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Uršić, Stanko; Zorc, Branka; Pilepić, Viktor; Vikić-Topić, Dražen

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β-Methyl-D-glucofururonohydroxamic Acid, the First Sugar-Hydroxamic Acid and its Iron(III) Complexes in Solution

Stanko Uršić, Branka Zorc, Viktor Pilepić, and Dražen Vikić-Topić+*

Faculty of Pharmacy and Biochemistry, University of Zagreb, 41000 Zagreb, A. Kovačića 1, Croatia

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β-methyl-D-glucofururonohydroxamic acid sodium salt, the first sugar-hydroxamic acid was synthesized starting from γ-lactone of β-methyl-D-glucofururonoside and characterized by its physical properties and ^{13}C NMR and IR spectral data. Investigation of the coordination ability of β-methyl-D-glucofururonohydroxamic acid toward the iron(III) in solution reveals the existance of sequential complexation reactions, giving mono-, bis- and tris-(hydroxamato)iron(III) complexes. The equilibrium quotients Q_n for the formation of iron(III) complexes with β-methyl-D-glucofururonohydroxamic acid in solution and at ionic strength of 2.0 M ($NaClO_4$ or $NaNO_3$) were determined. The equilibrium quotients Q_n, defined as $Q_n = [FeL_n]^{(3-n)+}[H^+]^n/[Fe^{3+}][HL]^n$ are as follows: Q_1, 34.59 (0.34); Q_2, 206.20 (14.86) and Q_3, 0.019 (0.007). The values of λ_{max} and the molar absorption coefficient at λ_{max} in visible spectra of mono- and tris-β-methyl-D-glucofururonohydroxamato complexes are 495 nm and 1067(4) mol^{-1} dm^3 cm^{-1} and 425 nm and 2680 mol^{-1} dm^3 cm^{-1}, respectively. These figures are very similar to the corresponding values for the other aliphatic hydroxamate.

INTRODUCTION

The exceptional coordination ability of hydroxamic acids toward the iron(III) and many other metal ions is probably the most salient feature of these weak organic acids.[1] Also, hydroxamic acids represent an important class of siderophores.[2] Siderophores are low molecular weight multidentate ligands which serve as ferric ion specific chelators of biological importance. Many of the naturally occurring siderophores are

* Author to whom correspondence should be addressed.
+ Present address: Laboratory of Analytical Chemistry, National Institutes of Health, Bethesda, MD 20892, USA. Permanent address: Ruđer Bošković Institute, 41000 Zagreb, Croatia

actually hydroxamic acids. The current interest in the chemistry of hydroxamic acids[3–6] arises, along with their role as siderophores and a model system for natural siderophores, from the variety of their industrial and pharmaceutical applications.

In this work, we describe the synthesis of β–methyl–D–glucofururonohydroxamic acid and the chelation of iron(III) ion by this new hydroxamate ligand. The coordination ability of this hydroxamic acid toward the iron(III) ion and the spectral characteristics of its complexes with this ion in solution are very similar to other aliphatic hydroxymates, while the other properties of interest for the siderophore chemistry and biology remain to be the subject of further investigation.

RESULTS AND DISCUSSION

β–methyl–D–glucofururonohydroxamic acid sodium salt (**2**) was synthesized starting from γ-lactone of β-methyl–D–glucofururonoside (**1**) and characterized by its m.p.,

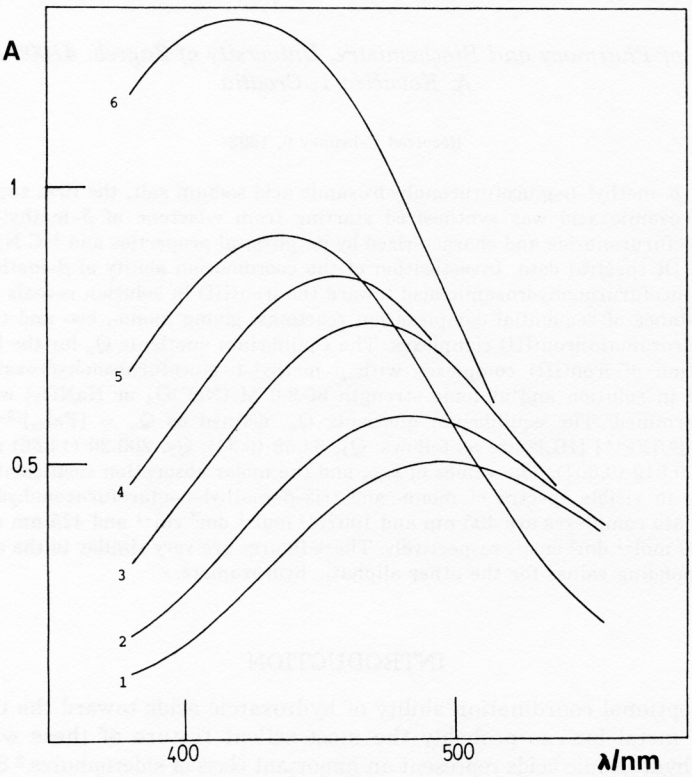
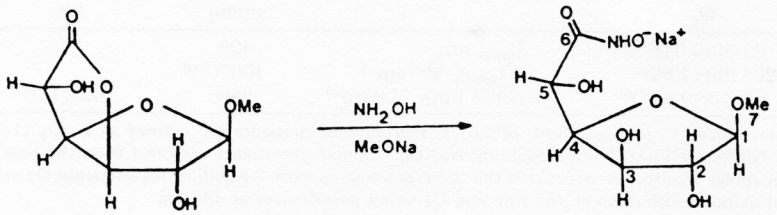


Figure 1. Spectrophotometric titration of β–methyl–D–glucofururonohydroxamatoiron(III) complexes. Conditions: **1**, $[Fe^{3+}]$ = 46.3 mM, [HL] = 1.24 mM, $[H^+](HClO_4)$ = 100 mM; **2**, $[Fe^{3+}]$ = 0.36 mM, [HL] = 1.78, pH = 2.00; **3**, $[Fe^{3+}]$ = 0.279 mM, [HL] = 1.38 mM, pH = 3.00; **4**, $[Fe^{3+}]$ = 0.245 mM, [HL] = 1.21 mM, pH = 3.99; **5**, $[Fe^{3+}]$ = 0.23 4mM, [HL] = 1.16 mM, pH = 5.03; **6**, $[Fe^{3+}]$ = 0.488 mM, [HL] = 9.76 mM, pH = 6.20 (HL = hydroxamic acid). The observed absorbance was multiplied, for clarity, by a factor that makes (seemingly) the total iron concentration equal in all spectra. Other conditions are as described in Experimental.

elemental analysis, specific rotation, ^{13}C NMR and IR spectra. ^{13}C NMR spectra of (**2**) in D_2O are consistent with the furanose type structure.[7,8] The one bond C–H coupling constant of anomer carbon atom (C–1, 173.6 Hz) corroborates the β-form, *i.e.* pseudo axial orientation of carbon-hydrogen bond.[9]



1 2

Formula 1

The synthesized hydroxamate (**2**) gives, as expected, hydroxamate complexes in water acidic solution of iron(III) ions. Examination of the pH dependence of the visible spectra of iron(III) complexes with hydroxamate (**2**) reflects the presence of sequential complexation reactions (Figure 1). At low pH values (pH ≤ 1) and in the presence of sufficient excess of ferric ions, only mono(β–methyl–D–glucofururonohydroxamato)iron(III) complex with λ_{max} at 495 nm (molar absorbance 1067 M^{-1} cm^{-1}) was formed (Figure 2). Such behaviour is characteristic of monohydroxamate, both in the position of λ_{max} and the magnitude of molar absorbance. As the pH is raised above 1.0, bis(hydroxamato-)iron(III) complex of hydroxamate (**2**) is formed, and on going to pH = 6.8 (in the presence of excess of hydroxamate ligand), the visible spectrum of the orange-yellow tris(hydroxamato)iron(III) complex exhibits the maximum absorbance at 425 nm.

Equilibrium quotients Q_1, Q_2 and Q_3 for the formation of the corresponding iron(III)-hydroxamato complexes (Eq. 1–3) were computed using the spectrophotometric titration data, and are shown in Table I.

$$Fe^{3+} + HL \rightleftharpoons FeL^{2+} + H^+ \qquad (1)$$

$$Q_1 = \frac{[FeL^{2+}]\,[H^+]}{[Fe^{3+}]\,[HL]}$$

$$Fe^{3+} + 2\,HL \rightleftharpoons FeL_2^{+} + 2H^+ \qquad (2)$$

$$Q_2 = \frac{[FeL_2^{+}]\,[H^+]^2}{[Fe^{3+}]\,[HL]^2}$$

$$Fe^{3+} + 3\,HL \rightleftharpoons FeL_3 + 3H^+ \qquad (3)$$

$$Q_3 = \frac{[FeL_3]\,[H^+]^3}{[Fe^{3+}]\,[HL]^3}$$

(HL = β–methyl–D–glucofururonohydroxamic acid)

TABLE I

Equilibrium and spectral data for β–methyl–D–glucofururonohydroxamato iron(III) complex formation

Equilibrium[a,e] quotient Q_n		Molar absorbances and λ_{max} of β–methyl–D–glucofururonohydroxamatoiron(III) complexes			
n	Q_n		mono	bis	tris
1	34.59(0.34)[b]	λ_{max}, nm	495		425
2	206.20(14.86)[c]	$\varepsilon(\lambda_{max})$, $M^{-1}cm^{-1}$	1067(4)[d]		2680
3	0.019(0.007)[c]	ε(465 nm), $M^{-1}cm^{-1}$	986	1623(13)[d]	2388

[a] In water, at 25 °C, ionic strength of 2.0 M. Equilibrium quotients are defined as in Eq. (1–3). [b] Ionic medium: $NaClO_4/HClO_4$. [c] Ionic medium: $NaNO_3$. [d] Molar absorbance obtained from the best fit. Other values for molar absorbance were from the corresponding spectra. [e] Equilibrium quotients Q_2 and Q_3 were computed using absorbances at 465 nm, and Q_1 using absorbances at 495 nm.

We have no value for the ionization constant of β–methyl–D–glucofururonohydroxamic acid. However, one may assume that its pK_a is probably similar to other aliphatic hydroxamic acids, *i.e.* about 9. Using this value, the overall formation constant for tris-(β–methyl–D–glucofururonohydroxamato)iron(III) complex may be estimated to be of the order of 10^{25} M^{-3}. The marked stability of this complex is apparent*. In addition, the solution containing this complex at pH = 6.56 (0.17 mM Fe^{3+} and 3.3 mM of hydroxamate) shows no change in either pH or absorbance for 16 hours. Solutions of mono complex at pH = 1.0 are of similar stability.

There is a question about the potential coordination of any hydroxyl group along with the hydroxamate group in chelation of iron(III) with β–methyl–D–glucofururonohydroxamic acid. It was reported that in the iron(III) complex of naturally occurring citrate-containing dihydroxamic acid schizokinen, deprotonated hydroxyl and carbonyl group of the citrate moiety are coordinated to the metal ion.[10] Ordinarily, the tendency of the hydroxyl group to ionize is markedly enhanced by coordination to metal, but the α-hydroxyl group of α-hydroxycarboxylic acids may sometimes remain protonated after coordination. Our results support the existence of the normally expected mono-, bis-, and tris–(hydroxamato)iron(III) complexes in solution. Following this observations, it seems that the coordination of hydroxyl group, for example in C–3 position, is not such a case, at least under the conditions employed. However, if coordination of the undissociated hydroxyl group had occurred, such a conclusion should have been questioned. At this stage, we have no information indicating this chelation mode of our ligand.

The β–methyl–D–glucofururonohydroxamic acid is, to our knowledge, the first synthesized hydroxamic acid with carbohydrate moiety attached to the carbon of hydroxamic functionality. It should be noted that, for over twenty years, the only really clinically useful drug for the treatment of iron overload has been a natural siderophore

* It should be noted that the equilibrium quotient Q_1 was determined using $NaClO_4$ and Q_2 and Q_3 using $NaNO_3$ to achieve the ionic strength of 2.0 M. However, although nitrate is not noncoordinating like perchlorate, it is much less coordinating than, for example, chloride. The formation constants Q_1, for mono(acetohydroxamato)iron(III) complex in 1.0 M NaCl and 2.0 M $NaClO_4$, are 28.8 and 67.7, respectively.[11,12] Therefore, there is probably no large difference between equilibrium quotients in $NaClO_4$ and $NaNO_3$.

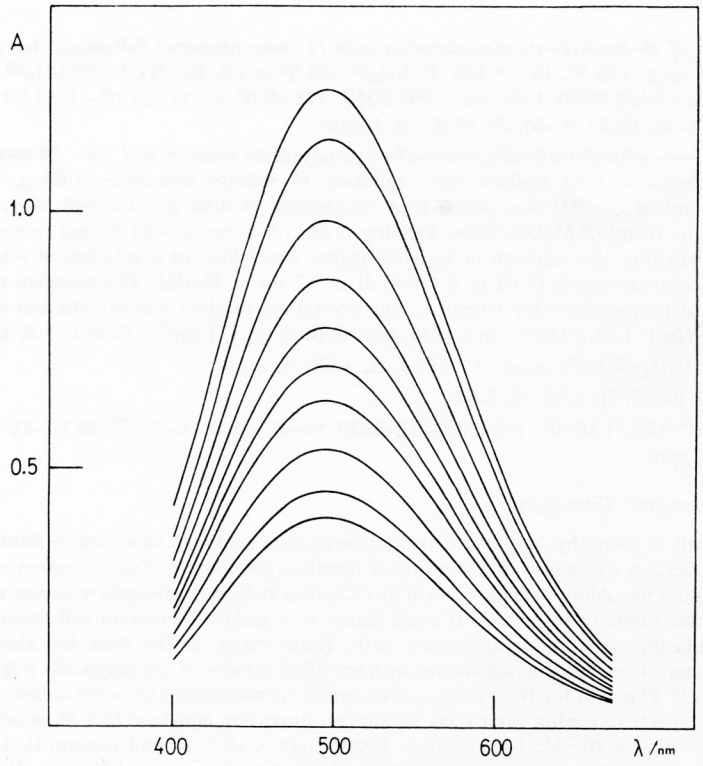


Figure 2. Spectrophotometric titration of β–methyl–D–glucofururonohydroxamatoiron(III) complexes. Total iron concentrations fall in the range 1.67–46.3 mM. $[H^+]$ ($HClO_4$) = 0.1 M. [HL] = 1.24 mM in all cases. Ionic strength = 2.0 M ($NaClO_4$ + $HClO_4$).

desferrioxamine.[1,2,13] There is a crucial need for an orally active drug for the treatment of transfusional iron overload.[13] Among others, the aim of this work is to come nearer the design of such iron chelator.

EXPERIMENTAL

Reagents and Apparatus.

All reagents were of analytical grade purity. Solvents were redistilled prior to use. D–glucurono–6,3–lactone was from Fluka. The acidity of stock iron(III) solution was determined by passing an aliquot through a cation exchange resin in the acid form and standardized using the known molar absorbance of ferric ion.[14a,b] Ionic strength was kept constant in all equilibrium experiments by adding $NaClO_4$ or $NaNO_3$. Specific rotation data were taken on the Opton polarimeter. The pH measurements were made with a Corning digital pH meter equipped with an Orion Ross 8102 combination glass electrode filled with 2.0 M $NaClO_4$ or $NaNO_3$. The meter was standardized by three standard buffers (pH = 1.00, 4.08, and 7.00). The electronic spectra were recorded on a Pye-Unicam Sp 8–100 spectrophotometer. IR spectra were taken on a Perkin-Elmer 457 spectrometer. ^{13}C NMR spectra were recorded on a JEOL FX–90 Q spectrometer, operating in the pulse FT mode at 22.5 MHz.

Synthesis.

γ-Lactone of β–methyl—D—glucofururonoside (1) was prepared following the procedure of Owen *et al.*[15a], *m.p.* 139 °C, lit.[15a] 139 °C. $[\alpha]_D^{20}$ –60.6° (*c* 1.0, EtOH). lit.[15b] $[\alpha]_D^{23}$ –61.° (*c* 1.0, EtOH). IR(KBr): 3460, 3360, 1785 cm^{-1}. ^{13}C NMR: 178.05 (C–6), 110.27 (C–1), 84.20 (C–4), 78.67 (C–3), 77.03 (C–2), 69.81 (C–5), 56.10 (C–7) δ/ppm.

β–Methyl—D—glucofuranosideuronic—N—hydroxyamide sodium salt (2) - (β-methyl—D—glucofururonohydroxamic acid sodium salt). Solution of sodium metoxyde (1.60 g, 70 mmol of sodium in 40 ml of MeOH) was added to a suspension of 4.86 g, 70 mmol of hydroxylamine hydrochloride in 10 ml of MeOH. After keeping it for 1.5 hours at –20 °C and removing sodium chloride by filtration, the solution of hydroxylamine was added to a solution of γ-lactone of β–methyl–D–glucofururonoside (1.03 g, 5.4 mmol) in 17 ml of MeOH. The reaction mixture was kept at ambient temperature for 19 hours. The crystalline product was filtered and washed twice by methanol. Yield: 1.12 g (85%), *m.p.* 135–145 °C (decomp.). $[\alpha]_D^{20}$ –12.4° (*c* 1.0, H_2O).

Analysis: $C_7H_{12}NO_7Na$ calcd.: C, 34.29; H, 4.93; N, 5.71.

Found: C, 34.57; H, 4.89; N, 5.54.

^{13}C NMR:* 166.31 (C–6), 109.65 (C–1), 82.51 (C–4), 80.42 (C–2), 75.45 (C–3), 69.64 (C–5), 56.10 (C–7) δ/ppm.

Spectrophotometric Titrations.

Sodium salt of β–methyl–D–glucofururonohydroxamic acid was dissolved in dilute perchloric acid and this solution was added to a solution of iron(III) perchlorate. Concentration of hydronium ions was adjusted by adding perchloric acid or CO_2-free sodium hydroxyde solution to the initial sample. Samples (usually 15 or 25 ml) were placed in a jacketed titration cell maintained at 25 °C by a circulating constant-temperature bath. Refinement of the data for absorbance and hydrogen ion concentration was performed by a modified version of the originally published CORNEK program.[16] The data for the evaluation of equilibrium quotient Q_1 were collected by recording ten visible spectra keeping the hydrogen ion concentration constant (0.1 M by adding $HClO_4$, Fe^{3+} was 1.67–46.3 × 10^{-3}M, hydroxamate ligand 1.24 × 10^{-3} M and constant). Data for the other equilibrium quotients were obtained from thirty-five spectra at different pH, adjusted by addition of sodium hydroxyde solution. Antilog of the measured pH was introduced in the refinement instead of the hydrogen ion concentration, in the range of 1.38 < pH < 6.79. The molar absorbances of mono(hydroxamato)iron(III) complex were obtained from the best fit, and the molar absorbances of tris(hydroxamato)iron(III) complex were obtained from the spectra of the tris complex at pH = 6.3 (0.488 mM Fe^{3+}, 9.77 mM hydroxamate ligand) and pH = 6.7 (0.394 mM of Fe^{3+}, 32.80 mM of hydroxamate). In both cases, the maximum absorbance was at 425 nm, and the values of molar absorbances were 2667 and 2680 $mol^{-1}dm^3cm^{-1}$, respectively.

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* In fact, these data should be related to the anion of β–methyl–D–glucofururonohydroxamic acid, which is present at least 99% in 0.2 M solution of (**2**) in deuterium oxide (taking pK_a of hydroxamic acid to be about 9, as usual for aliphatic hydroxamates).

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SAŽETAK

β-metil-D-glukofururonohidroksamska kiselina, prva hidroksamska kiselina izvedena od šećera i njezini kompleksi sa željezom(III) u otopini

Stanko Uršić, Branka Zorc, Viktor Pilepić i Dražen Vikić-Topić

Sintetizirana je natrijeva sol β-metil-D-glukofururonohidroksamske kiseline, polazeći od γ-laktona β-metil-D-glukofururonozida, te karakterizirana fizičkim svojstvima, ^{13}C NMR i IR spektrima. Ispitana je koordinacijska sposobnost β-metil-D-glukofururonohidroksamske kiseline prema ionu u otopini željeza(III). Utvrđeno je da u tri uzastopne reakcije nastaju mono-, bis- i tris(hidroksamato)željezo(III) kompleksi. Ravnotežni kvocijenti Q_n za stvaranje kompleksa željeza(III) s β-metil-D-glukofururonohidroksamskom kiselinom u otopini pri ionskoj jakosti 2,0 mol/L, definirani kao $Q_n = [FeL_n]^{(3-n)}\,[H^+]^n/[Fe^{3+}][HL]^n$ iznose Q_1 = 34.59 (0,34); Q_2 = 206,20 (14,86); Q_3 = 0.019 (0.007). Ti kvocijenti, kao i vrijednosti molarnih apsorbancija za pojedine komplekse slični su odgovarajućim vrijednostima za alifatske hidroksamate.