d, $J_{1,2}$ 3.5Hz) and two additional weak doublets at δ 5.42 (d, $J_{1,2}$ 1.8Hz), 5.41 (d, $J_{1,2}$ 3.65Hz), not included in TABLE 1 clearly indicate the presence of four anomeric α linkages with different ratios. The two broad doublets of similar intensity displayed at δ 4.75 (J = 8.4Hz) and 4.78 (J = 8.4Hz) were assigned as H-1β protons. As indicated by the coupling constant J and integration values, the broadening of the latter signals is not only due to virtual coupling produced by coupling with H-2, but also probably to the presence of other conformer of minor intensity. The first interpretation of the signals observed in ^{1}HNMR of **(3)** pointed out to anomeric equilibrium of more than one conformer. Because anomerization is related only to a hemiacetal carbon, hence there can be mutarotaion in the GalNH$_2$ residue of **(3)** and the same is not true for the GlcpA residue that can not interconvert because of the C-1 acetal carbon. Anomeric equilibrium in a deuterated aqueous solution of chondrosine **(2)** has been studied previously by ^{1}HNMR studies and results indicated an α/β ratio of 65:35[15]. Furthermore, literature values of equilibrated solution of GalNH$_2$.HCl in D_2O had indicated an α:β ratio of 59:41[16].

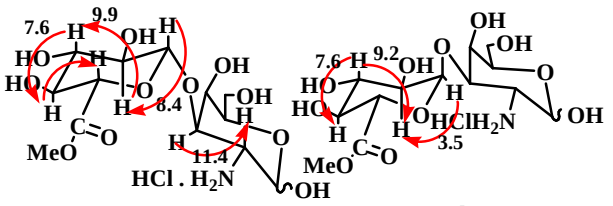
^{13}CNMR spectroscopy is a valuable tool in analyzing the composition of equilibrated mixtures and in detection of the minor components[178]. ^{13}C NMR spectrum at 125MHz displayed a clear picture of the equilibrated solution of ester **(3)** in D_2O and its anomeric

TABLE 1: ^{1}H NMR spectroscopy (500MHz, δppm, D_2O) data of ester (3)

H	α anomer	m	J	Hz	β-anomer	m	J	Hz
H-1	5.33	d	$J_{1,2}$	3.5	4.78	d	$J_{1,2}$	8.4
H-2	3.62	dd	$J_{2,3}$	11.0	3.31	dd	$J_{2,3}$	11.4
H-3	3.98	dd	$J_{3,4}$	3.0	3.97	dd	$J_{3,4}$	3.1
H-4	4.12-4.08	m			4.12-4.08	m	-	
H-5	3.96	dd	$J_{5,6}$	4.0	3.95	dd	$J_{5,6}$	3.7
2H-6	3.58-3.56	m			3.58-3.56	m	-	
MeO	3.66	s			3.66	s	-	
H-1'	4.11	d	$J_{1',2'}$	7.7	4.10	d	$J_{1',2'}$	7.7
H-2'	3.45	dd	$J_{2',3'}$	9.5	3. 41	dd	$J_{2',3'}$	9.5
H-3'	3.55	dd	$J_{3',4'}$	5.1	3.55	dd	$J_{3',4'}$	5.1
H-4'	3.93	dd	$J_{4',5'}$	6.8	3.93	dd	$J_{4',5'}$	6.8
H-5'	3.58-3.56	m			3.58-3.56	m	-	

TABLE 2: ^{13}C NMR spectroscopy (125MHz, δppm, D_2O) data of ester (3)

C	Estim.	Anomer-1	Anomer-2	Anomer-3	Anomer-4
CO	172.0	171.10	171.04	171.16	171.13
C-1	100.1	103.71	103.80	103.77	89.44
C-2	50.1	49.83	49.79	49.86	49.73
C-3	71.8	71.97	71.13	-	-
C-4	65.3	67.48	67.41	67.38	-
C-5	74.8	74.79	74.45	74.38	-
C-6	65.5	61.05	60.99	69.95	-
MeO	52.7	53.19	53.10	52.87	52.82
C-1'	99.6	103.63	92.98	92.88	89.37
C-2'	73.8	72.62	72.52	-	-
C-3'	72.4	75.09	75.02	94.96	-
C-4'	68.2	70.25	70.17	-	-
C-5'	78.9	77.55	77.49	77.45	77.39



(3a)
Methyl [b-D-Glc*p*A-(1-3)
-a,b-D-GalNH$_2$]

(3b)
Methyl [a-D-Glc*p*A-
(1-3)-a,b-D-GalNH$_2$]

Figure 1: Two isomers of chondrosine methyl ester with a- and b-glucuronidic linkages, respectively, undergoing mutarotation on the GalNH2 reducing end and selected 3J coupling constants (Hz)

composition. The configurations at C-1 and C-1' were assigned based on the chemical shift (δ) values. As shown in TABLE 2, signals at δ 103.71, 103.63, 103.80 and 103.77 ppm were diagnostic for all β anomers. Strong signals at δ 92.98, 92.88, 89.44, 89.37 ppm and other four weak peaks, not included in TABLE 2, resonated at δ 89.57, 89.32, 89.24 and 89.21ppm, respectively, were diagnostic for all α anomers.

Apparently, this data not only indicated the $^4C_1$ chair conformation of the disaccharide **(3)** in its four possible anomers in solution (i.e., αα, αβ, βα and ββ configurations), but it also unambiguously proved the presence of α-(1→3)-glucuronidic linkage, which is not in agreement with the literature data[8]. To this point, an important question raised; Can α/β designations be made for the GlcpA residue in **(3)** ? As mentioned above the GlcpA residue can not interconvert in aqueous solution because acetals do not show mutarotation and in order for anomerization to happen the disaccharide would subsequently have to hydrolyze first then anomeric conversion and reformation of glycosidic bond. Therefore, it is rational to conclude that the GlcpA anomers either generated in an early step during the preparation of chondrosine itself or both are naturally occurring products. Surprisingly, the published spectral data of methyl hyaluronate, the closely analogue of **(3)**, showed similar α-glucuronidic linkage[10]. The observing major signals in the ^{1}H and ^{13}CNMR spectra of **(3)** are thus corresponding to a mixture of two isomers, Figure 1, one with an β-(1→3)-linkage **(3a)** and one with a α-(1→3)-linkage **(3b)** which are undergoing mutarotation on the GalNH$_2$ reducing end. The additional minor peaks resonated at δ 89.57-89.21 ppm range may be assigned as the C-1 signals of α and β types of furanose forms of the mutarotated GalNH$_2$ residue. Similar small values of $J_{1,2}$ mentioned above have been reported for 1,2-trans-2-amino-2-deoxy-β-D-galactofuranose hydrochloride[16].

In summary, we report the first experimental evidence for the presence of two combinations of mutarotated condrosine methyl ester in aqueous solution one with a $\alpha_1 \rightarrow 3$ linkage and one with a $\beta_1 \rightarrow 3$ linkage. The α/β designation can be made for the GlcpA residue in **(3)**. Since the glycosidic linkage of GlcpA residue can not interconvert in aqueous solution, anomers are either generated in an early step during the preparation of chondrosine itself or both are natural products.

EXPERIMENTAL

General methods

^{1}H and ^{13}C NMR spectra were recorded using JEOL at 500 MHz and 125MHz respectively in D$_2$O with the chemical shifts are expressed on the δ scale in parts per million (ppm). Both positive-ion and negative-ion modes of mass spectral analysis were performed on an Agilent MSD Trap-SL mass spectrometer equipped with an electrospray ion source. Samples were dissolved in 1:1 water/methanol and introduced into ion source at a flow rate of 6μl/min. Nebulizer pressure was set to 15 psi and the dry nitrogen gas was used at a flow rate at 5L/min and the dry temperature was at 325^{0}C.

(Methyl β-D-glucopyranosyluronate)-(1→3)-2-amino-2-deoxy-D-galactopyranose hydro-chloride salt **(3)**. Acetyl chloride (0.75mL, 10.5mmol) was added to a stirred suspension of chondrosine **(2)**(3.00g, 8.44mmol) in MeOH (90mL) at 0°C then left for 4d at - 5°C. tert-BuOH (20mL) was added and the solution evaporated to dryness. The crude product was dissolved in water (150mL), filtered through Celite and the solvent was evaporated. Recrystallization by dissolution in MeOH (20mL) and 1:1 MeOH/i-PrOH (40 mL) was added followed by the addition of more i-PrOH (120mL). The solids were removed by filtration and discarded and the filtrate was evaporated to dryness. The resulting solid was dissolved in MeOH and it

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was re-precipitated by addition of Et_2O. The amorphous white solid was filtered off and dried in vacuo to give 1.4g of **(3)**, (40%). ^1H NMR (500 MHz, D_2O); see TABLE 1. ^{13}C NMR (125 MHz, D_2O) see TABLE 2. MS (ES): Calcd for $C_{13}H_{24}ClNO_{11}$ (405.5); Found: +MS m/z 370.1 $(M-Cl)^+$(100%), -MS m/z 404.5 $(M-1)^+$(100%).

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