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EXCESS THERMODYNAMIC PROPERTIES OF AQUEOUS SOLUTIONS OF L-FUCOSE AND L-RHAMNOSE AT 25°

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ABSTRACT

The excess thermodynamic properties of aqueous solutions of L-fucose and L-rhamnose have been determined by means of microcalorimetry and isopiestic gravimetry. The data suggest that solute–solute interactions are unfavoured in binary solutions of these hydrophilic compounds, the solute–solvent interactions being predominant. These results lead to the conclusion that the specific function that the corresponding monomeric units seem to have, in some cases, in the reciprocal recognition of polysaccharide chains requires the presence of complexing ions (*e.g.*, Ca^{2+}) and/or specific conformations of the segments of the chains involved in the process.

INTRODUCTION

The excess thermodynamic properties of aqueous solutions of mono- and oligosaccharides and their derivatives have, at present, been little studied. However, there are many data regarding the limiting properties of these solutions[1–4]. Nevertheless, detailed knowledge of the properties of concentrated and moderately concentrated solutions of these substances may be useful for formulating at least qualitative hypotheses on the molecular interactions occurring in solution (apart from solute–solvent). Moreover, these solutions represent models that are more realistic, than those that are infinitely dilute, for studying the interactions of polysaccharide molecules or the saccharide chains of glycoproteins.

L-Fucose (**1**) and L-rhamnose (**2**) are two 6-deoxyhexoses of biological importance. L-Fucose is a constituent of tragacanth gum and can be obtained by hydrolysis of the cell walls of many marine algae[5] or of blood polysaccharides, and from mucin and glycoproteins of frog or sea-urchin spawns[6]. L-Rhamnose is present as glycosides in plant pigments, plant gums, and mucilages[6,7]. L-Fucose seems to play a specific role in the reciprocal recognition of some glycoproteins in physiological conditions (*e.g.*, in the fertilisation of *Ascidians*[8,9]). The stable forms of the two

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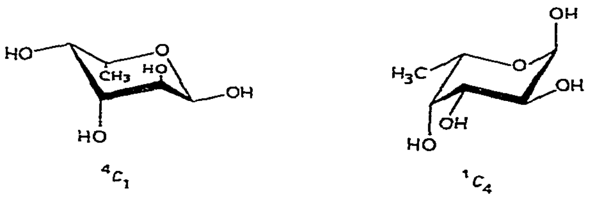
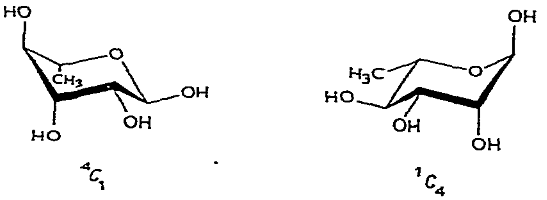
sugars in solution are pyranoid. By contrast with the hexoses and pentoses, the D isomers rarely occur in natural products.



1, α-L-Fucose



2, α-L-Rhamnose

We now report on a calorimetric and isopiestic study of binary aqueous solutions of L-fucose and L-rhamnose.

EXPERIMENTAL

α-L-Fucose and α-L-rhamnose (Sigma) were dried and used without further purification. All solutions were freshly prepared by weight before each set of experiments, using twice-distilled, de-gassed water. An LKB 2107–II2 standard batch-microcalorimeter was used. The experimental heat Q, obtained by integration and normalisation of the thermogram, was converted into the heat of dilution, ΔH^{dil} ($\mathrm{J.kg^{-1}}$ of solvent) or into the relative, molar, apparent enthalpy change $\Delta\Phi_L$ ($\mathrm{J.mol^{-1}}$) by the following relation:

$$-Q/n_2 = \Delta H^{\mathrm{dil}}(m_i \rightarrow m_f)/m_f = \Delta\Phi_L(m_i \rightarrow m_f), \qquad (1)$$

where n_2 is moles of the solute, and m_i and m_f the initial and final molalities of the solutions.

Isopiestic gravimetric measurements were performed by using an apparatus and a methodology previously discussed[10,11]. The practical osmotic coefficients were obtained from the molalities and from the known values of the osmotic coefficients of the KCl reference solutions:

$$\phi = \frac{2\,\phi_R\, m_R}{m_f}, \qquad (2)$$

where m_f is the molality of the sugar solution, and ϕ_R and m_R refer to the KCl reference solution after the equilibrium is reached. The data of Robinson and Stokes[12] were used for KCl.

THERMODYNAMICS

The excess thermodynamic properties of a binary solution of a non-electrolyte can be represented as virial expansions as a function of the molality[1,13-15]:

$$Y^E = Y - Y_w^0 - mY_x^0 = y_{xx}m^2 + y_{xxx}m^3 + \cdots, \qquad (3)$$

where Y is the thermodynamic property (free energy G, enthalpy H, entropy S, volume V, *etc.*); Y^E is the corresponding excess quantity, referred to an amount of solution containing m moles of solute and 1 kg of water; Y_w^0 is the value of the standard property for 1 kg of water; and $\bar{Y}_x$ is the limiting, partial molal function of the solute. The coefficients y_{xx}, y_{xxx}, *etc.* can be evaluated from the experimental data. Hence, the excess free-energy coefficients are obtained by interpolating the osmotic coefficients[15] ϕ:

$$RT(\phi - 1) = g_{xx}m + 2g_{xxx}m^2 + 6g_{xxxx}m^3 + \cdots \qquad (4)$$

The excess enthalpy coefficients are given by interpolation of the changes of the relative, molar apparent enthalpy[16]:

$$\Delta\Phi_L = h_{xx}(m_f - m_i) + h_{xxx}(m_f^2 - m_i^2) + \cdots \qquad (5)$$

The entropic terms are given by the differences:

$$s_{xx} = (h_{xx} - g_{xx})/T; \quad s_{xxx} = (h_{xxx} - g_{xxx})/T. \qquad (6)$$

Eq. *3* is defined at 1 atm pressure and using the molality scale. As a consequence, for the ideal solution, $g_{ii} = -M_1RT/2 \cdot 1000$ (and not zero), where M_1 is the molar mass of the solvent in kg.mol^{-1}.

According to the McMillan–Mayer approach[13,17-20], the coefficients g_{xx}, g_{xxx}, *etc.* of the excess free-energy G^E characterise the interactions between pairs, triplets, *etc.* of solute particles. For instance, g_{xx} is related to the second virial coefficient B* of the osmotic pressure:

$$\pi/kT = \rho + B^*\rho^2 + C^*\rho^3 + \cdots, \qquad (7)$$

where ρ is the number of solute particles per cubic centimeter, and to the corresponding pair-interaction coefficient e_{xx} of the excess internal energy. These two virial coefficients, B* and e_{xx}, have a well-defined mechanico-statistical significance, and they can be evaluated directly by theoretical models:

$$\lim_{\rho \to 0} B^* = \frac{-1}{2} \int_0^\infty [g(r) - 1] 4\pi r^2 dr =$$

$$= \frac{-1}{2} \int_0^\infty \{\exp[W(r)/RT - 1]\} 4\pi r^2 dr \qquad (8)$$

and

$$e_{xx} = \int_0^\infty \frac{\delta[W(r)/kT]}{\delta[1/kT]} g(r)\, 4\pi r^2 dr. \qquad (9)$$

In Eqs. *8* and *9*, W(r) is the potential of average force and g(r) is the pair correlation function for two particles whose centers of mass are separated by the scalar distance r. If the two molecules are not spherically symmetrical, the functions to be integrated must be previously averaged over all possible reciprocal orientations of the two molecules. However, the virial coefficients take into account implicitly the solute–solvent interactions and the changes of the overall state of the solvent occurring in the concentration process. Unfortunately, in aqueous solutions of polar non-electrolytes, the solute–solvent interactions are of the same order of intensity as those of solute–solute. Then, the analyses of the excess thermodynamic properties, in terms of molecular interactions, must be made with caution and looking for qualitative responses.

RESULTS AND DISCUSSION

The experimental osmotic coefficients, the molalities of the relative solutions, and the data for the isopiestic reference solutions are reported in Tables I and II.

TABLE I

OSMOTIC COEFFICIENTS OF L-FUCOSE AT 25°

m_{KCl}	ϕ_{KCl}	m_{Fuc}	ϕ_{Fuc}
0.4351	0.9004	0.7427	1.0549
0.4021	0.9014	0.7116	1.0186
0.4093	0.9011	0.7140	1.0331
0.4798	0.8993	0.8304	1.0392
0.3942	0.9018	0.6767	1.0506
0.3345	0.9047	0.5745	1.0536
0.3452	0.9042	0.5991	1.0418
0.2986	0.9067	0.5146	1.0521
0.5217	0.8985	0.8975	1.0446
0.8947	0.8971	1.4908	1.0768
1.2179	0.8988	1.9920	1.0990
1.5142	0.9028	2.4376	1.1216

TABLE II

OSMOTIC COEFFICIENTS OF L-RHAMNOSE AT 25°

m_{KCl}	ϕ_{KCl}	m_{Rha}	ϕ_{Rha}
0.7553	0.8969	1.2641	1.0718
0.5780	0.8977	0.9765	1.0628
0.9021	0.3862	0.6498	1.0723
0.4537	0.8999	0.7674	1.0640
0.2915	0.9070	0.4975	1.0631
0.3055	0.9062	0.5203	1.0640
0.8234	0.8970	1.3991	1.0558
0.6191	0.8975	1.0748	1.0340
0.5548	0.8980	0.9360	1.0646
0.8085	0.8970	1.3469	1.0768
0.4590	0.8985	0.8082	1.0203
0.3944	0.9020	0.6951	1.0237

TABLE III

HEATS OF DILUTION IN WATER OF L-FUCOSE AT 25°

m_i	m_f	$-\Delta\phi_L$ (J.mol^{-1})
1.0629	0.3191	482.21
0.3191	0.2040	75.77
1.0629	0.6794	259.69
0.6794	0.1813	340.68
1.0629	0.5214	358.62
0.2360	0.1463	64.63
0.4564	1.5200	706.90
0.4564	0.1521	209.94
0.4564	0.2916	113.64
0.9505	1.5200	363.59
0.9505	0.2849	453.95
0.9505	0.5933	233.01
0.3320	1.5200	784.29
0.2162	0.3320	82.07
0.0841	0.2657	130.61
0.1713	0.2657	67.17

The heats of dilution, as a function of the initial and final molalities, are given in Tables III and IV. The data were interpolated by a least-squares method. The coefficients obtained are reported in Table V along with their 95% confidence limits. The "best" values of the interaction coefficients are given by the polynomial expansions of highest degree whose coefficients still exceed their own 95% confidence limits. As a consequence of this criterion, it is found that, for the excess free-energy, only the second virial coefficient is significant.

TABLE IV

HEATS OF DILUTION IN WATER OF L-RHAMNOSE AT 25°

m_i	m_f	$-\Delta\phi_L$ ($J.mol^{-1}$)
1.3005	0.8152	304.7
1.3005	0.3945	566.0
0.8152	0.2553	359.8
0.8152	0.5086	194.7
1.3701	0.3978	581.9
1.3701	0.4035	601.6
1.3701	0.8039	346.3
0.4035	0.1289	182.0
0.4035	0.2494	105.3
0.8039	0.2467	360.6
0.8039	0.4510	225.0
0.6677	0.3686	198.5
0.3686	0.1993	119.0

TABLE V

VIRIAL COEFFICIENTS[a] OF THE EXCESS ENTHALPY, FREE-ENERGY, AND ENTROPY FOR AQUEOUS SOLUTIONS OF 6-DEOXY-L-HEXOSES AT 25°

	h_{xx}[b]	h_{xxx}[c]	g_{xx}[b]	$(-Ts_{xx})$[b]
L-Fucose	685 ±32	−38 ±20	140 ±34	−545
L-Rhamnose	700 ±16	−21 ±10	131 ±36	−569

[a] According to the Expansion: $Y^E = y_{xx}m_x^2 + y_{xxx}m_x^3 + \ldots$ [b] Units: J.mol^{-1} (mol.kg^{-1})$^{-1}$. [c] Units: J.mol^{-1} (mol.kg^{-1})$^{-2}$.

The data reported underline the strict similarity of the properties of the two isomers: the values of the coefficients of the excess properties are the same within the confidence limits. The positive value of the g_{xx} coefficients is remarkable for its biological implications. In fact, it is an indication of the absence of any favourable interaction (even solvent "mediated" or promoted) between pairs of L-fucose or L-rhamnose molecules in their binary aqueous solutions. It is evident from Table V that the values of g_{xx} are determined mainly by the enthalpic contributions, which prevail over the entropic contributions. This behaviour is similar to that of sugars (in particular D-xylose) and other hydrophilic molecules that interact easily with water without distortion of the geometry of the solvent[21,22]. Hence, the introduction of the methyl group on C-5 is not enough to change the overall hydrophilic character of the pyranoid ring.

The excess thermodynamic properties, as a whole, show enthalpy–entropy compensation, a well-known effect that is particularly evident in aqueous solution[23].

In the present case, the compensation is partial, as for many non-electrolytes in water, and less remarkable than for non-methylated monosaccharides[22].

The analogies between the excess thermodynamic properties of the 6-deoxyhexoses and those of other monosaccharides permit the two sets of substances to be dealt with in a similar way. All of the limiting thermodynamic properties[1-4], as well as the spectroscopic ones[24-28], show qualitatively that the aqueous solutions of sugars are controlled predominantly by the solute–solvent interactions. Some models[29,30] deal with the excess free-energy of the solutions of glucose and sucrose on the basis of a quasi-ideality approach. The unique processes in solution are assumed to be hydration equilibria, which, at increasing dilution, are shifted towards an increase of hydration. These models, however, cannot describe quantitatively other properties. Raman evidence[31] suggests an enhancement in the structure of the solvent water due to an increase of the number, more than the intensity, of hydrogen bonds, in the proximity of the sucrose molecules.

This is in agreement with the properties of the solutions of L-fucose and L-rhamnose. The signs and magnitude of the enthalpy and entropy changes in the concentration process can be qualitatively correlated with a retrogradation of the hydration. In other words, part of the hydration water in the cosphere of the solute relaxes from a less enthalpically and entropically rich state to the richer bulk-state, which determines the signs and relative values of the h, s, and g coefficients. Probably the anomeric and other interconversions between isomeric forms, as well as conformational changes (from $^4C_1$ to $^1C_4$, for instance) could contribute to the values of the excess properties. In any case, this contribution seems not to be important, given the similarity with the properties of di- and tri-saccharides, which do not experience many of these isomeric equilibria.

A quantitative interpretation of the values of the excess properties requires a detailed knowledge of the state of the hydration water on the hydrophilic and hydrophobic sites of the solute. At present, this is far from being achieved. However, the stereochemistry of the two isomeric 6-deoxyhexoses allows some hypotheses. The ratio between the axial and equatorial hydroxyl groups of the two α-anomers is 2:2, both in the $^4C_1$ and $^1C_4$ conformers. This observation can explain the strict similarity of the properties of L-fucose and L-rhamnose, since water involved in the hydrophilic hydration seems to be in a specific state, depending on the stereochemistry of the site[1,24-27]. In contrast, water involved in the hydrophobic hydration of the methyl groups, for instance, seems to be in a state independent of the stereochemistry. The compatibility with the solvent (the equatorial groups enter very easily into the structure of water) in turn prevents favourable solute–solute interactions for these solutes.

The present data suggest that a favourable interaction and a specific, reciprocal recognition between pairs of molecules or residues of L-fucose and similar compounds can occur only in the presence of other agents. These can be a complexing, bivalent, metallic ion[32] (*e.g.*, Ca^{2+}) and/or more-severe stereochemical constraints, such as those imposed by a particular overall conformation of the relevant segments of the glycoprotein chains. Moreover, as a consequence of the incorporation of the monomer in the polysaccharide chain, a conformation of the 6-deoxyhexose ring or an anomeric form can be stabilised, which is different from that prevailing for the monomer in solution.

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