Host−Guest Interactions between β-Cyclodextrin and the (*Z*)-Phenylhydrazone of 3-Benzoyl-5-phenyl-1,2,4-oxadiazole: The First Kinetic Study of a Ring−Ring Interconversion in a "Confined Environment"

Susanna Guernelli, Maria Francesca Laganà, and Domenico Spinelli*

Dipartimento di Chimica Organica "A. Mangini", Via S. Donato 15, I-40127 Bologna, Italia

Paolo Lo Meo, Renato Noto,* and Serena Riela

Dipartimento di Chimica Organica "E. Paternò", Viale delle Scienze Parco d'Orleans 2, I-90128 Palermo, Italia

spinelli@alma.unibo.it

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The effect of β-cyclodextrin (β-CD) on the mononuclear heterocyclic rearrangement of the (*Z*)-phenylhydrazone of 3-benzoyl-5-phenyl-1,2,4-oxadiazole (**1**) in aqueous borate buffer at pH = 9.6 has been analyzed at temperatures ranging from 293.15 to 313.15 K. The trend of the absorption spectra of **1** as a function of time has been accounted for with the formation of two different 1:1 complexes between β-CD and **1**, the first, "unreactive" complex being formed faster than the "reactive" one. The occurrence of negative activation enthalpy values for the studied interconversion evidences the kinetic relevance of inclusion processes. Computational models elaborated using the MM2 molecular mechanics force field give an idea of the relative importance of the different complexes, additionally helping us to formulate a suitable reaction scheme.

Introduction

Cyclodextrins (CDs) are very promising materials in several fields: their actual or potential uses in pharmaceuticals, foods, cosmetics, or chemicals are summarized in some recent monographs.[1] Furthermore, cyclodextrins have been extensively studied as enzyme models[2] and as reagents or catalysts for regio- and stereoselective synthesis.[3]

Cyclodextrins are also widely used as hosts to form inclusion complexes with small- and medium-sized organic molecules,[4] thus dissolving compounds otherwise water insoluble. Stabilization of the complex is achieved by means of van der Waals forces, hydrogen bonding, decrease of strain energy, and release of high-energy water molecules from cavities.[5] Changes in physicochemical properties, as well as in reactivities, result from such host−guest interactions.[6] Chemical reactions pertaining to the included guest may take place, and the effects of inclusion on the reactivity vary widely depending on the guest, the CD, and the reaction examined. The CD may take part directly in the reaction interacting with the substrate through one (or more) hydroxylic groups (or their conjugate bases, depending on the pH) placed on the secondary rim.[7] In some cases, the reaction rate is greatly reduced,[8] a result that has led to the use of CDs as stabilizers; in other cases, CDs increase the rate of processes by affecting enthalpic and/or entropic factors. Finally, it must be remembered that the host CD can merely provide a confined environment that is less polar than the bulk solvent,[9] thus operating as a totally different reaction medium.

In this paper, we report the results of a kinetic study regarding the effect of β-CD on a mononuclear heterocyclic rearrangement (mhr),[10] a well-known process that allows interconversion, under fixed constrictions, of

* Corresponding author. Phone: +39 051 243218. Fax: +39 051 244064.

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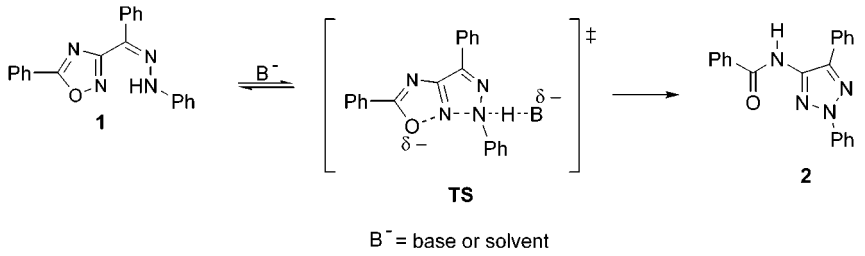
Scheme 1



heterocyclic derivatives and shows interesting applications as well as intriguing mechanistic aspects;[10] the reaction examined was the rearrangement of the (*Z*)-phenylhydrazone of 3-benzoyl-5-phenyl-1,2,4-oxadiazole (**1**) into 4-benzoylamino-2,5-diphenyl-1,2,3-triazole (**2**) in aqueous borate buffer at pH = 9.6, at different *β*-CD concentrations, and at temperatures ranging from 293.15 to 313.15 K. The course of reaction has been followed spectrophotometrically (Scheme 1).

The mechanism of the above reaction can be classified as an internal nucleophilic displacement (S_Ni) by nitrogen (the >NH group of the phenylhydrazono moiety) onto nitrogen (the N-2 atom of the 1,2,4-oxadiazole ring). Mhrs have been extensively studied in organic solvents (acetonitrile, benzene, dioxane, ethyl acetate, and methanol)[10,11] or in a dioxane−water (50/50, v/v) mixture[10,12] because of the very low solubility of substrates in water. Recently, a kinetic study of the **1** to **2** rearrangement has been carried out in the presence of a nonionic surfactant.[13] CDs, as well as micellar aggregates, are able to dissolve compound **1** in water, and this allows collection of data for an mhr in an aqueous medium. The reaction rate strongly depends on the solvent (the reaction occurring more easily in a polar than in an apolar solvent) and on the medium acidity. At $pS^+ = 11.9$ (corresponding, in dioxane−water, to a proton concentration equivalent to pH 9.6 in water), it has been found that the rearrangement of **1** occurs through a general-base-catalyzed mechanism.[10,12] As a matter of fact, data collected[12b] at various pS^+ and buffer concentrations (sodium borate−boric acid) fit well with Hammett's criterion[14] for the identification of this kind of catalysis. Therefore, in **TS** (see Scheme 1), B^- represents every base present in the reaction medium (in this case, OH^- and $H_2BO_3^-$).

Results and Discussion

Addition of **1** to a water solution of excess *β*-CD (the range of **1**:*β*-CD molar ratios from 1:2 to 1:10 has been investigated) affords a rapid solubilization of **1** (otherwise insoluble in water) with formation of an "unreactive" 1:1 complex (λ_{max} 293 and 393 nm). Then, this first complex turns into a second "reactive" one (about 150 min are required; λ_{max} 299 and 425 nm) that "slowly" (in the experimental conditions of Figure 2, $t_{1/2} > 13$ h; i.e. the time of formation of the second complex is quite lower than that necessary for the rearrangement) rearranges into **2**.

Curves at five wavelengths (in the 380−420 nm spectral region) showing the time-dependent absorption change of **1** are reported in Figure 1. As can be seen, during the first period (about 150 min, with no significant dependence on the *β*-CD concentration), the curve of absorbance as a function of time can show or not show a maximum depending on the wavelength (see, e.g., the curves registered at 420 and 380 nm, respectively). The observed behavior appears to be a consequence of the trend of the absorption spectra reported in Figure 2: such spectra present a fair isosbestic point, while the absorption maximum undergoes a moderate hypochromic and a strong bathochromic shift from about 393 to 425 nm (Figure 3).

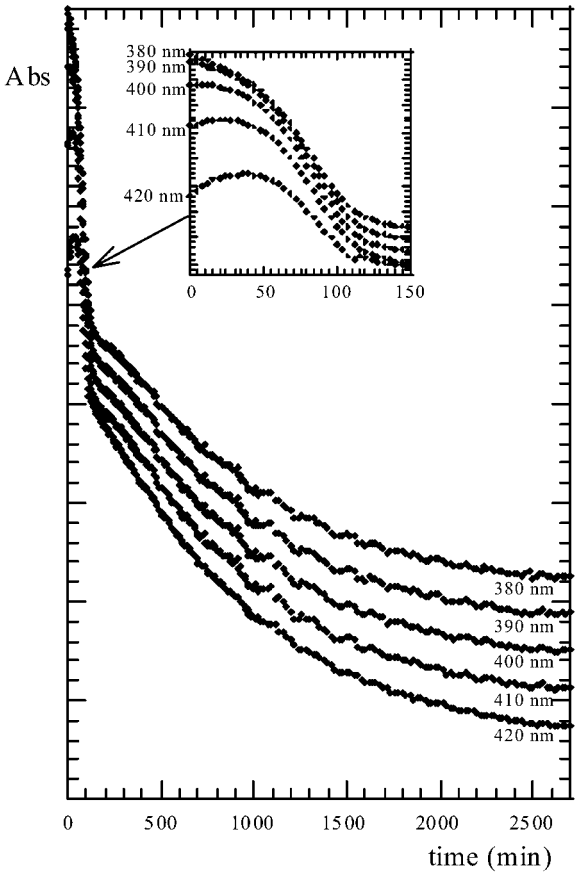


Figure 1. Absorption curves vs time for a typical kinetic run ([**1**] = 0.05 mM, [*β*-CD] = 0.5 mM, T = 313.15 K). Curves are suitably offset; an enlargement of the first period (0−150 min) is shown.

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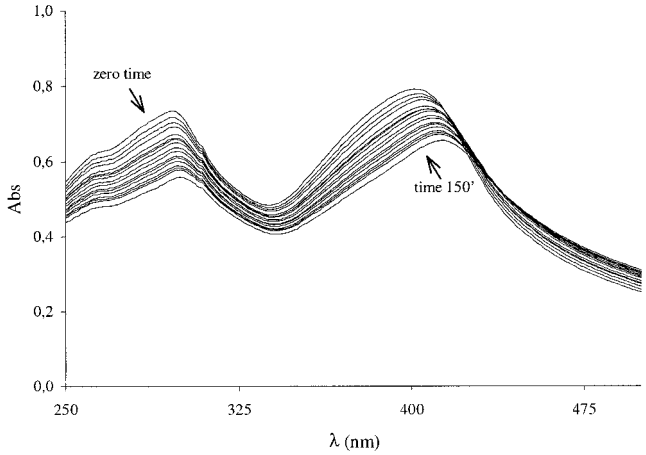


Figure 2. Absorption spectra ([**1**] = 0.05 mM, [β-CD] = 0.1 mM, T = 313.15 K): variation observed in the first period (0−150 min).

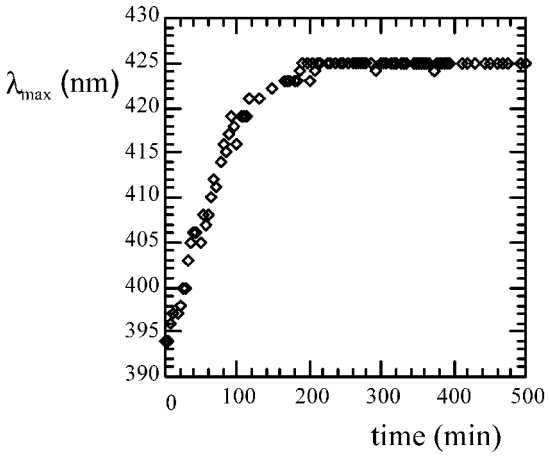


Figure 3. Plot of the absorption maximum wavelength vs time for a typical kinetic run ([**1**] = 0.05 mM, [β-CD] = 0.5 mM, T = 313.15 K).

It must be remarked that in different dioxane−water mixtures (from 20/80 dioxane−water up to pure dioxane, see Experimental Section), the absorption maximum remains unchanged at 366 ± 2 nm, while it shifts to 376 nm[11] in the less polar benzene. The shift observed in the presence of β-CD is so marked that it could be hardly related to only a simple "solvent effect" due to the inclusion into the lipophilic CD cavity; it seems rather related to some specific host−guest interactions consequent to binding.

In the second period of the reaction (hereinafter referred to as the "regular period"), λ_{max} is nearly constant (see Figure 3) and the absorption decays exponentially as expected for a simple pseudo-first-order process. In Table 1, the values for the pseudo-first-order rate constant relevant to the regular period are reported at several β-CD concentrations in the 293.15−313.15 K temperature range.

The plot (Figure 4) of k_{obs} vs β-CD concentration clearly shows a Michaelis−Menten-type trend, and curve fitting allows estimation of a value of 3×10^3 M^{-1} for the binding constant. Interestingly, the k_{obs} values determined in the presence of β-CD ($k_{obs} = 1.5 \pm 2.2 \times 10^{-5}$ s^{-1} at 313.15 K in the $0.1 \pm 2 \times 10^{-3}$ M concentration range of β-CD) are much lower than that measured in dioxane−water ($k_{obs} = 5.46 \times 10^{-3}$ s^{-1} at $pS^+ = 11.9$ and 313.15 K);[12]

Table 1. Observed Kinetic Constants and Activation Parameters for the "Regular Period"

[β-CD] × 10^3 (mol)	T (K)	k_{obs} × 10^5 (s^{-1})[a]	$\Delta H^{\ddagger}$ (kJ/mol)	$\Delta S^{\ddagger}$ (J mol^{-1} K^{-1})
0.1	293.15	13.7		
0.1	303.15	4.15	−88 ± 2	−619 ± 8
0.1	313.15	1.47		
0.3	313.15	1.68		
0.5	313.15	1.97		
0.8	313.15	2.02		
1.5	313.15	2.08		
2.0	293.15	9.25		
2.0	303.15	3.37	−56 ± 4	−514 ± 33
2.0	313.15	2.23		

[a] Data are obtained ([**1**] ≈ 0.05 mM) as mean values on at least three independent runs and are reproducible to within ±5% uncertainty.

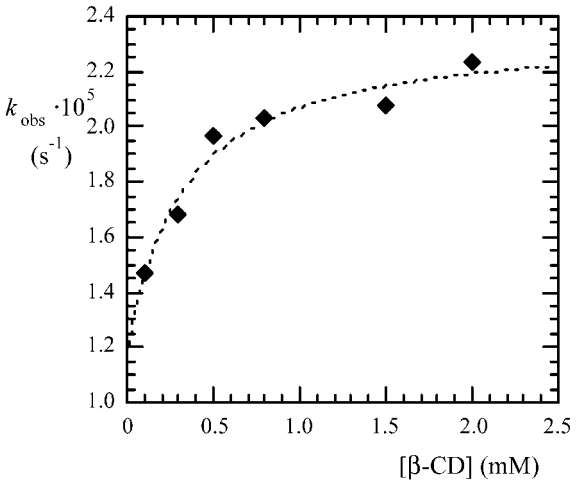


Figure 4. Plot of the observed rate constant at 315.15 K vs β-CD concentration.

this large kinetic effect (rate ratios = 230−360) can be explained on the basis of the fact that the reaction (occurring via a transition state more polar than the reagents) is disfavored by a nonpolar reaction medium such as the cavity of β-CD (see above).

The importance of this effect on an mhr can be evaluated by comparing the rate of rearrangement in dioxane−water and in benzene with bases of similar strength such as borate or phenoxide[12b] and piperidine,[11] respectively; differences in pK_a are lower than two units, accounting, on the grounds of the Brönsted relationship previously calculated by us[12b] for the mhr of **1**, for an approximately 10-fold rate-constant variation. Nonetheless, the pathways catalyzed by borate (or phenoxyde) ions in dioxane−water (50/50, v/v) or by piperidine in benzene show a much higher (>10^4) rate ratio, the reaction in the polar solvent being very strongly favored. Then, the rearrangement in β-CD occurs at reaction rates intermediate between those observed in benzene and in dioxane−water; an observation that can be accounted for considering that the cavity of β-CD has a lipophilic character that is intermediate between those of dioxane−water and benzene. Moreover, another unfavorable effect played by the formation of **1**:β-CD complexes should be the stabilization of the substrate by means of its inclusion, due to hydrogen bonding, lipophilic interactions, and so on.

A factor that could intriguingly affect the reactivity of **1** is its ability to give intramolecular hydrogen bonding

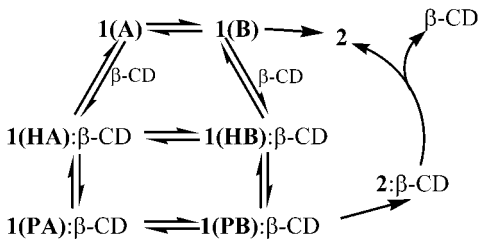
between the phenylhydrazonic proton and the nitrogen atoms of the 1,2,4-oxadiazole ring. As a matter of fact, comparing IR and ^{1}H NMR spectra of the (*Z*)- and (*E*)-isomers of the phenylhydrazone of 3-benzoyl-5-phenyl-1,2,4-oxadiazole evidences the occurrence of an intramolecular hydrogen bond in the (*Z*)-isomer in solvents such as Nujol or chloroform.[15] This observation has been confirmed also by MM2 calculations that additionally indicate that such an intramolecular hydrogen bond occurs also in the **1**:*β*-CD complex; therefore, the stabilization caused by intramolecular hydrogen bonding could be, at least in part, responsible for the "low" reactivity of **1** in the mhr herein.

Surprisingly, as evidenced by the kinetic data in Table 1, in the regular period, the rate constant increases by lowering the temperature! The analysis of Arrhenius plots at [*β*-CD] = 0.1 mM and at [*β*-CD] = 2 mM thus leads to the unexpected result of negative activation enthalpies (−88 ± 2 and −56 ± 4 kJ/mol, respectively) as well as largely negative activation entropies (−619 ± 8 and −514 ± 33 J mol^{-1} K^{-1}, respectively). These results should be compared with those for the base-catalyzed reactions in dioxane−water at the same proton concentration (i.e., at $pS^+ = 11.9$, see above), for which[12] $\Delta H^{\ddagger} = +91$ kJ/mol and $\Delta S^{\ddagger} = +1$ J mol^{-1} K^{-1}. All these apparent anomalies can be easily explained considering the preliminary formation of complexes between **1** and the *β*-CD host: the experimental activation parameters are thus composed values affected by the contribution of complexation, whose $\Delta H°$ values are usually large and negative.[5a] The increase in the $\Delta H^{\ddagger}$ value (less negative value) on going from [*β*-CD] = 0.1 mM to [*β*-CD] = 2 mM indicates that the interconversion is favored on increasing the *β*-CD concentration, i.e., the contribution deriving from the negative free energy of complexation becomes less important with respect to the free energy of reaction. Consequently, different trends for k_{obs} as a function of *β*-CD concentration have been found at different temperatures.

In our opinion, the experimental data herein account for the existence in the reaction system of at least two different host−guest complexes, the former unreactive (λ_{max} at 393 nm) and rapidly formed, and the latter reactive (λ_{max} at 425 nm) and slowly formed. As a matter of fact, the presence of an isosbestic point in the spectra of the first reaction period accounts for the slow conversion of the first adduct into another species that cannot be the product **2** (which is formed instead during the regular period and does not absorb at high wavelengths). This species may well be a second complex with the host. Actually the fast and easy dissolution of **1** in water[16] requires the fast formation of a complex; we can therefore suppose that this first complex cannot easily rearrange into **2** but turns into a "reactive form" that affords **2** during the regular period. In principle, such a "reactive adduct" could be formed directly from free **1** and the *β*-CD host, but the possibility can be rejected on the basis of spectroscopic and kinetic data.

Moreover, the formation of a reactive substrate−*β*-CD complex with 1:2 stoichiometry (**1HPB**) cannot be excluded on the grounds of the observed k_{obs} trend. Anyway, it would have the 5-phenyl group (**P**) and the phenylhydrazono moiety (**H**) into two different host cavities, the latter insertion disfavoring the rearrangement process (see below). Thus, as no decrease of the observed reaction rate was detected by increasing the *β*-CD concentration, we could definitely rule out a 1:2 stoichiometry for the reactive complex.

Scheme 2



On the other hand, direct evidence for the formation of a 1:1 complex was obtained by electrospray ionization mass spectrometry [ESIMS] experiments, a technique that can give[17] useful information on complex formation. Because of the relatively low desolvation temperature and applied voltage, the formation of weak complexes can also be detected. Thus, a feeble signal for the 1:1 (**1**:*β*-CD) complex could be observed, while no signal for a 1:2 complex could be seen even in the presence of a large excess of *β*-CD. Accordingly, the above-reported hypotheses are summarized in Scheme 2.

We also used computational models in order to get further insight into the course of the reaction. Using the molecular mechanics force field MM2,[18] we elaborated models for both the guest (**1**) and some possible complexes with *β*-CD. The isolated guest can assume two different conformations (**A** and **B**) due to rotation around the single bond between C-3 and the phenylhydrazono moiety (Scheme 3). Only conformer **B** is able to rearrange (as only in **B** is the >NH group of the phenylhydrazono moiety able to attack the N-2 atom of the 1,2,4-oxadiazole ring; see the structure of the above-reported **TS**), while calculations show that it is slightly less stable than the other one. For each conformer, at least two different host−guest complexes can be supposed, namely, with either the 5-phenyl group (**P**) or the phenylhydrazono moiety (**H**) coming into the host cavity. Steric energies for the conformers and the related complexes are reported in Table 2.

The more stable complex is calculated to be that bearing the guest in the conformation **B** suitable for the rearrangement and with the 5-phenyl ring inside the cavity and the heterocyclic ring at the rim of the cavity itself [complex **1**(**PB**)]. Thus, the inclusion in the *β*-CD cavity is able to reverse the order of stability of the conformers. Similar calculations on the triazole **2** predict that its inclusion in the *β*-CD cavity leads to complexes at least 30 kJ/mol less stable than those with **1**. Similarly, the interaction of the more stable complex of **1** with a second *β*-CD unit to form a 1:2 stoichiometric complex leads to a stabilization lower than that for the 1:1 complex itself. However, stability data give only a

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(16) In the reaction conditions, **1** is not soluble unless in the presence of *β*-CD.

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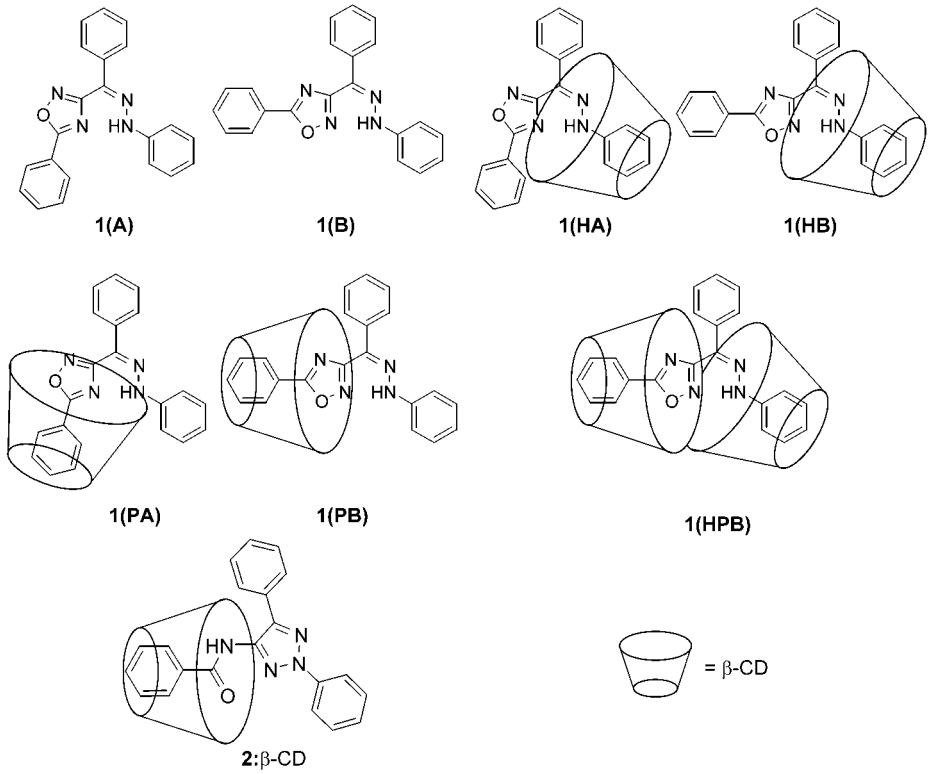
Scheme 3[a]



[a] Structures are not intended to give a precise picture of the actual minimized structures (or of how deeply the guest moieties are included) and only indicate the host−guest ineraction mode.

Table 2. Steric and Stabilization Energies (kJ/mol) for 1 and 2 and for Their Inclusion Complexes with *β*-CD

	isolated	complex **P**		complex **H**	
guest	E_{ster} (kJ/mol)	E_{ster} (kJ/mol)	ΔE_{stab}[a] (kJ/mol)	E_{ster} (kJ/mol)	ΔE_{stab}[a] (kJ/mol)
1(A)	25.0	179.3	226.2	190.8	214.7
1(B)	26.0	173.3	231.2	205.7	200.8
2	65.8	247.5	197.5		

	isolated	complex with 2 *β*-CD units	
guest	E_{ster} (kJ/mol)	E_{ster} (kJ/mol)	ΔE_{stab}[b] (kJ/mol)
1(HPB)	26.0	334.4	219.4

[a] Calculated as E_{ster}(complex) − E_{ster}(guest) − E_{ster}(*β*-CD), and keeping E_{ster}(*β*-CD) = 380.5 kJ/mol. [b] Calculated as E_{ster}(complex 1:2) − E_{ster}(complex 1:1) − E_{ster}(*β*-CD).

measure of relative concentrations of complexes and it must be stressed that the stability constant ($K = k_c/k_d$, where k_c and k_d are the rate constants for the formation and the decomposition of the complex, respectively) and rate of formation (k_c) of the complex do not necessarily follow, for different substrates, the same order; i.e., the most stable complex need not be the fastest to form. So, we think that at a first instance, the formation of the unreactive complexes [**1**(**HA**) and **1**(**HB**)] occurs, with the phenylhydrazono moiety included into the host cavity (in **H** complexes, the guest entering into the host has lower steric requirements than when entering into **P** complexes). This hypothesis is in agreement with the fact that the smaller the guest molecule, the faster the formation and the decomposition of the complex.[6a] Subsequently, the formation of complexes with the 5-phenyl group inside the cavity [unreactive **1**(**PA**) and reactive **1**(**PB**)] occurs (Scheme 3). The difference in structures between complexes **H** and **P** seems to be able to justify the different λ_{max} and log ϵ observed during the "first" period. Eventually, **2** leaves the host cavity after its formation.

Why is the **1(HB)** complex unreactive? The reason is linked to its structure, of course. MM2 calculations indicate that the >NH group of the phenylhydrazono moiety is too deeply embedded into the host, having neither sufficient space nor freedom to interact with either bases present (which, for their hydrophilicity, are not able to enter the host) and with the N-2 of the 1,2,4-oxadiazole ring to furnish **TS**. In contrast, in **1(PB)**, the >NH group is outside the host and then "free" to lead to the **TS** of the S_Ni reaction. Interestingly, the behavior in the presence of α- and *γ*-cyclodextrin seems to be significantly different and is still under investigation.

Conclusion

The ability of water solutions of *β*-CD to dissolve the (*Z*)-phenylhydrazone of 3-benzoyl-5-phenyl-1,2,4-oxadiazole (**1**) has furnished the opportunity of studying its ring−ring interconversion in water at pH 9.6 in a large range of *β*-CD concentrations.

The course of the reaction is strongly affected by the presence of *β*-CD: it shows an "induction" period, which can be related to the fast formation of a first unreactive complex [**1(HA)** and/or **1(HB)**] that allows the solubilization of **1** (whose λ_{max} shifts to 393 nm). It is then converted into a second reactive one (the λ_{max} now shifts to 425 nm), which gives the usual mhr furnishing **2**. Comparing the reactivity herein with that observed in other systems the following trend can be evidenced: k_{obs} in benzene < k_{obs} in *β*-CD < k_{obs} in dioxane−water (1:230−360:>10^4).

The plot of k_{obs} vs [β-CD] shows a typical Michaelis−Menten trend that well explains the experimental thermodynamic parameters.

The results of molecular mechanism force field MM2 calculations give support to our interpretation of spectroscopic and kinetic data.

Experimental Section

Materials. Pure (*Z*)-phenylhydrazone **1** (free from the (*E*)-isomer) was synthesized and purified according to literature methods.[12,15b] All other products were purchased and used without further purification. Samples of β-CD used for kinetic measurements were dried prior to use by keeping them for 48 h in a drying apparatus in vacuo at 90 °C over phosphorus pentoxide and stored in the same apparatus at 40 °C. Stock solutions of borate buffer at pH 9.6 were prepared and used within a few days after being tested.

Kinetic and Spectroscopic Measurements. Reaction systems were prepared by introducing with a microsyringe the suitable amount of a mother solution of the substrate in dioxane into a buffered and thermostated solution of β-CD. Spectra were recorded at due times in the range 200−500 nm. The absorbances (Abs) at 380, 390, 400, 410, and 420 nm were plotted vs time and showed a complex trend in the first period, while a simple exponential decay was observed during the regular period (see Figures 1−3). Thus, the curves related to the "second" period were processed both by the Guggenheim method[19] and by fitting them directly into the equation Abs = $a + b\exp(-kt)$, keeping *a*, *b*, and *k* as fitting parameters. We found that the calculated values for the kinetic constant *k* with the two different methods and for the five absorbance curves were all in excellent agreement within the uncertainties. KaleidaGraph 3.0.1 software (Abelbeck Software) was used to perform data processing.

The wavelength of the absorption maximum for different dioxane−water mixtures (from dioxane−water 20/80 (v/v) up to pure dioxane) was measured. Practically, the value of λ_{max} stays unchanged by decreasing the amount of dioxane ($\lambda_{max} = 366 \pm 2$ nm).

ESIMS Experiments. Spectra were recorded on a single-quadrupole mass spectrometer operating at 4000 *m*/*z*. A Hamilton syringe driven by a Harvard pump was used for direct injection of the sample into the mass spectrometer at a rate of 15 μL/min. A capillary voltage of 3.3 kV and a cone voltage of 20 V were applied for the experiments with the **1**−β-CD complex dissolved in water−acetonitrile (50/50, v/v); a desolvation temperature of 120° C was used.

Calculations. MM2 calculations were performed by means of the CS Chem3D Pro 5.0 software package (Cambridgesoft Corporation). Structures of the guest conformers were fully optimized; evaluation of the energy profile for the rotation around the single bond linking the heterocycle and the hydrazono moieties, by means of the "dihedral driver" algorithm, leads to the conclusion that the torsional barrier is ∼25 kJ/mol. Models of the guest−β-CD complexes were elaborated on the basis of the "quenced dynamics" (QD) method introduced by Lipkowitz.[20] For each complex, the MD algorithm within the Chem3D software was used to obtain a "simulation pool" (simulations were performed at 300 K for 500 ps) from which starting point models were randomly sampled and allowed to undergo geometry optimization by simulated annealing; in this way only a limited amount of energy minima are found. An energy cutoff of 0.1 kcal/mol was used for calculations.

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