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# Spectroscopic characterization and thermal behavior of two sugar α-amino acids

M.A. Díaz-Díez [a,*], F.J. García-Barros [b], A. Bernalte-García [b], C. Valenzuela-Calahorro [b]

[a] *Departamento de Química Analítica y Electroquímica, Facultad de Ciencias, Universidad de Extremadura, 06071 Badajoz, Spain*

[b] *Departamento de Química Inorgánica, Facultad de Ciencias, Universidad de Extremadura, 06071 Badajoz, Spain*

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**Abstract**

The carbohydrate α-amino acids, 2-amino-2-deoxy-D-glycero-D-talo-heptonic acid and 2-amino-2-deoxy-D-glycero-L-gluco-heptonic acid, have been characterized by means of both spectral (IR) and thermal (TG and DSC) methods. The acid–base characteristics of the amino acids have been studied at 298 K and ionic strength $I = 0.1$ M in aqueous solution.

*Keywords:* Amino acid; DSC; HGa; HMa; IRS; TG

## 1. Introduction

It is known that among the compounds that inhibit glycoprotein synthesis there is a great number of polyhydroxylate substances that also possess nitrogen atoms [1] and which, owing to their molecular structure, could behave as polydentate ligands to form five- or six-membered ring metallic chelates. If the molecular structures of glycoproteins and the inhibitors referred to are considered, it is possible that competition between protein and inhibitor may arise in the presence of metal cations in order to combine with the cation and thus form complexes which

* Corresponding author.


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would allow its possible mechanism of action to be developed. If such coordination is necessary for the glycoprotein to act on a biological level, its action will not be developed in the case where the inhibitor shows greater affinity than the protein at the moment of coordinating with the metal ion, and vice versa.

Glycoproteins are macromolecules and therefore present low solubility both in water and non-polar solvents. For this reason, they are not easy to study. However, it is possible to obtain information on the interaction between such cations and the monomer glycoaminoacids that make up glycoproteins.

These reasons seemed sufficiently interesting to initiate a study of the interaction between glycoaminoacids and cations of some of the transition elements. In this paper, results are reported on the behavior of 2-amino-2-deoxy-D-glycero-D-talo-heptonic acid (HMa) and 2-amino-2-deoxy-D-glycero-L-gluco-heptonic acid (HGa).

## 2. Experimental

2-Amino-2-deoxy-D-glycero-D-talo-heptonic acid (HMa) and 2-amino-2-deoxy-D-glycero-L-gluco-heptonic acid (HGa) were prepared according to the method of Galbis et al. [2] and recrystallized twice from double-distilled water.

For potentiometric titrations, sodium hydroxide stock solutions were prepared with freshly boiled water previously saturated with purified nitrogen. They were standardized against potassium hydrogen phthalate and used as titrant. Stock solutions of ligands were stored under a nitrogen blanket and they were conditioned in $NaClO_4$, which was used to achieve an ionic strength of 0.1 M.

All the remaining chemicals proved to be sufficiently reliable to be used without further purification.

### *2.1. Apparatus and measurements*

Chemical analyses of C, H and N were performed by means of microanalytical methods using a Perkin-Elmer 240C microanalyzer.

Thermoanalytical data were obtained from TG, DTG, and DSC curves. These were recorded on a Mettler TA-3000 system with a Mettler TG 50 thermobalance and a Mettler DSC 20 differential scanning calorimeter. The atmospheres used were air (flow, 200 ml $min^{-1}$) or nitrogen (flow, 200 ml $min^{-1}$; purity, 99.99% v/v) for TG and DSC runs. The heating rate was 10°C $min^{-1}$ with sample weights around 10 mg for TG and 3 mg for DSC.

Infrared (IR) spectra were obtained from KBr pellets (4000–450 $cm^{-1}$) using a Perkin-Elmer FT-IR 1720 spectrophotometer.

Potentiometric measurements were made at $25 \pm 0.1$°C with a Radiometer TTT80 titrator with an ABU80 autoburette and a pHM82 pH-meter with G2040C and K4040 glass and calomel electrodes, respectively. The pH measured was converted to hydrogen ion concentration by the method of Irving et al. [3]. Nitrogen was bubbled through the samples to ensure the absence of oxygen and carbon dioxide.

### 2.2. Data treatment

In order to ascertain the ionization constants, numerical calculations were performed using the general minimizing program MINIQUAD [4]. This program considered that the best set of equilibrium constants is that which minimizes the error square sum $U$

$$U = \sum_{N_p} (E_{cal} - E_{exp})^2 \quad (1)$$

where $N_p$ represents the number of experimental points, $E_{cal}$ is the e.m.f. calculated by the computer and $E_{exp}$ is the experimental e.m.f. value. The values obtained were subsequently refined by the SUPERQUAD program [5] with $\sigma_E = 0.3$ (mV error) and $\sigma_V = 0.005$ (volume error). All the calculations were carried out on a Sperry Computer S.O.-1771 (Computer Center, University of Extremadura).

## 3. Results and discussion

The analytical results allow the following molecular formulae to be put forward for the HMa and HGa amino acids: $C_7H_{15}NO_7$ (HMa) and $C_7H_{15}NO_7 \cdot H_2O$ (HGa), coinciding with those indicated in an earlier work [2]. HMa was seen to be particularly hygroscopic.

### 3.1. Thermal study

The thermal behavior of the HMa and HGa amino acids in air and nitrogen is identical up to about 270°C; above this, considerable differences emerge, namely pyrolysis of the sample in $N_2$ flow, and combustion in air.

In the region of temperatures up to 270°C, two clearly differentiated effects can be observed in the TG–DTG curves. The first of them (in the 70–120°C interval for HMa and 160–200°C for HGa) can be attributed to the loss of water attached by hydrogen bonding to the –OH of the glycosidic chain and which corresponds to the loss of a water molecule per mole of HGa amino acid and 0.25 water molecules per mole of HMa amino acid. These molecules of water are more strongly retained than in liquid water, because the enthalpy corresponding to such effects is 53.9 kJ $mol^{-1}$ $H_2O$ for HMa and 60.5 kJ $mol^{-1}$ $H_2O$ for HGa, greater than that of the vaporization of water, 43.6 kJ $mol^{-1}$ $H_2O$. However, the water in the HMa amino acid is eliminated more easily than that in the HGa amino acid; thus, heating in an oven to 110°C would be sufficient, confirming the analytical result that HMa is anhydrous whereas HGa is a monohydrate.

At higher temperatures, a second effect appears in the TG–DTG curve (in the 190–250°C interval for HMa and 200–240°C for HGa) which is seen as a marked inflection of the curves. This effect may be attributed to pyrolysis of the sugar chain with an irreversible loss of water. This second effect also involves, as is deduced from the DSC curves, a noticeable endothermic effect and, simultaneously with the

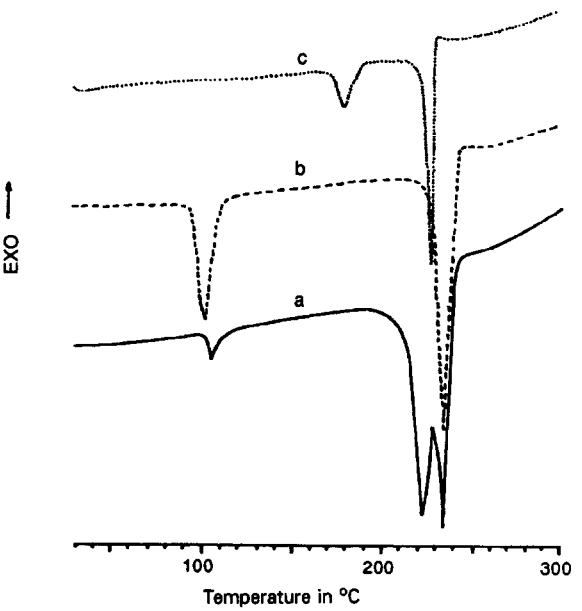


Fig. 1. DSC curves (in nitrogen atmosphere): (a) HMa; (b) "aged" HMa; (c) HGa.

irreversible loss of water, melting of the aforementioned sugar chain is produced. In addition, it can be observed (Fig. 1) that whereas this effect appears to be simple for HGa, for HMa it appears split into two effects, which could be interpreted as the –OH groups in the HMa chain not being equivalent from an energy point of view at the moment of their pyrolysis/elimination. However, this splitting was not observed when the DSC of aged HMa was recorded about a year later.

In view of this, the TG–DTG and DSC of both the "aged" amino acids were recorded again. For HGa, no difference was observed. For HMa, in addition to the change mentioned above, an increase is detected in the amount of water corresponding to the first effect, passing from 0.25 molecules of $H_2O$/mol HMa to a molecule of $H_2O$/mol of amino acid, an amount identical to that of the HGa amino acid. This suggests that the crystals initially obtained from the HMa amino acid were in a metastable phase, with a small amount of water and differentiation of the –OH groups in the glycosidic chain; with time and due to the absorption and greater presence of water in the crystalline structure, this phase evolves by molecular regrouping to another more energetically stable phase in which there is no differentiation between the –OH groups.

### *3.2. Infrared spectra*

In the 3400–2700 $cm^{-1}$ range, HMa shows two bands (3408 and 3338 $cm^{-1}$) and a shoulder (3233 $cm^{-1}$) assigned to $\nu$(O–H). These values are relatively low to be assigned to free hydroxyl groups. Thus, the bands may correspond to stretching

vibrations belonging to hydroxyl groups that interact by means of intermolecular hydrogen bonds; however, the value at which the shoulder appears suggests that the said hydrogen bond must be intramolecular between two hydroxyl groups situated in adjacent carbons presenting a "gauche" or slanted spatial conformation, a five-membered ring being formed. This is important in the study of amino acids derived from sugars, because these bonds are more stable than the corresponding intermolecular ones and they are formed at their expense. Furthermore, the intramolecular bonds reduce intermolecular association and consequently, they possess effects contrary to the intermolecular hydrogen bond on the physical and chemical properties that depend on these associations (melting and boiling points, solubility, etc.).

Because the band intensities are greater than that of the shoulder, it is reasonable to think that most hydroxyl groups are seen to form intermolecular hydrogen bonds, whereas a minority must form intramolecular hydrogen bonds. Likewise, these data suggest that there are no free hydroxyl groups but that all of them are associated through one kind of bond or another by a hydrogen bonding.

In the case of HGa, two strong bands appear situated at 3435 and 3148 $cm^{-1}$; the first is assignable to free –OH groups and the second could correspond to stretching vibrations in hydroxyl groups with intramolecular hydrogen bonds.

The presence of strong intramolecular bonds in the HGa amino acid, justified in view of the arrangement of the –OH groups in the galactose chain, could explain the fact that this compound is much less soluble in water than HMa, in which the hydroxyl groups, most of them interacting in the solid phase by means of intermolecular hydrogen bonds, can be solvated more easily.

The stretching vibration $\nu(NH_3^+)$ could be attributed to the peaks at 3124 $cm^{-1}$ (HMa) and 3065 $cm^{-1}$ (HGa). This assignment seems to be confirmed by the fact that such peaks do not appear in the complexes of these amino acids with metal ions, where the amino group seems to be in the form $-NH_2$.

In the range between 1700 and 1300 $cm^{-1}$, bands ($\nu(COO^-)$, $\delta(NH_3^+)$, etc.) are found which correspond to the bonds whose energy can be affected to a considerable degree by the coordination of the ligands to a metal ion. The major bands observed in this region are summarized in Table 1.

From the study of the bands in this area it may be concluded that the behavior of the HMa and HGa amino acids is similar to that of other α-amino acids, although HMa shows a $\nu$(C–O) band at 1389 $cm^{-1}$, a value which is unusually low.

Table 1
Major infrared data ($cm^{-1}$)

| Assignment | HMa | HGa | Assignment | HMa | HGa |
|---|---|---|---|---|---|
| $\delta_d(NH_3^+)$ | 1636 | 1622 | $\nu_a(COO^-)$ | 1599 | 1590 |
| $\delta_s(NH_3^+)$ | 1517 | 1543 | $\nu_s(COO^-)$ | 1389 | 1403 |
| | | | $\Delta\nu$ | 210 | 187 |

When the amino acid spectra are compared with those of the corresponding carboxylic acids, a strengthening of the carbon–oxygen bonds would be expected, as well as a consequent shift in the $\nu$(C=O) and $\nu$(C–O) stretching vibration bands to higher wavenumber values. However, in most cases this shift is not observed and this seems to be due to the existence of different interactions among the amino acid molecules. Of all the different kinds of interactions (electrostatic, hydrogen bond, hydrophobic or Van der Waals), it seems that in the case of $\alpha$-amino acids the most important one is the electrostatic interaction, also described as a reinforced ionic bond, in which the ionic bond between the $C–O^-$ group and the $NH_3^+$ group is strengthened with a significant covalent contribution [6]. This covalent contribution must lead to a weakening of the $C–O^-$ bond and, therefore, to a shift of $\nu$(C–O) to lower wavenumbers.

This relatively anomalous behavior of HMa, when $\nu$(C–O) is shown at such low wavenumbers, could be explained by referring to the state of the hydroxyl groups in the mannose chain, which participate mainly in intermolecular hydrogen bondings. These bonds will give rise to a greater closeness between the carboxylate and amine groups of contiguous molecules, which strengthens the reinforced ionic bond (optimum distance between 2 and 3 Å), both in its ionic part as well as the hydrogen bond. As we have already stated, the result of this strengthening is a clear decrease in $\nu$(C–O) and a certain increase in the value of $\nu$(C=O), giving rise to a marked increase in $\Delta\nu$. In the case of the HGa amino acid, the interaction between the chains is not so enhanced, a value of $\Delta\nu$ being obtained which is only slightly greater than that generally found for the $\alpha$-amino acids [7].

All the assignments suggest that amino acids exist in a solid phase, like dipolar ions (zwitterions), in which the hydroxyl groups from the sugar chain interact through hydrogen bonds that are fundamentally intermolecular.

### *3.3. Acid–base character*

The potential biological activity of the HMa and HGa amino acids is what led us to study their behavior in an aqueous medium as ligands with relation to metal cations. Because the complex formation reactions may be considered, in most cases, as acid–base reactions, it is clearly interesting to carry out a study of the acid–base behavior of each of the amino acids, HMa and HGa, in an aqueous solution.

In the present work, the study in question was carried out by means of potentiometric methods. The titration curves for the HMa and HGa amino acids were analyzed using the MINIQUAD and SUPERQUAD programs [4,5], models of dissociation processes being established in which the possible existence of anionic ($Ma^-$, $Ga^-$), neutral (HMa, HGa) and protonated ($H_2Ma^+$, $H_2Ga^+$) species was considered. The experimental results show that only the species $Ma^-$ and HMa (or $Ga^-$ and HGa) are present in the aqueous solution of these amino acids. The values obtained for the formation constants of HMa and HGa ($\beta_{11}$) by means of the above-mentioned programs are given in Table 2, where the values for $pK_a$, $\sigma$, $R$ and $\chi^2$ are also shown. In this table, it can be observed that both calculation methods lead to practically similar $\beta_{11}$ values.

Table 2
Formation constants ($\beta_{11} \times 10^{-8}$) and $pK_a$ of HMa and HGa (0.1 mol $dm^{-3}$ ($NaClO_4$), $T = 298$ K)

| Program | | HMa | HGa |
|---|---|---|---|
| MINIQUAD | $pK_a(\sigma)$ [a] | 8.84(2) | 9.27(2) |
| | $\beta_{11} \times 10^{-8}$ | 6.92 | 18.51 |
| | $R$ [b] | 0.0023 | 0.0035 |
| SUPERQUAD | $pK_a(\sigma)$ [a] | 8.87(2) | 9.26(1) |
| | $\beta_{11} \times 10^{-8}$ | 7.41 | 18.20 |
| | $\chi^2$ | 11.29 | 12.42 |

[a] Standard deviations given by the programs. [b] $R$ is the Hamilton $R$-factor calculated by MINIQUAD.

It is worth pointing out that the $pK_a$ value for HMa is unusually low (8.84) as compared with that of other amino acids [8], which may be a result of the formation of intermolecular hydrogen bonds which bring the molecules closer together with a resulting weaking of the N–H bond on interaction between the hydrogen atom of the latter and the oxygen atom of a carboxylate group of another contiguous molecule.

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