Electronic Supporting Information

Calcium Complexation and Acid-Base Properties of L-Gulonic Acid, a Diastereomer of D-Gluconic Acid

Bence Kutus[1,4], Gábor Peintler[2,4], Ákos Buckó[1,4], István Pálinkó[3,4], Pál Sipos[1,4]*

[1] Department of Inorganic and Analytical Chemistry, University of Szeged, Dóm tér 7, H–6720 Szeged, Hungary

[2] Department of Physical Chemistry and Material Science, University of Szeged, Rerrich Béla tér 1, H–6720 Szeged, Hungary

[3] Department of Organic Chemistry, University of Szeged, Dóm tér 8, H-6720 Szeged, Hungary

[4] Material and Solution Structure Research Group, University of Szeged, Institute of Chemistry, Aradi Vértanúk tere 1, H–6720 Szeged, Hungary

* Corresponding author, fax: +36–62–420–505, email: sipos@chem.u-szeged.hu

Assignation of the ^{1}H and ^{13}C NMR spectra and protonation of Gul^{-}

The assignment of all proton and carbon signals was obtained from the one-dimensional ^{1}H NMR spectrum, as well as the ^{1}H–^{13}C HSQC (Heteronuclear Single Quantum Coherence) spectrum of NaGul solutions (Figure S1 and S2). When alkaline medium was employed, more resolved spectra with smaller line widths were obtained; similar observations were made for Gluc^{-} [1]. The proton-proton coupling constants at neutral and alkaline solutions are shown in Table 1. The absolute values of the NMR parameters obtained by us were compared with those published in ref. [2]. Our ^{1}H and ^{13}C NMR chemical shifts are uniformly displaced upfield by *ca.* 0.2 ppm and 2.0 ppm, respectively. The reason of this displacement is most probably associated with the way of using D_2O inside the samples contrary to our protocol (described above). The reported values of the $J_{H,H}$ constants were practically identical with the largest difference of 0.2 Hz. The only conspicuous difference was between the $^{3}J_{H4,H5}$ values (5.0 and 1.3 Hz, respectively). Additionally, a repetition was made resulting the same coupling constants (the largest deviation between the two sets of data was 0.1 Hz). (It can be seen that the coupling constants were only slightly sensitive to the presence of NaOH, which possibly associated with the deprotonation of one of the OH groups.) The order of the carbon peaks according to decreasing chemical shift (C1, C2, C5, C3, C4, C6) matches with the reported ones, too.

On the ^{13}C NMR spectrum of a close-to-neutral NaGul solution (Figure 2), six well-defined peaks for the six carbon atoms could be observed. When the pH of this solution was changed to *ca.* 2 by adding HCl to the system, the ^{13}C NMR chemical shifts of C1, C2, C4 and C5 moved significantly upfield (the extent of this variation changed in the order of C1 > C2 >> C4 ≈ C5), while those of C3 and C6 remained practically unchanged (or, if any, they moved downfield; see the lower spectrum in Figure 2). This is due to the protonation of the carboxylate group with the decreasing pH. The systematic change of the chemical shift for these peaks shows that the deprotonated and protonated forms are in fast exchange on the NMR timescale at 25 °C, which is a common property of carbohydrate derivatives. It is interesting to note that, beside C1 and C2, the extent of these pH-dependent displacements in the carbon signals of Gul^{-} (as in case of Gluc^{-} [1]) shows no correlation with the proximity to the protonation site. This implies that the shielding factor of these nuclei is very sensitive not only to the protonation of the COO^{-} group, but to the simultaneous conformational change as well.

Table S1. $J_{H,H}$ coupling constants (in Hz) of 0.15 M Na-L-gulonate in **water** and in 0.5 M NaOH solution and at T = (25 ± 1) °C, respectively.

		$J_{H,H}$ (Hz)					
		H2	H3	H4	H5	H6	H6’
$J_{H,H}$ (Hz)	H2		**5.3**/5.1				
	H3	**5.3**/5.1		**3.0**/2.8			
	H4		**3.0**/2.8		**4.9**/4.7		
	H5			**4.9**/4.7		**3.7**/4.1	**6.7**/6.3
	H6				**3.7**/4.1		**11.7**/11.7
	H6’				**6.7**/6.3	**11.7**/11.7	

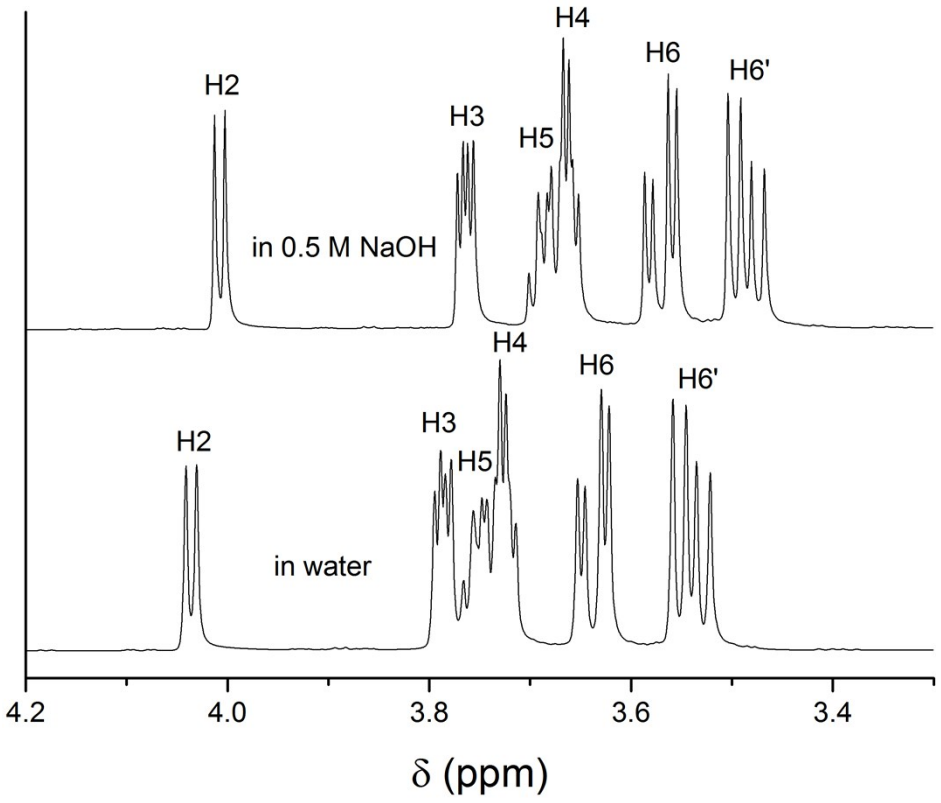

Figure S1. ^{1}H NMR spectrum of 0.15 M Na-L-gulonate in water (pH ~ 7) and in 0.5 M NaOH solution with peak assignments at T = (25 ± 1) °C.

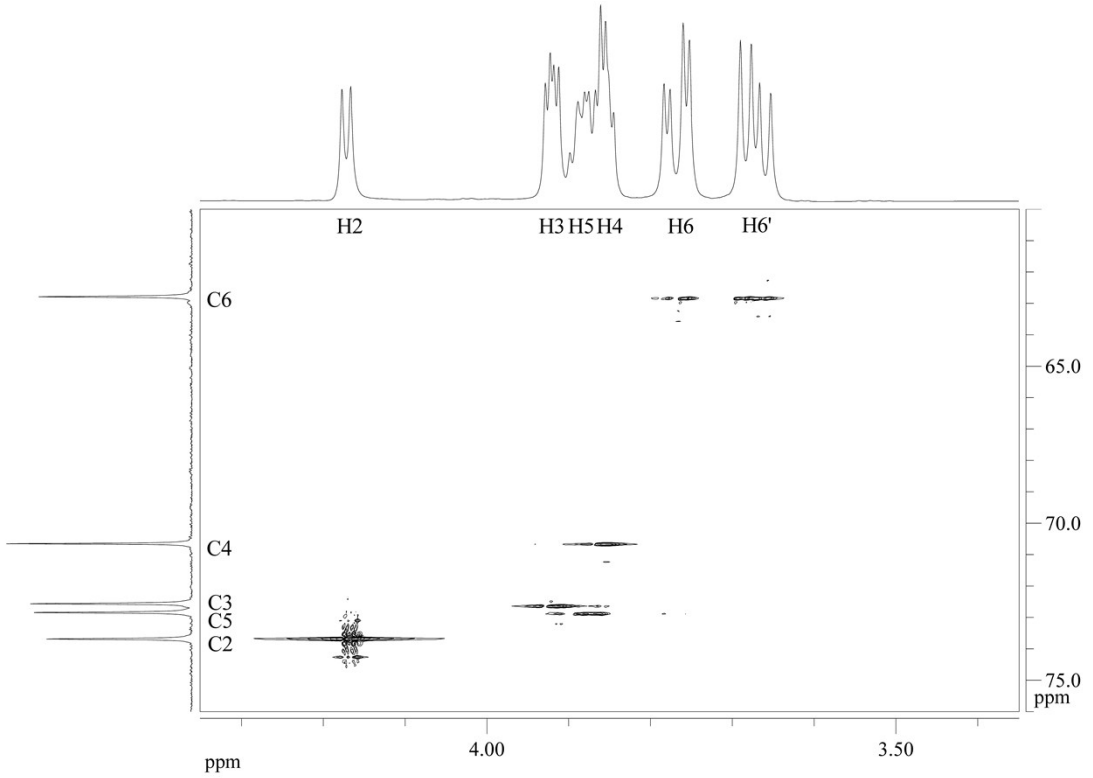

Figure S2. The $^{1}H–^{13}C$ HSQC spectrum of an aqueous solution containing 0.5 M NaGul solution at T = (25 ± 1) °C.

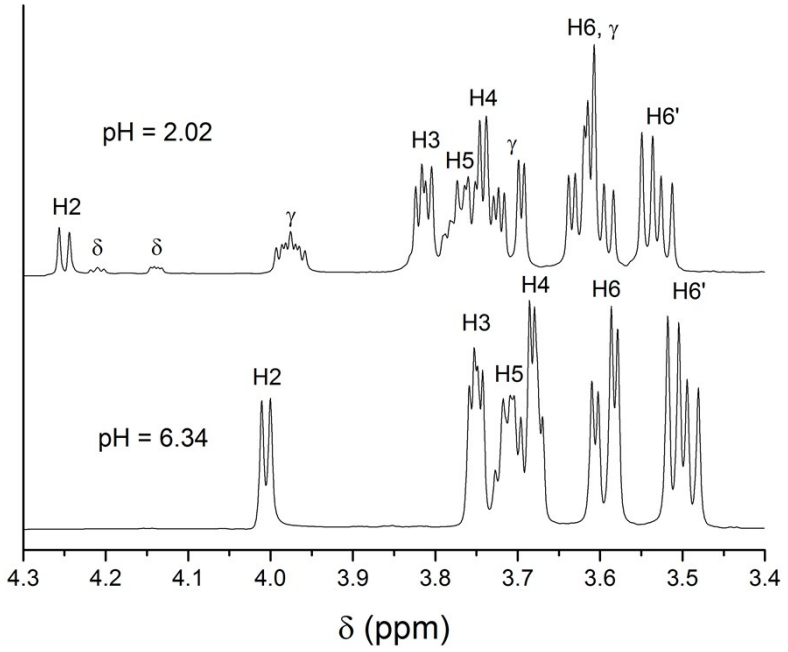

Figure S3. ^{13}C NMR traces of a close-to-neutral (lower spectrum) and an acidic (upper spectrum) at T = (25 ± 1) °C, I = 1 M with $[Gul^{-}]_T$ = 0.200 M. The actual pH of the solutions is shown in the graphs. Spectra were recorded and the actual pH measured after 4 days of preparation. The letters γ and δ on the upper graph indicate carbon signals corresponding to the γ- and δ-lactones of HGul, respectively. For more experimental details, see text.

Definitions used in the freezing point depression measurements and their evaluations. Freezing point depression is a colligative property of a solution, which is calculated according to Blagden's law:

$$\Delta T_f = K_f \cdot m$$

where ΔT_f is the freezing point depression defined as the difference between the freezing point of the solvent and the solution, K_f is the cryoscopic constant (which is 1.86 °C·kg·mol^{-1} for water) and m is the molality of the solute, which may be replaced by the practically identical molar concentration values for relatively diluted solutions. For a system containing n different species (denoted as X) with the analytical concentration of $[X]_T$, the theoretical freezing point depression ($\Delta T_{f,theo}$) is proportional to the sum of the concentrations:

$$\Delta T_{f,theo} = K_f \cdot \sum_{i=1}^{n} [X_i]_T$$

If the number of solute particles decreases because of any association, *e.g.,* complex formation process, the measured freezing point depression ($\Delta T_{f,meas}$), being a colligative property, also decreases. Thus, $\Delta T_{f,meas}$ will be proportional to the sum of the equilibrium concentrations of species X:

$$\Delta T_{f,meas} = K_f \cdot \sum_{i=1}^{n} [X_i]$$

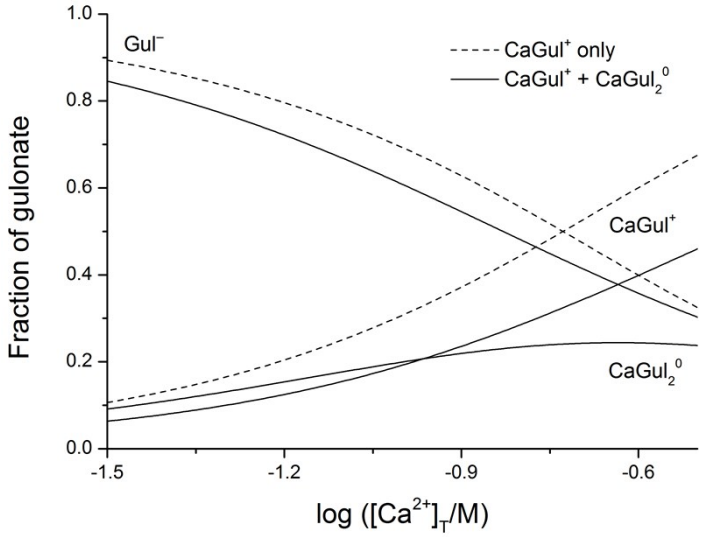

Figure S4. Distribution of gulonate among the various aqueous species in presence of calcium, as a function of log ($[Ca^{2+}]_T$/M) at $[Gul^-]_T$ = 0.2 M. The dashed and solid lines correspond to the fitted chemical model including $CaGul^+$ only (log $K_{1,1}$ = 1.06) and $CaGul^+$ + $Ca(Gul)_2^0$ together (log $K_{1,1}$ = 0.88, log $\beta_{1,1}$ = 1.51), respectively. Experimental conditions: I = 1 M NaCl, T = (25 ± 1) °C.

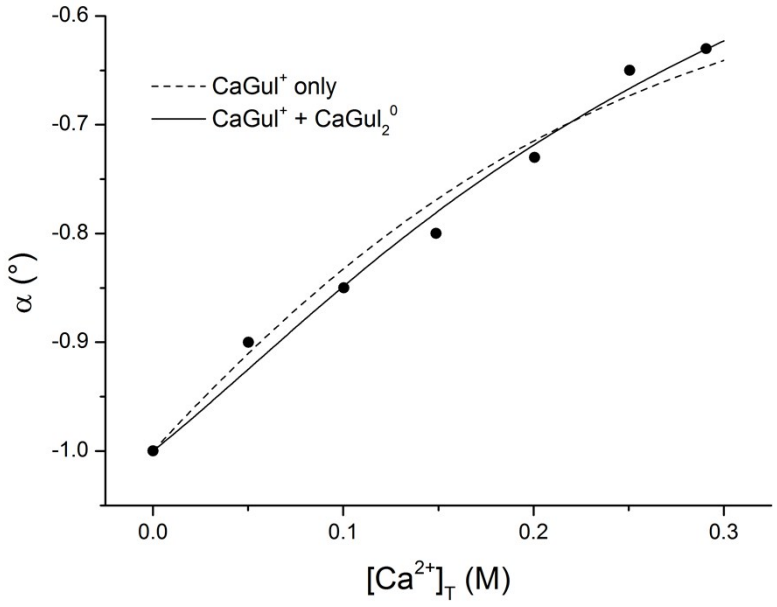

Figure S5. Variation in the optical rotation (α in °) of solutions containing $[Gul^-]_T$ = 0.2 M and $[Ca^{2+}]_T$ = 0.290 M as a function of $[Ca^{2+}]_T$. The dashed and solid lines correspond to the fitted chemical model including $CaGul^+$ only (log $K_{1,1}$ = 1.06) and $CaGul^+$ + $Ca(Gul)_2^0$ together (log $K_{1,1}$ = 0.88, log $\beta_{1,1}$ = 1.51), respectively. Experimental conditions: I = 1 M NaCl, T = (25 ± 2) °C

Table S2. Stability constants for protonation and complex formation reaction of L-gulonate determined in this study or reported earlier with respect to the ionic strength. For comparison, equilibrium constants are given for D-gluconate. All of the given log β values were obtained at 25 °C. For more experimental details, see the text and the cited references, respectively.

		L-Gul$^-$			D-Gluc$^-$		
Reaction[a]	**I (M)**	**log β**	**Method**[b]	**Ref.**	**log β**	**Method**[b]	**Ref.**
H + L = HL	→ 0	3.68[c]	POT	3	3.77 ± 0.02[c]	POT, POL	6
		3.48 ± 0.02	POT	4	3.50 ± 0.03	POT	4
	0.1				3.30 ± 0.02	^{13}C NMR	7
		3.20 ± 0.10[d] (3.20 ± 0.05)	^{1}H NMR	this study	3.23 ± 0.01	^{1}H NMR	8
	1	3.19 ± 0.03[d] (3.19 ± 0.02)	^{13}C NMR		3.24 ± 0.01	^{13}C NMR	
					3.30 ± 0.1	POT	9
M + L = ML	0.1	1.6[e]	POT	5	1.6	POT	5
	0.5				1.1 ± 0.1	POT	10
		0.88 ± 0.02	POT		1.15 ± 0.09	TITR, POT	11
	1	1.1 ± 0.1[d] (1.12 ± 0.01)	^{1}H + ^{13}C NMR	this study	1.03 ± 0.01	^{13}C NMR	8
	2	0.90 ± 0.01	POT		0.75 ± 0.06	^{13}C NMR	
	3	0.88 ± 0.01	POT		0.79 ± 0.07	^{13}C NMR	
	4	0.84 ± 0.01	POT		0.85 ± 0.05	^{13}C NMR	
M + HL = ML + H	1	– 2.31 ± 0.04[f]		this study	– 2.22 ± 0.01[g]	^{13}C NMR	8
M + 2L = ML$_2$	0.5				1.88 ± 0.08	POT	10
	1	1.51 ± 0.03		this study			
	2	1.49 ± 0.03					
	3	1.41 ± 0.03					
	4	1.49 ± 0.02					
ML + L = ML$_2$	0.5				0.78 ± 0.13[g]	POT	10
	1	0.63 ± 0.03[f]		this study			

[a] For the sake of simplicity, charges are omitted in the given reactions.
[b] POT = potentiometry, POL = polarimetry, NMR = Nuclear Magnetic Resonance, TITR = titration with EDTA.
[c] Calculated by means of protonation constants (expressed with concentrations) and activity coefficients.
[d] Suggested value by the authors. The result of the best fit is given in parenthesis.
[e] Determined for D-gulonate.
[f] Calculated by using log K_p = 3.19, log $K_{1,1}$ = 0.88 and log $\beta_{1,2}$ = 1.51.
[g] Calculated from the date given in refs. [8,10].

References

1. A. Pallagi, É. G. Bajnóczi, S.E. Canton, T. Bolin, G. Peintler, B. Kutus, Z. Kele, I. Pálinkó, P. Sipos, *Environ. Sci. Technol.*, **48**, 2014, 6604–6611.
2. M. L. Ramos, M. M. Caldeira and V. M. S. Gil, *Carbohyd. Res.,* 2000, **329,** 387–397.
3. P. A. Levene and H. S. Simms, *J. Biol. Chem.,* 1925, **65**, 31–47.
4. T. Gajda, B. Gyurcsik, T. Jakusch, K. Burger, B. Henry and J. J. Delpuech, *Inorg. Chim. Acta,* 1998, **276**, 130–140.
5. M. van Duin, J. A. Peters, A. P. G. Kieboom, H. van Bekkum, *Carbohyd. Res.*, 1987, **162**, 65–78.
6. Y. Pocker and E. Green, *J. Am. Chem. Soc.*, 1973, **95**, 113–119.
7. Z. Zhang, P. Gibson, S. B. Clark, G. Tian, P. L. Zanonato and L. Rao, *J. Sol. Chem.*, 2007, **36**, 1187–1200.
8. A. Pallagi, P. Sebők, P. Forgo, T. Jakusch, I. Pálinkó and P. Sipos, *Carbohyd. Res.,* 2010, **345,** 1856–1864.
9. Z. Zhang, S. B. Clark, G. Tian, P. L. Zanonato and L. Rao, *Radiochim. Acta*, 2006, **94**, 531–536.
10. M. Masone and M. Vicedomini, *Ann. Chim. (Rome)*, 1981, **71,** 517–523.
11. M. Vavrusova, M. B. Munk and L. H. Skibsted, *J. Agr. Food Chem.*, 2013, **61**, 8207–8214.