Biolog. J. Armenia, *Special issue: Cyclodextrins*, 2001

THERMODYNAMIC INTERACTIONS OF MODEL ALLELOPATHIC COMPOUNDS (POLYPHENOLS) WITH α- AND β-CYCLODEXTRIN

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The present thermodynamic study is an initial attempt to check the possible efficiency of cyclodextrins in trapping allelopathic substances and therefore to reduce their phytotoxic effect. As a first choice, phenols have been chosen as models for complexation study. The equilibrium constants for the complexation of the polyphenols with α- and β-cyclodextrins have been determined. In many occasions the stoichiometry of the complex formed can also be obtained.

Данное термодинамическое исследование – начальная попытка проверить возможность продуктивности циклодекстринов в связывании аллелопатических веществ и таким образом привезти к снижению их фитотоксического эффекта. Первым выбором явились фенолы как модели для изучения комплексообразования. Определены константы равновесия для комплексообразования полифенолов с α- и β-циклодекстринами. Во многих случаях могут быть получены стехиометрии образованных комплексов.

Ներկայացված թերմոդինամիկական ուսումնասիրությունը առաջին փորձն է, ստուգելու ալլելոպատիկ նյութերի հետ ցիկլոդեքստրինների կապման արդյունավետության հնարավորությունը, որը այդպիսով կբերի այդ նյութերի ֆիտոտոքսիկ ազդեցության նվազմանը: Որպես համալիրագոյացման ուսումնասիրության մոդելներ առաջինն ընտրվել են ֆենոլները: Որոշվել են հավասարության հաստատունները α- և β-ցիկլոդեքստրինների հետ պոլիֆենոլների համալիրագոյացման համար: Շատ դեպքերում կարող են ստացվել առաջացած համալիրների ստեխիոմետրիաները:

Introduction

Cyclodextrins are cyclic oligosaccharides consisting of several glucopyranose units which are joined together by α(1-4) linkages. The natural cyclodextrins have six, seven, or eight glucopyranose units, respectively known as α-, β-, and γ-cyclodextrin. The oligosaccharide ring forms a torus with the primary hydroxyl groups lying on the narrow end of it, and the secondary hydroxyl groups of the glucose residues on the wider end. The cavity of the torus provides a relatively hydrophobic environment which allows to entrap (or complex) organic compounds [1-6]. The complexation is noncovalent and the driving force for the complex formation has been discussed in terms of van der Waal's interactions, hydrogen bonding, hydrophobic interactions, electrostatic effects, the release of high energy water molecules and of steric strains. To analyse these different contributions several families of guests have been the subject of systematic thermodynamic studies. Among them we can mention alcohols [7-9], phenols [10], acids [11,12], monosubstituted benzene

derivatives [13], surfactants [14], bile salts [15,16], amines [12,17], cyclohexane derivatives [18], phenethylamines, ephedrines [19], etc.

This ability of forming stable inclusion compounds has led to some technological applications of the cyclodextrins, as for instance in drug delivery systems, food technology, etc. [2] Probably because their relatively high prize till recently, less attention has been paid to the applications in agriculture, and related fields. A common problem for the propagation *in vitro* of some plants is that they become readily brown and do not germinate. Although the reasons are not clear, it has been related to the formation of allelopathic substances [20] in the cultive medium. To reduce the phytotoxic effect of these substances, active carbon has been used but it is rather ineffectiveness. Among the allelopathic substances we can mention naphtoquinones, coumarines, phenols and polyphenols, cinnamic acid and derivatives, etc.

Parallel to *in vitro* investigations, the present thermodynamic study is an initial attempt to check the possible efficiency of cyclodextrins in trapping allelopathic substances and therefore to reduce their phytotoxic effect. As a first choice, phenols have been chosen as models for the complexation study as they are very common. Furthermore, only one study has been published for the complexation of the polyphenols chosen here (see Figure 1 for structures), and only the equilibrium constants have been determined [21].

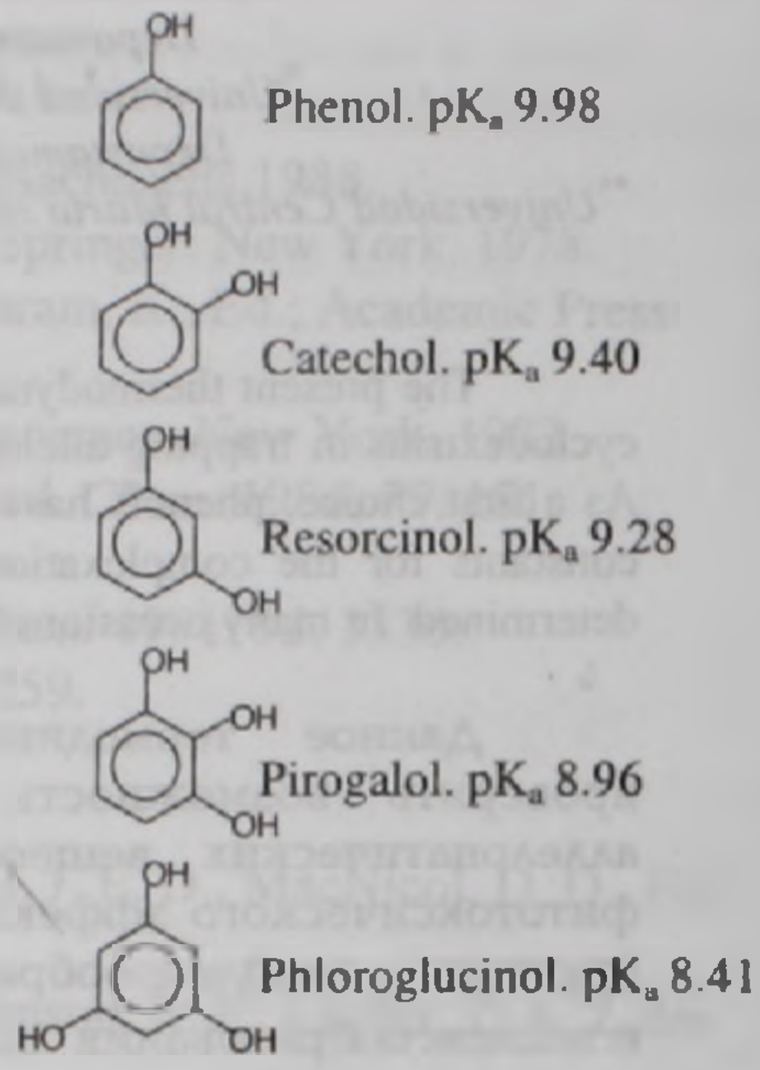

Figure 1. Structure of phenols

For the purposes of the present study, Isothermal Titration Calorimetry (ITC) has been chosen as the right experimental technique as it allows the determination of enthalpy, entropy and free energy (and the associated equilibrium constant) changes, in a single experiment. In many occasions the stoichiometry of the complex formed can also be obtained.

Calorimetry

The description of the thermodynamical background can be found elsewhere [22]. Here only the essential equations are provided.

As both host (any cyclodextrin) and guest (any phenol) have been used as titrating agents or as the sample substrates, the following nomenclature is preferred: M is the sample substrate (i.e. the reagent in the sample cell of the calorimeter), X is the titrating agent (i.e. reagent which is in the syringe) and MX is the complex formed. The association constant for the formation of a 1:1 inclusion complex (eq. 1), K can be written as in eq. 2

$$M + X \rightarrow MX \qquad [1]$$

$$K = \frac{\Theta}{(1-\Theta)[X]} \qquad [2]$$

where $\Theta = [MX]/M_t$, [X], and [MX] are the equilibrium concentrations of titrating and complex, respectively, and M_t is the initial concentration of the sample. Therefore

$$X_t = [X] + \Theta M_t$$

Combining previous equations, we arrive at

$$\Theta^2 - \Theta\left[1 + \frac{X_t}{M_t} + \frac{1}{KM_t}\right] + \frac{X_t}{M_t} = 0$$

The heat of association is

$$Q = \Theta M_t \Delta H^\circ V_0$$

where ΔH° is the enthalpy change associated to the formation of the complex and V_o is the total volume of the solution

The combination of previous equations gives

$$Q = \frac{M_t \Delta H^\circ V_0}{2}\left[1 + \frac{X_t}{M_t} + \frac{1}{KM_t} - \sqrt{\left(1 + \frac{X_t}{M_t} + \frac{1}{KM_t}\right)^2 - \frac{4X_t}{M_t}}\right]$$

The computer program Origin was used to calculate the equilibrium constant and the standard molar enthalpy of reaction from a titration experiment. The standard molar Gibbs energy of reaction, ΔG°, and standard molar entropy of reaction, ΔS°, were calculated from the measured equilibrium constant and standard molar enthalpy of reaction ΔH°, *i.e.*, by the equation

$$\Delta G^\circ = -RT \text{ Ln } K = \Delta H^\circ - T\Delta S \qquad [3]$$

The standard deviations of the thermodynamical parameters shown in Table 1correspond to the average of 4-6 independent runs, and are normally a little higher than those derived from the fit of a single experiment.

Experimental section

The phenols used in this work were purified by standard procedures. α-cyclodextrin was from Wacker and β-cyclodextrin from Roquette and were used, as well as other substances, without further purification. All solutions were freshly prepared for each experiment and degassed. Cyclodextrins and phenols were prepared in phosphate buffer 0.05 M from sodium monophosphate and sodium hydroxide. pH was checked after each calorimetric titration and no variations were observed from the initial pH. At this pH all phenols studied here are in their neutral form as the pH of 6.9 used for the experiments is well removed from the pK's of phenols (Figure 1) and therefore, the complication of having the phenols in their anionic forms is avoided. The phosphate buffer used to maintain the pH constant does not interact with cyclodextrins [19].

Enthalpies of dilution of the titrant and sample were determined in separate experiments by titrating the phosphate solution into the sample solution or by adding the

titrating reagent into phosphate solution, in exactly the same conditions of the real experiment (i.e., identical concentration of reagents, number of dilutions and volume added) and the associated calorific effects were added point by point. In a typical experiment 30 additions of 10 μL of the titrating agent (80 mM when it was a phenol or 13 mM when it was a cyclodextrin) were added over an initial volume of 1.374 mL of the sample (4 mM when it was a cyclodextrin or 1.5 mM when it was a phenol). All experiments were carried out at 303 K and at de 400 rpm.

Bruker Instruments AC300 and AMX500 were used to measure NMR spectra.

Results and Discussion

Data from a representative titration experiment (phenol 80 mM and β-CD 4 mM) are given in Figure 2. Similar curves were obtained for the rest of the systems studied here.

The shape of the titration curve [22] yields the required information concerning the thermodynamics of binding. The form is usually signalled by the dimensionless quantity C given by

$$C = K M_t$$

(K expressed in $dm^3 mol^{-1}$). For high C values (>500) almost all moles of the titrating injected into the sample cell are bond to the substrate. The curve is sigmoidal and the inflexion point correspond to the stoichiometry of the complex formed. For lower values of C, only a small fraction of the injected substrate binds to the sample and this fraction decreases with increasing in injection number. The titration plot becomes less informative and confidence in the estimates of K and enthalpy of binding decreases.

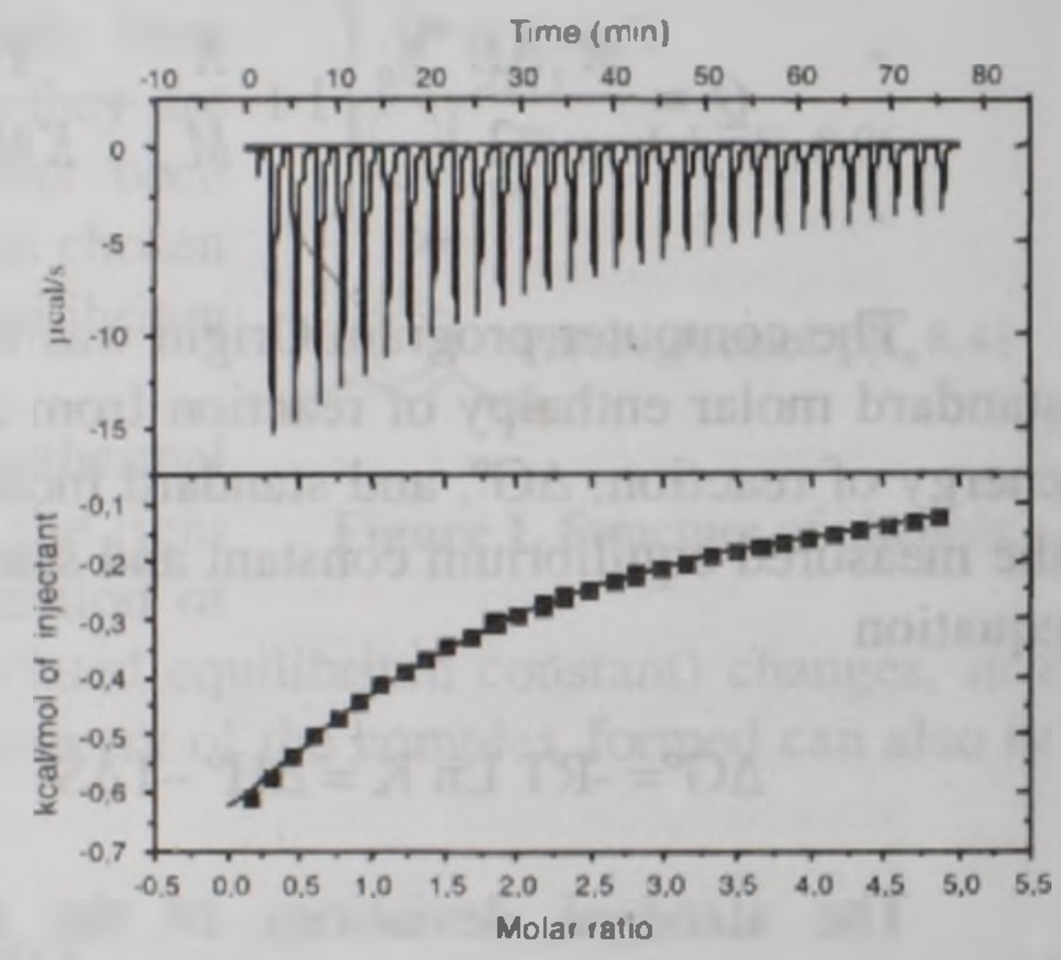

Figure 2. Calorimetric data for the titration of phenol (80 mM) into β-cyclodextrin [phosphate] = 0.05 M; pH 6.90; T=303 K. 30 injections of 10 μL.

Figure 2 clearly shows that the curve is far from being sigmoidal. By taking into account that the maximum M_t concentration used was 80 mM, it is expected that the equilibrium constant for the formation of the complex must be lower than 100 $dm^3 mol^{-1}$ (this is valid for all the phenols studied here). This is confirmed by the quantitative analysis carried out. The obtained results are presented in Table 1.

Table 1. Thermodynamic quantities for the reactions guest+cyclodextrin→complex

	β-cyclodextrin			α-cyclodextrin		
	$-\Delta H$ kJ mol^{-1}	$-\Delta S$ J $mol^{-1}K^{-1}$	K dm^3mol^{-1}	$-\Delta H$ kJ mol^{-1}	$-\Delta S$ J $mol^{-1}K^{-1}$	K dm^3mol^{-1}
Phenol	12.2 ± 0.13	5.2 ± 0.5	67.7 ± 1.1	18.4 ± 1.1	41.0 ± 3.7	10.9 ± 0.6
Catechol	17.8 ± 0.5	33.2 ± 1.7	20.4 ± 0.7	19.6 ± 5.3	54 ± 18	3.8 ± 1.1
Resorcinol	18.3 ± 0.08	24.7 ± 0.7	74.5 ± 0.6	25.0 ± 0.8	68.6 ± 2.7	5.3 ± 0.2
Pirogalol	18.6 ± 0.4	37.6 ± 1.3	17.9 ± 0.4	5.3 ± 1.0	3.2 ± 3.7	5.9 ± 1.2
Phloroglucinol	43.6 ± 1.8	112.0 ± 6.0	47.2 ± 2.3			

As can be seen from Table 1, formation of the inclusion complexes of phenols with both α- and β-cyclodextrin are exothermic driven as the enthalpies are negative and the entropies are unfavourable (negative) in all cases.

The enthalpy values in Table 1 compare favourably with those published for the complexation of p-substituted phenols with both α- and β-cyclodextrin, since values ranging from –10 (hydroquinone) to –27 kJ mol^{-1} (p-nitrophenol) have been published at pH 4.2 [10] (see Table 2). In this table, values obtained at pH > 9, where the phenols or even the cyclodextrins can be (at least partially) in their anionic forms, are omitted.

Table 2. Thermodynamic quantites for the formation of the complexes formed between p-substituted phenols and α- and β-cyclodextrin

Complex	$-\Delta H^{o}$ kJmol^{-1}	ΔS^{o} J $mol^{-1}K^{-1}$	K $dm^{3}mol^{-1}$	Reference
p-nitro–β–CD	14.9	-11.0	955	23
p-nitro-α-CD	27.1	-47	200	10
p-nitro-β-CD	13.4	-1 ± 2	260	
p-chloro-α-CD	20.1	-20	292	10
p-chloro-β-CD	11.9	10	410	
p-hydroxy-α-CD	10	-7 ± 7	24	10
p-hydroxy-β-CD	17.1	-18	113	
Phenol-α-CD	10.2	2 ± 2	37	10
Phenol–β–CD	12.2	4 ± 1	94	
p-bromo-α-CD	25.6	-31	710	10
p-bromo-β-CD	12.2	15 ± 2	860	
p-methyl-α-CD	17.7	-29	37	10
p-methyl-β-CD	12.5	4 ± 1	250	
m-nitro–β–CD	10.2	-6.3	661	23
phenol-α-CD	7.52	65	16000	24
phenol-β-CD	10.9	29	2500	

Less agreement does exist with the published values for the entropy. All the values obtained in this paper for the complexation of different polyphenols with both α- and β-cyclodextrin are negative. For the complexation of p-substituted phenols with α-cyclodextrin, the values range from 2 (phenol) to -47 (p-nitro-phenol) J mol^{-1} K^{-1}, while in the present paper values ranging from –3 (pirogalol) to –69 (resorcinol) J mol^{-1} K^{-1} have been obtained, i.e., both sets of values fall into characteristic ranges. For complexes with β-cyclodextrin Table 1 shows values ranging from –5 (phenol) to –112 (phloroglucinol) J mol^{-1} K^{-1}, while positive (from 15 J mol^{-1} K^{-1} for p-bromophenol) and negative values (-18 J mol^{-1} K^{-1} for p-hydroxyphenol) have been obtained for p-substituted phenols (Table 2) [10]. In some cases, the values are very close to zero (phenol, p-nitrophenol, p-methylphenol) with very high standard deviations. These high errors arise in part from the low values (close to zero) for the change in entropy for the complexation by cyclodextrins and by the fact that the entropy is calculated from equation 3 and therefore is greatly affected by the value used for the equilibrium constant. The value for the equilibrium constant itself is affected by a high dispersion as Table 3 illustrates for the complexation of p-nitrophenol with β-cycldoextrin for which values ranging from 130 to 1000 dm^{3} mol^{-1} have been published.

Table 3. Published Equilibrim Constants (K/ dm^3mol^{-1}) for the reaction p-nitrophenol + cyclodextrin → complex

α–CD	160		200	126		170	160	172; 204
β–CD	407	955	260	1000	130	220		185
Reference	21	23	10	24	26	27	28	29

Equilibrium constants in Table 1 are lower than those found in the literature (see Tables 2 and 4).

Table 4. Published Equilibrium constants for the formation of different complexes between the phenols studied in this paper and β-cyclodextrin

Guest	K/ dm^3mol^{-1}	Reference
Catechol, 20 °C	109	21
Resorcinol	117	21
Phenol	120; 95; 105	25
Phenol	129	26

Table 2 clearly shows that, except for pirogalol, the ΔS^o values for the complexation of polyphenols with α-cyclodextrin are more negative than those for the complexation with β-cyclodextrin. This fact is in agreement to the one observed by Rekharsky *et al* [18] for cyclohexanol, cis-1.2-cyclohexanol and trans-1.2-cyclohexanol, since negative values were observed for their complexation with α-cyclodextrin but positives ones for their complexation with β-cyclodextrin. This is also the case of cyclic alcohols (except for cyclooctanol) [12], aliphatic alcohols [7], negatively charged alkyl carboxylates and protonated amines [12], phenethylamines, ephedrines and related substances [19].

Table 1 also shows that, except for pirogalol, the ΔH^o values are more negative for the complexation of polyphenols with α-cyclodextrin than with β-cyclodextrin. As the equilibrium constants are always higher in the case of β-cyclodextrin, it is clear that the difference in ΔS^o between both cyclodextrins is the principal thermodynamic factor behind the stronger binding of these substances to β-cyclodextrin compared to α-cyclodextrin. With a few exceptions this is again in agreement to what has been observed for other systems such as cycloalcohols [12,18], phenethylamines, ephedrines, and related substances [19], and p-substituted phenols [10]. In this comparison we have only taken into account those papers in which values for the complexation of the substances with both cyclodextrins were studied. Linear alcohols are not considered as according to Masui and Mochida [7] complexation of 1-butanol, 1-pentanol and 1-hexanol with β-cyclodextrin is entropy driven (ΔH^o and ΔS^o are both positive).

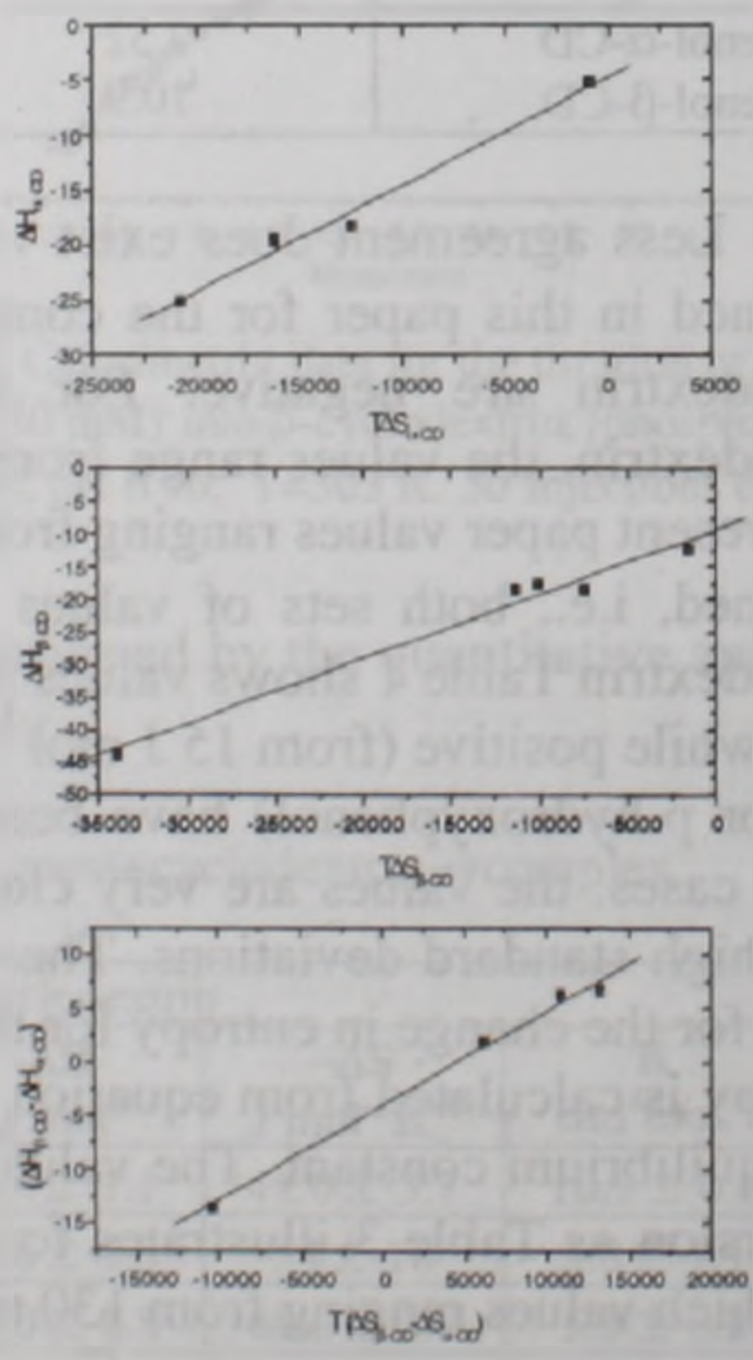

Figure 3. Enthalpy-Entropy Compensation Plot

From the entropic point of view alone, the complexation of these guests with both cyclodextrins

is unfavorable, but the entropic loss is compensated by the gain from the release of water molecules bound in and around of the cyclodextrin cavity and the guests. Highly negative values of the complexation entropy have been rationalized in terms of the decreased number of trapped water molecules that can be released upon complexation [30]. Obviously it does exist some enthalpy-entropy compensation in these complexation reactions.

Compensatory enthalpy-entropy relationships have already been observed in previous thermodynamic studies when complexing different families of compounds with cyclodextrins [10,12,14,18,19,24,30,31]. Figure 3 illustrates this relationship for the formation of complexes studied here. Slopes and intercepts are given in Table 5. Obviously, the slopes are not statistically different from 1, which means that the enthalpic gain/loss from any changes in host, guest, or solvent is perfectly canceled out by the entropic loss/gain arising from structural changes in the inclusion compound produced. This has been related to the global reorganization of the original hydrogen bond network upon complexation. Guests with hydroxyl groups, as the ones studied here, must be heavily solvated through dipole-dipole and hydrogen bonding interactions in water. The reorganization of the hydrogen-bond network in the host-guest complex has to play a significant role in the complexation [30]. This helps us to inferred the structure of the complexes, as it is commented on below.

The value equal to one observed for the slope implies that the isoequilibrium constant corresponds to the experimental temperature used in this work, *i. e.*, 303 K. This temperature is similar to other values found in the literature [10,12,18,19,30-32]. The intercept values, which correspond to hypothetical reactions without change in the entropy, are clearly negative. Close to zero values or negative ones have also been obtained by Inoue *et* al [30], Rekharsky *et al* [19], and Liu *et al* [31] for different families of compounds.

Table 5. Slope and intercept of the Enthalpy-Entropy Compensation Plots

Reaction	Intercept/ kJ mol^{-1}	Slope	r^2
guest + α-CD → α–complex	-4.8 ± 1.3	0.98 ± 0.09	0.98
guest + β–CD → β–complex	-9,4 ± 1.3	0.99 ± 0.08	0,98
α–complex + β–CD → β–complex + α–CD	-4.0 ± 0.5	0.87 ± 0.05	0.99

Following Bertrand *et al* [10] the compensation between entropic and enthalpic effect is most readily seen in terms of the exchange reaction 4, in which the effects of solvation of the free phenols in water, and the difference in solvation of the cyclodextrins is a fixed contribution for all the family of compounds.

α-complex + β-CD → β-complex + α-CD [4]

The slope and intercept of the ΔH° vs ΔS° plot are given in Table 5. The intercept is very close to the value of -3.6 kJmol^{-1} obtained by Bertrand *et al* for a set of p-substituted phenols and not far from those obtained by Rekharsky *et al* [19] (=-1.9 kJmol^{-1}) for phehethylamine, ephedrines and related substances, and cyclohexane derivatives (=-7.5 kJmol^{-1}). For primary and secondary alcohols, Rekharsky *et al* [32] have found a positive value for the intercept (4.2 kJ mol^{-1}). Again the high value for the slope indicates that the enthalpy-entropy compensation is essentially complete. The corresponding isoequilibrium temperature, 265 K is lower than those found by Bertrand *et al* [10](= 360K) or Rekharsky *et al* [12,18] but close to those obtained by Rekharsky *et al* [19,32] (= 251 K and 274, respectively).

The most frequent mode of complexation of guests to cyclodextrins consists of insertion of the hydrophobic portion of the guest into the cyclodextrin cavity while the polar groups of the ligand remain solvent exposed and near (depending on the size and structure of the guest) of the hydroxyl rims. In the present case, the hydrophobic part of the guest, *i. e.*, the phenyl ring is small enough to fit inside of both cyclodextrin cavities. On the other hand, Rekharzsky *et al* [19], have found that the OH group, provides a significant enthalpy stabilization for the ligand-cyclodextrin complex, although this stabilization can be almost cancelled by unfavorable entropy changes (see above). These authors have also found that simple additivity of the thermodynamic quantities due to the addition of an OH group to the aromatic ring only works in a few examples. Finally, in the present case the hydroxyl polar groups of the guests could form hydrogen bonds with the primary or secondary hydroxyl groups of the cyclodextrin, if the structure of the complex adopts a favorable conformation. The nature of the hydration cosphere of the guest molecule plays an important and often fundamental role in the formation of a stable inclusion complex [8] and therefore the formation and breaking of hydrogen bonds will also play an important role in the gain in entropy for water relaxed from the hydration cospheres to the bulk from both the guest and host.

On the other hand, the general tendency of the ΔH^{o} values in Table 1, clearly indicates that enthalpy change becomes more negative with the increase of the number of hydroxy groups attached to the phenyl ring. The simplest conclusion is that the formation-breaking of hydrogen bonds have to play an important role in the thermodynamics of the complexation of the polyhydroxyphenols. This is also supported by the results from Bertrand *et al* [10] who concluded that the "hydroquinone complexes seem to differ somewhat form the other p-substituted phenol complexes and that the hydroxyl group appears reluctant to enter the hydrophobic cavity of α-cyclodextrin while the strong interaction of the hydroquinone molecule with β-cyclodextrin could be due to some preferred orientation of the hydroquinone molecule penetrating the cavity and forming strong hydrogen bonds with hydroxyl groups at both the top and bottom of the cavity". Finally it is expected that, if they exist, the steric effects play a similar role all over the set of compounds.

With all these comments in mind, in order to propose a structure for the complexes, the comparison between the resorcinol and pirogalol with β-ciclodextrin plays an important role. If the hydroxyl groups of the guest remain near of the hydroxyl groups of the cyclodextrin the most obvious structure should be the one showed in Figure 4 (the structure proposed with the hydroxy phenol groups near the primary rim of cyclodextrin is supported by ^{13}C NMR and ROESY experiments commented on below). The interactions between the cyclodextrin and the hydroxy groups in relative positions 1 and 3 should be the same for both guests, while the third one in pirogalol does not interact with the β-cyclodextrin. In this way, solvent rearrangements around this third hydroxyl group should no suffer almost any change in its environment when it is transferred from the bulk solvent into the cyclodextrin cavity. At the same time, the modifications experienced by the solvent in the hydration cospheres of the other two hydroxyl groups and the apolar phenyl ring should be very similar for both phenol derivatives, and the process of squeezing out the water molecules, when the guest penetrates the cavity will be the same in both cases. The global result is that the enthalpy and entropy changes in complexation have to be very similar for both compounds as it has been observed experimentally.

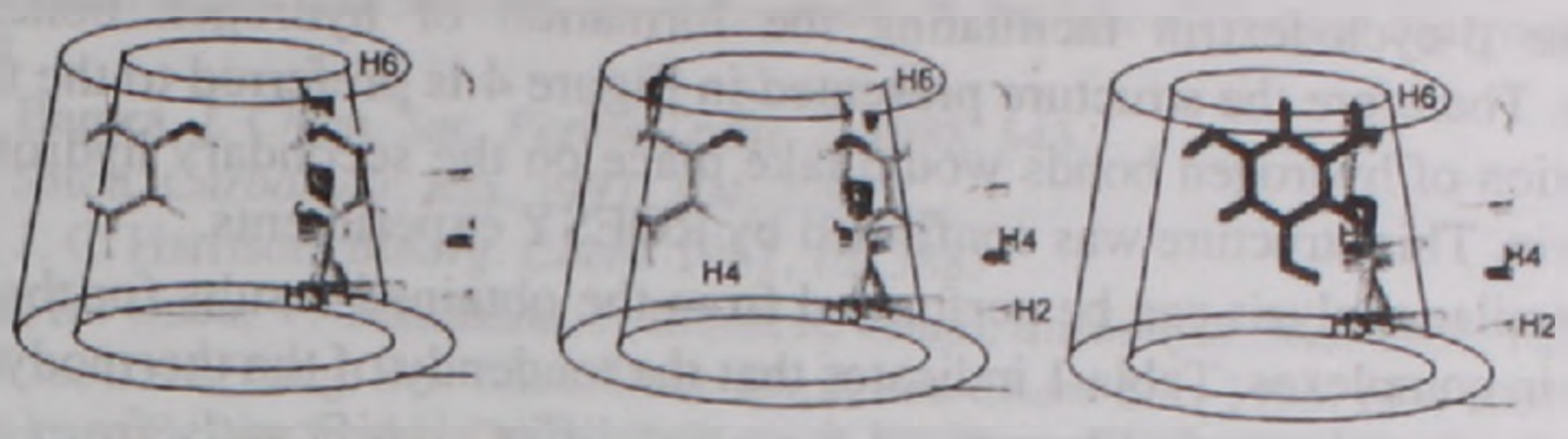

Figure 4. Schematic structures for β-cyclodextrin complexes with resorcinol, pirogalol, and phloroglucinol.

While accepting the previous structure we can propose a structure for the phloroglucinol-β-cyclodextrin complex. In this case, two of the three hydroxyl groups will present the same thermodynamic interactions as those commented on above, and the third one could form a new hydrogen bond with the other rim of the cyclodextrin in a similar way to that proposed by Bertrand *et al* for hydroquinone. Therefore, solvent rearrangements surrounding the phloroglucinol and, in less degree the cyclodextrin, must be much more important than in previous cases resulting in a much more negative entropy change. Furthermore, the phenyl ring is completely embedded into the hydrophobic cavity of the β-cyclodextrin facilitating van der Waals interactions resulting in an increase in the exothermicity of the reaction.

The similarity of the experimental thermodynamic quantities for the complexation of catechol with those of resorcinol and pirogalol (in fact, $\Delta H^{\circ}_{resorcinol} - \Delta H^{\circ}_{catechol} \approx \Delta H^{\circ}_{pirogalol} - \Delta H^{\circ}_{resorcinol}$) suggests that similar interactions and rearrangements have to be implied in the complexation process and therefore similar structures for all these complexes are expected. In this case, the structure of the guest allows the phenyl ring to enter deeper inside the cyclodextrin cavity.

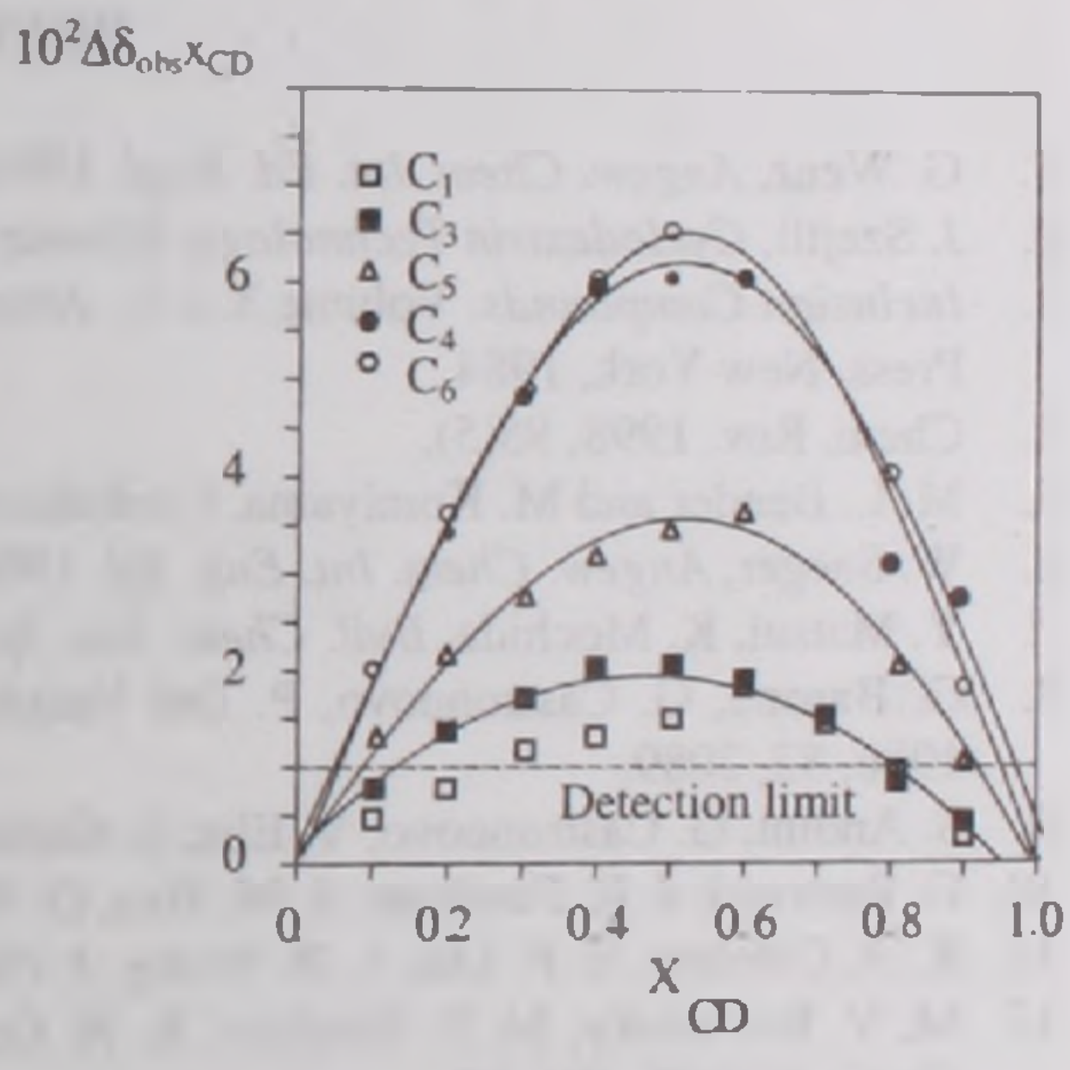

Figure 5. Job's plot corresponding to the chemical shift displacement of different carbons of β-cyclodextrin

Preliminary NMR experiments were carried out to confirm previous ideas. Figure 5 shows, in the form of a Job plot, the increments of the chemical shifts of the different carbons of the β-cyclodextrin as a consequence of the complexation of resorcinol. The maxima of the plot at $x_{\beta\text{-}CD} = 0.5$ confirm that the complex has a 1:1 stoichiometry which was used as a fixed parameter in the analysis of the experimental ITC data. These experimental data allow the determination of the equilibrium constant. However the obtained value is not shown since the standard deviation is very high as a consequence that the $\Delta\delta$ *vs* $x_{\beta\text{-}CD}$ plots are far from reaching a plateau at high β-cyclodextrin concentration, a typical finding for systems with low

equilibrium constants for the complex formation. The great chemical shift displacement for carbon 6 suggests that a strong guest-host interaction does exist near the primary hydroxyl rim of the β-cyclodextrin facilitating the formation of hydrogen bonds between both substrates. Therefore the structure presented in Figure 4 is preferred to the that one in which the formation of hydrogen bonds would take place on the secondary hydroxyl rim of the β-cyclodextrin. This structure was confirmed by ROESY experiments.

A similar analysis can be performed from the obtained results for the formation of α-cyclodextrin complexes. Table 1 indicates that the tendendy of the thermodynamic quantities in the series phenol-catechol-resorcinol does not differ significantly from the one obtained for β-cyclodextrin, the only exception being the complexation of pirogalol, since much less negative entalpy and entropy changes are observed. So in this case, it seems that the existence of a third hydroxyl group squeezes out the guest from the α-cyclodextrin cavity, resulting in a less favorable guest- α-cyclodextrin interaction and a less requirement for the rearrangement of the solvent surrounding the guest and α-cyclodextrin molecules. This hypothesis has to be confirmed by NMR experiments.

Acknowledgements. Authors thank Xunta de Galicia (Project PGIDT99PXI26201B) and CYTED (Project VIII.3) for financial support.

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