Structure and Photochemical Behavior of the Cyclodextrin Inclusion Complexes of the Benzoylthiophene-Derived Drugs Tiaprofenic Acid (= 5-Benzoyl-α-methylthiophene-2-acetic Acid) and Suprofen (= α-Methyl-4-(2-thienylcarbonyl)benzeneacetic Acid)

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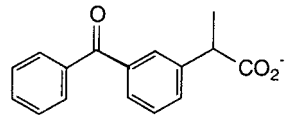
This paper is dedicated to Professor *André M. Braun* on the occasion of his 60th birthday

The effect of β-cyclodextrin (β-CD) on the excited-state reactivity of the two benzoylthiophene derivatives, tiaprofenic acid (TPA; **2**) and suprofen (SPF; **3**) in their carboxylate forms is studied. The presence of β-cyclodextrin does not affect the nature of the photoproduced transients and the photoproducts, but increases the photodegradation quantum yields of both drugs. The efficiency of the photodecarboxylation process is enhanced. This effect is rationalized in the light of the inclusion of **2** and **3** in the β-CD cavity, affecting the energy of the lowest excited states of the drugs. The structure of the complexes is determined by induced circular dichroism, and molecular-mechanics and dynamic Monte Carlo calculations. The photoreactivity of the decarboxylated photoproduct **7** of tiaprofenic acid (**2**) in presence of β-CD is also examined.

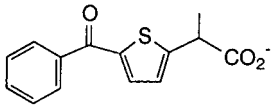
1. Introduction. – Cyclodextrins (CD) have been receiving the attention of chemists for many years because of their ability to associate within their cavities a large variety of molecules by noncovalent bonds [1]. Due to the change of environment, the physical and chemical properties of the complexed species can be strongly modified with a large spectrum of applications [2]. For example, CDs are used as carriers for the solubilization, stabilization, and increased bioavailability of the drugs and exhibit a significant potential for decreasing their local and systemic toxicity [3]. In particular, β-CD was suggested as a tool for decreasing adverse photoeffects against cell components occurring with nonsteroidal antiinflammatory (NSAID) drugs [4][5].

To elucidate the role of the cyclodextrins in the photochemistry of NSAID drugs and to rationalize the CD-induced protection effects, the photodecarboxylation mechanism of the β-CD inclusion complexes of ketoprofen (KPF; **1**) [6], suprofen (SPF; **3**) [7], and tolmetin (TM; **4**) [8] has been recently investigated by steady-state and time-resolved spectroscopic techniques. These studies showed that, in spite of the structural analogies in this series of molecules (all of them bear a benzophenone-like aromatic moiety and a substituent with a carboxy group in dissociated form at neutral pH), the effects of β-CD on the photochemical pathways are very diverse, so that the photostability of the included drug is not always improved. Inclusion of ketoprofen (**1**)

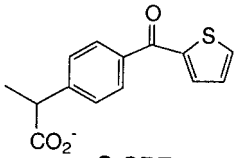
in β-CD strongly depresses the quantum yield of photodecarboxylation ($\Phi_{dc} = 0.75$ in water [9] and $\Phi_{dc} = 0.42$ in β-CD [6]). Moreover, an additional photoprocess, likely reductive, contributes to keep the global photodegradation quantum yield of the drug close to the high value of the homogeneous medium [6]. On the other hand, β-CD inclusion drastically increases the photodegradation quantum yield of suprofen (**3**) (at 298 K, $\Phi_{-SPF} = 0.076$ in neutral buffer [10] and $\Phi_{-SPF} = 0.23$ in β-CD [7]), while it decreases that of tolmetin (**4**) ($\Phi_{-TM} = 0.0062$ in H_2O and $\Phi_{-TM} = 0.0035$ in β-CD, [8]). Indeed, depending on the nature of the excited states involved and the structural features of the complexes, the β-CD macrocycle may either participate in a photoreaction as a reagent [6] or affect, *via* environmental factors, the deactivation pathways of the excited drug [8].



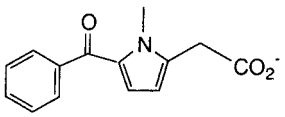
1 KPF



2 TPA



3 SPF



4 TM

In the present contribution, we report a study of the effects of inclusion in β-CD on the excited-state reactivity of some benzoylthiophene derivatives, tiaprofenic acid (TPA; **2**) and suprofen (SPF; **3**) in their carboxylate form. The photobehavior of the decarboxylated photoproduct **7** of tiaprofenic acid, *i.e.*, of DTPA, in presence of β-CD is also examined. The influence of β-CD on the photodegradation quantum yields and on the photoproduced transients of TPA (**2**) and SPF (**3**) is investigated. The structure of the inclusion complexes is determined by induced circular dichroism (ICD) and molecular-mechanics and dynamic Monte Carlo (DMC) calculations. The relationship between the excited-state reactivity and the structural features of the drug-CD associates is discussed.

2. Results. – 2.1. *Absorption and Circular Dichroism.* The absorption spectrum of TPA (**2**) ($2 \cdot 10^{-5}$ M) in 10^{-2} M phosphate buffer at pH 7.2 (λ_{max} at 266 (ε 8000 M^{-1} cm^{-1}) and 314 nm (ε 14000 M^{-1} cm^{-1}) and a weak tail extending up to 380 nm, see [11][12]) is affected by the addition of β-CD. With $5 \cdot 10^{-3}$ M β-CD, a slight hypsochromic effect (*ca.* -10%) and a shift to the blue of the maximum by 4 nm are observed. Similar changes are produced on the analogous absorption features of DTPA (**7**). These variations indicate that the aromatic chromophores experience a less polar environment, in agreement with the formation of inclusion complexes.

These species were characterized on the basis of the induced circular dichroism (ICD) spectra. The ICD signal of **2** (racemic mixture) and **7** in presence of β-CD is

positive and similar in the two compounds, in the whole absorption range (*Fig. 1, a*). The dependence of the signal amplitude on the β-CD concentration is in agreement with a complexation of 1:1 stoichiometry. The association constants were deduced by the application of the *Benesi-Hildebrand* treatment [13] (*Fig. 1, b*) and are reported in the *Table*[1]).

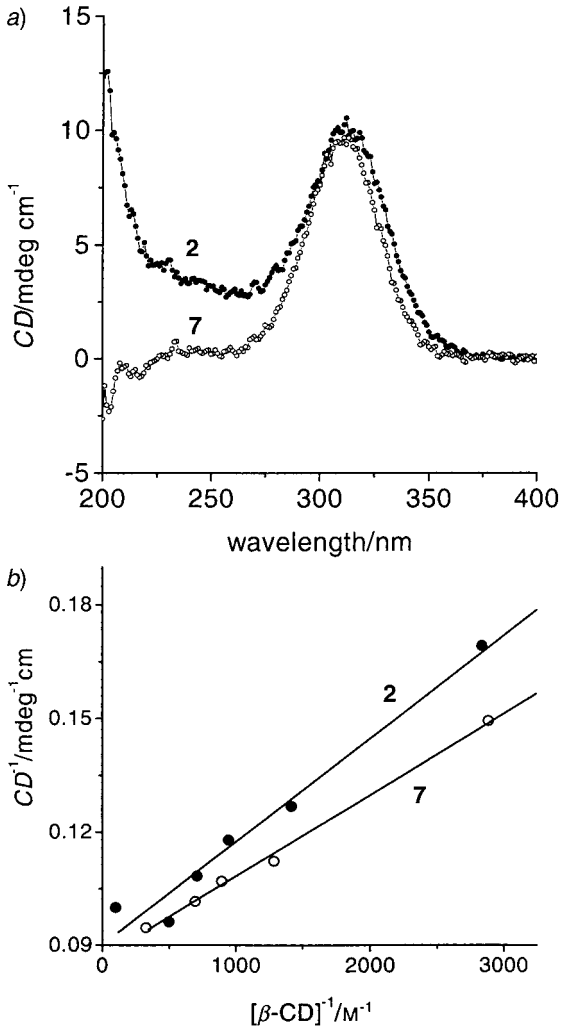


Fig. 1. a) *Induced circular dichroism (*ICD*) in $1\cdot10^{-4}$* M *TPA (***2***) and $1\cdot10^{-4}$* M *DTPA (***7***) in presence of $2\cdot10^{-3}$* M *β-CD in 0.01*M *phosphate buffer* (pH 7.2). b) Benesi-Hildebrand *plots of the* ICD *signal for* **2** (●) *and* **7** (○).

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[1]) From phosphorescence emission measurements at high concentrations of β-CD, there is evidence for the occurrence of aggregation or higher-order association phenomena. Thus, with both TPA (**2**) and DTPA (**7**), the β-CD concentration used in any experiment was $\leq 5\cdot10^{-3}$ M. Guest complexation percentages were anyway $\geq 90\%$.

Table 1. *Association Constants of SPF* (**3**), *TPA* (**2**), *and DTPA* (**7**) *Complexes with β-CD, in Neutral Phosphate Buffer* (0.01M) *at 298 K*

	SPF (**3**)	TPA (**2**)	DTPA (**7**)
K_{ass}/M^{-1}	1900 ± 200 [7]	3000 ± 500	5100 ± 200

The absorption and ICD features of the SPF-β-CD inclusion complex are similar to those of TPA, except for the long wavelength absorption λ_{max} at 300 nm, and were already described [7].

2.2. *Theoretical Calculations.* 2.2.1. *Calculation of Low-Energy-Complex Structures.* The computation of potential energies of the complexes between the guests and the host β-CD was performed by application of the block-diagonal-matrix-minimization method on an initial potential built using *Allinger*'s MM3-92 force field [14]. The fully minimized reference structure of β-CD, derived from crystallographic data [15], corresponds to 298.3 kJ/mol. The potential energy of the guest molecule is calculated as 54.6 kJ/mol and 26.1 kJ/mol for TPA (**2**) and SPF (**3**) respectively. These reference structures were used in all calculations described below.

Low-energy-complex geometries were found by applying a dynamic Monte Carlo (DMC) routine [16] within the program package MultiMize [17]. This method, combining potential energies calculated by a force field and free energies of solvation derived from continuum approximations, proved to give valuable structural results for cyclodextrin complexes in an aqueous environment [18–20]. Details of the calculation are presented in [18]. The contributions arising from solvation effects, and here mainly from hydrophobic interactions, are an important prerequisite for obtaining correct complex geometries [18].

The start geometries for DMC runs are defined by a random relative orientation of host and guest molecules within a distance of 5 Å. In each DMC step, this relative position of the host and guest is stochastically altered in the *x*, *y*, and *z* directions by a maximum of 0.5 Å, the guest molecules are rotated by a maximum of 5°, and the individual glucose units within the host molecule are also rotated by a maximum of 5°. Each stochastically generated structure is fully minimized within the force field and accepted according to the modified *Metropolis* criterion [16], *i.e.*, including both potential and solvation energies. The simulation temperature is kept constant at 300 K, and low-energy complex structures are obtained within 1000 DMC steps.

The number of accepted configurations was 543 and 507 for TPA (**2**) and SPF (**3**), with final complex energies of 336.9 and 302.9 kJ/mol respectively. A drawing of two low-energy complexes is displayed in *Figs. 2, a* and *3, a*. Both the potential energy as well as the free energy of solvation indicate a reasonable complex stability. However, TPA (**2**) is, to a certain extent, flexible in the cavity, *i.e.* a smooth complexation-energy surface allows at least a restricted dynamics of the guest in the host cavity. On the contrary, the SPF (**3**) complex is characterized by a deeper guest inclusion and more-defined equilibrium geometry.

2.2.2. *Calculation of the Induced Circular Dichroism.* To test the reliability of the geometries found, a calculation of the ICD for the generated energy minima was carried out, allowing a direct evaluation of the structural data with respect to experimental results by comparing calculated and experimentally determined ICD values. In the case of 1:1 complexes, the rotational strength arises from the interaction of the dipole

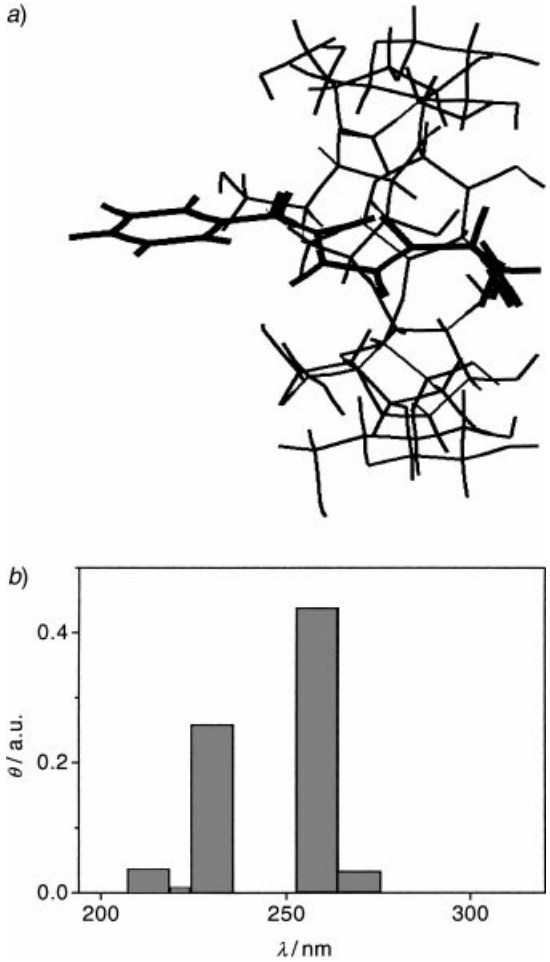

Fig. 2. a) *Ground-state conformation and* b) *calculated induced circular dichroism* (ICD) *of the TPA-β-CD inclusion complex*

transition moments of the guest excited states with those (at high energy) of the macrocycle. The pertinent expression is obtained by replacing the original dipole-dipole interaction scheme [21] in the *Kirkwood* equations with the polarizability of the bonds of the chiral macrocycle. According to this approximation, the equations of the rotatory strength for a transition $0 \rightarrow a$ are given by *Eqns. 1* and *2*, where e_{0a} and e_j are unit vectors along the transition moment μ_{0a} and parallel to the *j*-th bond, respectively; ν_{0j} and ν_a are the frequencies of the electric transitions of the host and the guest, that are located at a distance r_j, and α_{11}, α_{33} represent the bond polarizabilities at zero frequency, parallel and perpendicular to the symmetry axis of the bond *j*. In *Eqn. 1*, the energies and electric moments are calculated by a semiempirical quantum-mechanical method (CNDO/S). *GF* (*Eqn. 2*) gives the geometrical factor, which is based on the actual geometry of the β-CD – guest complex under consideration. The results, reported in

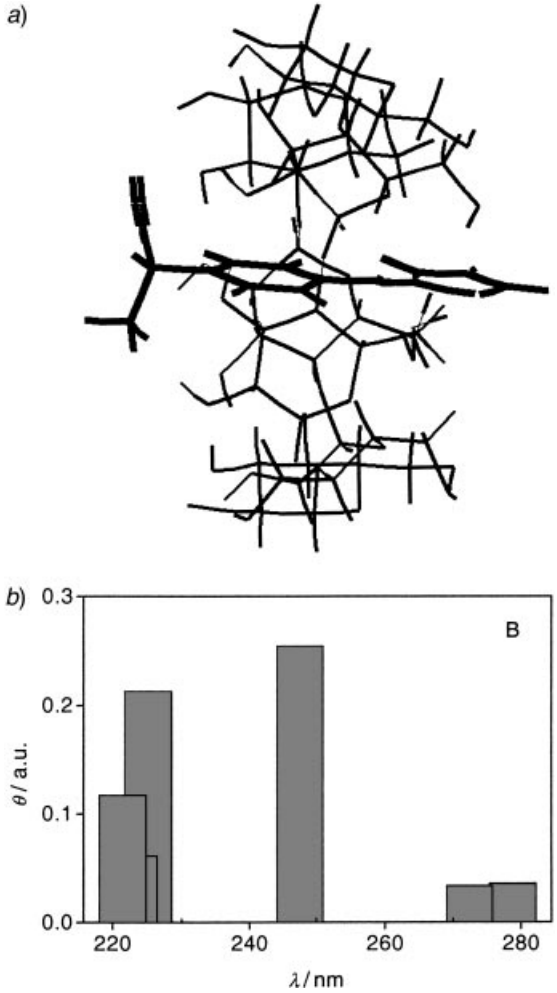

Fig. 3. a) *Ground-state conformation and* b) *calculated induced circular dichroism* (ICD) *of the SPF-β-CD inclusion complex*

Figs. 2, b and *3, b* for TPA (**2**) and SPF (**3**), respectively, show the presence of three positive contributions in the range 270–210 nm (for **2**) and 280–218 nm (for **3**), in agreement with the totally positive ICD of the complexes under examination. A totally positive spectrum is also found in correspondence with most accepted structures, indicating the formation of a steady and well-defined complex in both cases.

$$R_{0a} = \pi \nu_a \mu_{0a}^2 \sum_j \frac{\nu_{0j}^2 (\alpha_{33} - \alpha_{11})_j (GF)_j}{c(\nu_{0j}^2 - \nu_a^2)} \tag{1}$$

$$(GF)_j = \frac{1}{r_j^3} \left[e_{0a} e_j - \frac{3(e_{0a} r_j)(e_j r_j)}{r_j^2} \right] e_{0a} \times e_j r_j \tag{2}$$

2.3. *Steady-State Photolysis.* Comparative photolysis experiments were performed with $5 \cdot 10^{-4}$ M TPA (**2**) in phosphate buffer at pH 7.2 at 298 K in the absence and in the presence of β-CD ($5 \cdot 10^{-3}$ M; 95% complexation). The photodegradation was followed by HPLC as well as the concomittent formation of the photodecarboxylated product DTPA (**7**) under the same conditions. The percentage of consumed TPA (**2**) is linearly dependent on the duration of the irradiation up to *ca.* 20% conversion, so that the initial slopes of the plots reported in *Fig. 4, a* are proportional to the quantum yields of the photoprocess. It appears that the photodegradation quantum yield is higher by a factor of *ca.* 1.7 in the presence of β-CD. All the reacted TPA (**2**), both in the absence and in the presence of β-CD, is converted to DTPA (**7**), as confirmed by the very good mass balance obtained by detection of the photoproduct. With longer irradiations,

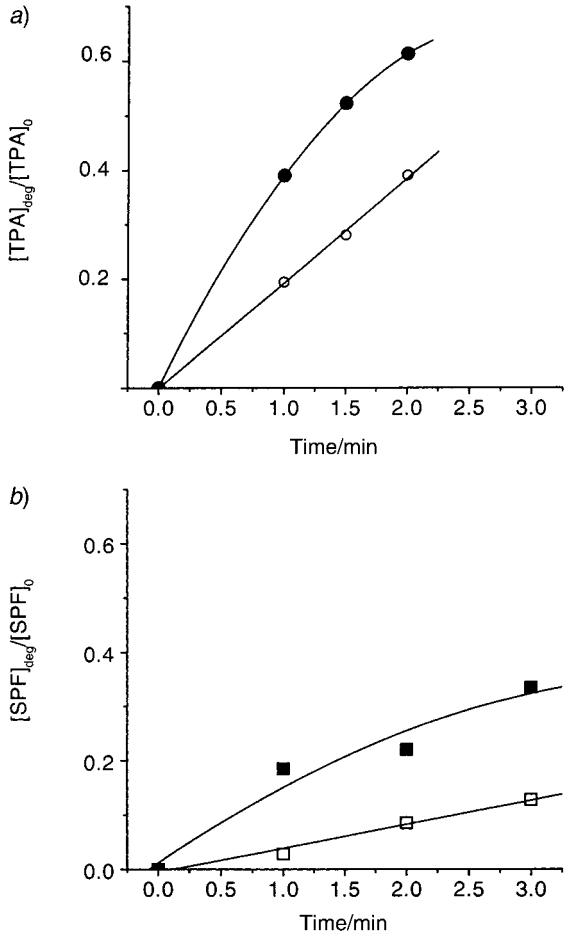

Fig. 4. *Consumed fraction of* a) *TPA* (**2**) *and* b) *SPF* (**3**) *upon irradiation in the absence* (○, □) *and in the presence* (●, ■) *of* $5 \cdot 10^{-3}$ M β*-CD in 0.01*M *phosphate buffer at pH 7.2.* Drug concentration: $5 \cdot 10^{-4}$ M. Irradiation conditions: see *Exper. Part.*

the DTPA formed does not account for all the drug consumed, but no further photoproducts were detected.

Comparative experiments of photolysis of TPA (**2**) and DTPA (**7**) in the presence of β-CD ($5 \cdot 10^{-3}$ M) under identical conditions (96% complexation) allowed to estimate the photodegradation quantum yield of the DTPA-β-CD complex to be one order of magnitude lower than that of the TPA-β-CD complex.

The course of the photolysis of $5 \cdot 10^{-4}$ M SPF (**3**) in the absence and in the presence of β-CD ($5 \cdot 10^{-3}$ M; 90% complexation) is reported in *Fig. 4, b*. From the initial slope, we derive that the quantum yield of photodegradation of the SPF-β-CD inclusion complex is higher by a factor of *ca.* 3 than that of free **3**. The absolute value of Φ^{CD}_{-SPF}, determined at $5 \cdot 10^{-5}$ M SPF in the presence of $5 \cdot 10^{-3}$ M β-CD, was found to be $\Phi^{CD}_{-SPF} = 0.22 \pm 0.02$, in agreement with a previous determination [7]. All the reacted drug is transformed into decarboxysuprofen (DSPF; **8**)[2]).

When the irradiation times are much longer, the correspondence between SPF reacted and DSPF formed is not quantitative. After 30 min of irradiation, *ca.* 25% of the drug was lost, but no further photoproducts were detected. The occurrence of a photoreaction involving the decarboxylated photoproduct was confirmed by direct photolysis of DSPF ($5 \cdot 10^{-4}$ M) in presence of β-CD ($5 \cdot 10^{-3}$ M). After 60 min of irradiation, *ca.* 50% of the starting material was lost. The quantum yield of DSPF photodegradation was estimated to be *ca.* ten times lower than that of SPF photodecarboxylation, used as reference.

2.4. *Laser-Flash Photolysis.* Excitation of Ar-saturated solutions of TPA ($1.22 \cdot 10^{-4}$ M) in presence of β-CD ($4 \cdot 10^{-3}$ M) in 0.01 M phosphate buffer at pH 7.2 and 298 K was performed by 355-nm laser pulses. Under these conditions, the percentage of drug associated to β-CD is *ca.* 92%. The absorbance variations reported in *Fig. 5, a*, are characterized by a broad VIS band with a maximum around 600 nm, a band in the UV region at *ca.* 380 nm, and a negative signal at *ca.* 315 nm, corresponding to the depletion of the ground state. Time evolution consists of disappearance of the VIS band and development of an intense band at 380 nm, whose decay occurs on a much longer time scale. Kinetic analysis of the time profiles up to *ca.* 10 μs gives best fits to a biexponential function with time constants $\tau_1 = 0.36 \pm 0.03$ μs and $\tau_2 = 1.4 \pm 0.2$ μs. These features are closely similar to those observed in buffer and attributed to the sequential formation of three species ([12], see Fig. *5, b*), although, in the system with β-CD, the spectral bands are broader, the intensity of the long-lived 380 nm transient is higher, and the value of τ_1 is significantly shorter (0.36 *vs.* 0.8 μs in [12]). Fairly good isosbestic points are recognizable in the time evolution of the spectra, at *ca.* 435 nm between the first and the second transient and at 395 nm between the second and the

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[2]) It had been reported in [7] that, in the inclusion complex, the quantum yield of decarboxylation is lower than that of SPF disappearence, *i.e.*, $\Phi^{CD}_{dc} \approx 0.11$ *vs.* $\Phi^{CD}_{-SPF} \approx 0.23$, respectively. We believe that the discrepancy between the previous and the present results is due to the completely different procedure used to quantify the photoproducts. The previous procedure involved acidification of the irradiated solution and extraction of the products with $CHCl_3$ prior to HPLC. The present method does not require any manipulation of the irradiated solutions. Thus, we have more confidence in the present indication that $\Phi^{CD}_{-dc} = \Phi^{CD}_{-SPF}$. We presume that the underestimation of the yields of DSPF (**8**) in [7] was due to incomplete extraction of the photoproduct from the β-CD. The starting compound, on the contrary, had been completely removed and, consequently, correctly quantified.

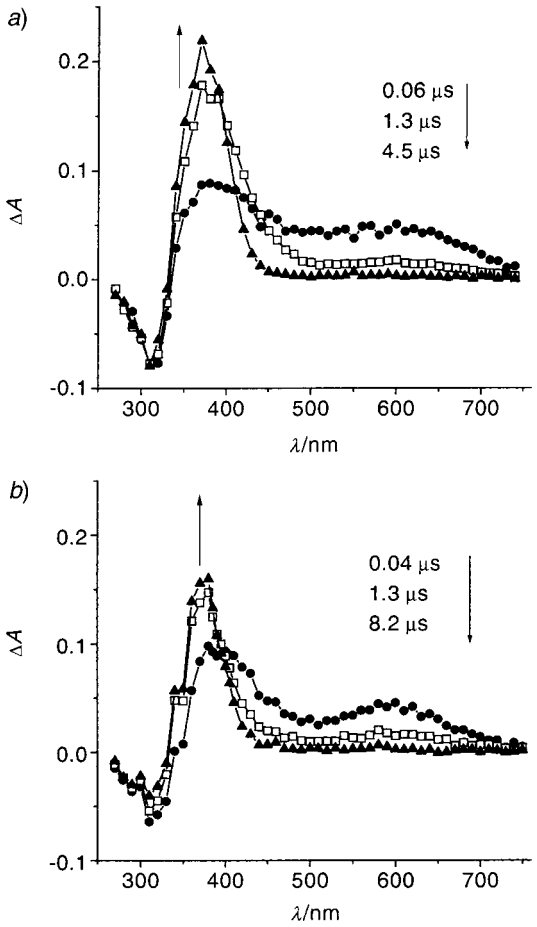
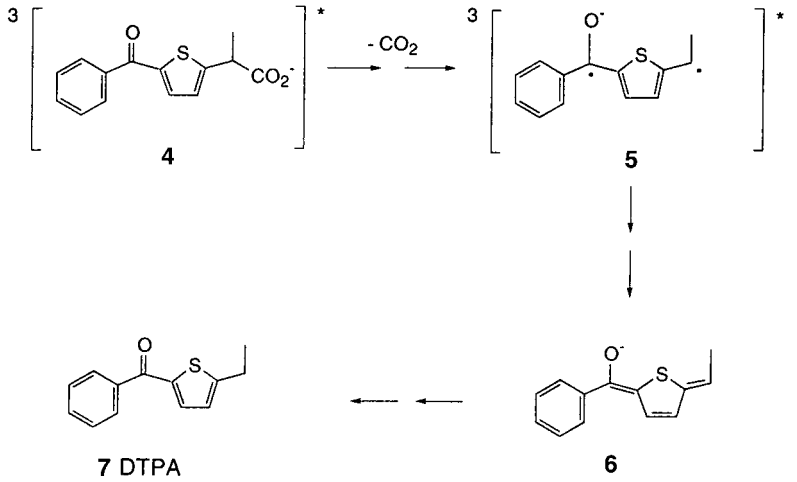


Fig. 5. *Absorbance changes observed in Ar-saturated, 0.01*M *phosphate buffer solution at pH 7.2, and 298 K, at several delays after a 3.7 mJ laser pulse at 355 nm:* a) *$1.22 \cdot 10^{-4}$* M *TPA* (**2**) *in presence of β-CD $4 \cdot 10^{-3}$* M (92% of **2** complexed) *and* b) *$1.22 \cdot 10^{-4}$* M *TPA* (**2**) *alone, from* [12].

third transient, to be compared with 398 and 390 nm, respectively, for the corresponding reactions in buffer [12]. The three transients were, therefore, assigned (see *Scheme*) to the lowest triplet of the complexed drug **4** ($\tau_1 = 0.36$ μs), to the decarboxylated triplet biradical **5** ($\tau_2 = 1.4$ μs), and to a further long-lived ground-state enolate intermediate (**6**).

Ar-Saturated, buffered solutions of DTPA (**7**; $1.25 \cdot 10^{-4}$ M) in the presence of β-CD ($3 \cdot 10^{-3}$ M; substrate *ca.* 94% complexed) were also examined. Excitation at 266 nm was used, due to the low absorption coefficient at 355 nm and the low solubility in H_2O of this compound. The difference spectrum in *Fig. 6*, with maxima at 370 and 610 nm, is attributed to the population of the triplet state of the inclusion complex on the basis of the close analogy with that of the free DTPA (**7**) [12]. A second species with an absorption maximum at 370 nm, a shoulder at 410 nm and an extended, structured band in the VIS region is evidenced. Indeed, the decay kinetics of the absorption changes is

Scheme. *Schematic Reaction Path of the Photodecarboxylation of TPA* (**2**) *in Aqueous Medium*



biexponential with lifetimes $\tau' = 3.5 \pm 0.4$ μs and $\tau'' = 12.0 \pm 2$ μs (see the inset of *Fig. 6*). The shorter lifetime is similar to that found in ^{i}PrOH [12] and is attributed to the triplet state of the DTPA-β-CD complex. The longer lifetime is assigned to the ketyl radical of DTPA, the corresponding spectral features being closely similar to those assigned to the same species in ^{i}PrOH [12].

The photobehavior of the SPF (**3**) system was in part described [7]. In *Fig. 7, a* are reported the transient difference spectra, obtained by excitation at 355 nm of Ar-saturated solutions of **3** ($3.5 \cdot 10^{-4}$ M) in the presence of β-CD (0.01M) in phosphate buffer (0.01M, pH 7.4), at 298 K (extent of SPF complexation > 95%). The absorbance

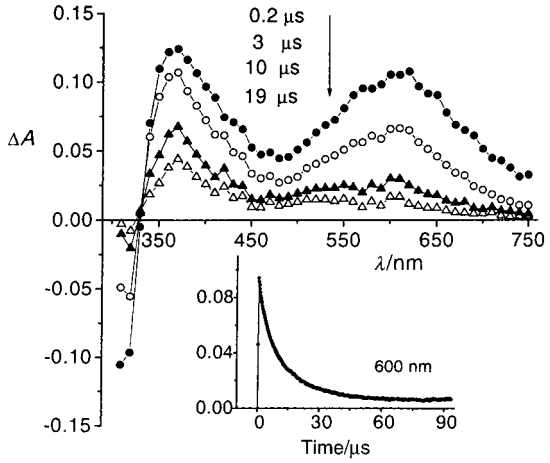


Fig. 6. *Absorbance changes observed in Ar-saturated, 0.01M phosphate buffer solution at pH 7.2 and 298 K, after a 4 mJ laser pulse at 266 nm: $1.25 \cdot 10^{-4}$ M DTPA* (**7**) *in presence of β-CD* ($3 \cdot 10^{-3}$ M; 94% of **7** complexed). Inset: time profile at 600 nm.

changes, very similar to those observed in aqueous medium under identical conditions and recalled from [7] for the sake of clarity (see *Fig. 7, b*), are attributed to the population of the triplet state of the SPF inclusion complex. The decay of the absorption in presence of β-CD is a good monoexponential function with time constant of 12 μs. At 49 μs delay, the difference spectrum is characterized by a maximum at 360 nm and a long, weak tail in the VIS (*Fig. 7, a*). The latter species compares well with that observed in aqueous medium 15 μs after the pulse (*Fig. 7, b*) and is assigned to a decarboxylated enol intermediate [7].

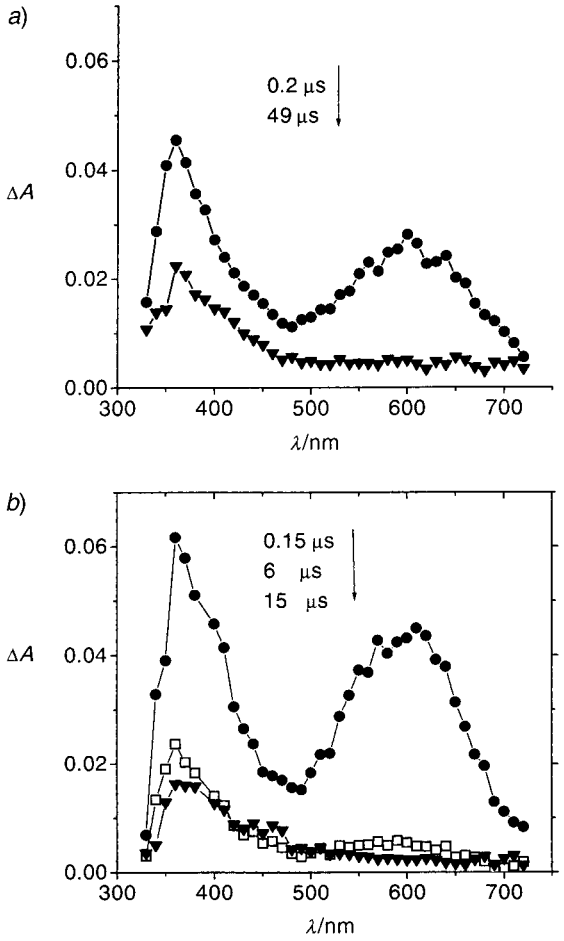


Fig. 7. *Absorbance changes observed in Ar-saturated, 0.01*M *phosphate buffer solution at pH 7.4 and 298 K, at several delays after a 2.3 mJ laser pulse at 355 nm:* a) *$3.5 \cdot 10^{-4}$* M *SPF* (**3**) *in presence of β-CD* (10^{-2} M; > 95% of **3** complexed) *and* b) *$3.5 \cdot 10^{-4}$* M *SPF* (**3**) *alone, from* [7].

3. Discussion. – Both absorption and ICD indicate a favorable interaction between the 2-benzoylthiophene moiety and the β-CD cavity, and indeed, the association constants K_{ass} are rather high (2000 – 5000 M^{-1}, *Table*). TPA (**2**) results only partially

included in β-CDx (see *Fig. 2, a*) with the thiophene and propanoic-acid moieties situated in a central position in the cavity, whereas the benzoyl moiety is located close to the primary rim of the β-CD, with the Ph ring protruding from the cavity. On the contrary, SPF (**3**) shows both the aromatic and carbonylic parts of the molecule well included in the cavity, with the thiophene ring located close to the primary rim and the propanoic-acid group protruding from the secondary rim (*Fig. 3, a*).

Photodecarboxylation is the only photoreaction in homogeneous aqueous medium for both TPA and SPF molecules. The mechanism of the process involves an upper triplet state, likely the second triplet of n,π^* character, higher in energy by 30–40 kJ/mol than the lowest triplet of π,π^* nature [7][11][12]. The results of steady-state photolysis, with perfect mass balance between the amounts of drug consumed and of the corresponding decarboxylated photoproducts, clearly indicate that photodecarboxylation is the only reaction also in the inclusion complexes. However, the quantum yields of the photoprocess appear affected by β-CD complexation. Indeed, in the TPA-β-CD complex, the quantum yield of photodegradation at 298 K appears sensibly higher (by a factor of *ca.* 1.7) than that of the free guest in the homogeneous medium (*Fig. 4*). By considering that the quantum yield of TPA photodegradation at 298 K in the absence of β-CD is $\Phi_{-\mathrm{TPA}} = 0.25$ [11], the quantum yield in the TPA-β-CD inclusion complex $\Phi^{\mathrm{CD}}_{-\mathrm{TPA}}$ is estimated to be *ca.* 0.43. Transient absorption by laser excitation helps to rationalize these findings. The signals exhibited by complexed TPA (*Fig. 5, a*) are quite similar in both intensity and spectral features to those of the free molecule (*Fig. 5, b*) and indicate that the triplet state of the drug is populated with similar efficiency ($\Phi_{\mathrm{ISC}} = 0.9$ [12]). Moreover, in agreement with the occurrence of photodecarboxylation as the only photoprocess, the subsequent transients are completely analogous to those in the absence of β-CD (*Fig. 5*). The triplet lifetime of **2** being shortened by β-CD complexation (from 0.8 μs [12] to 0.36 μs), the increase in the quantum yield of photodecarboxylation has to be correlated with an increased rate of the photoprocess itself. If, for k_{dc}, an *Arrhenius*-like dependence on the temperature is assumed and entropic terms are neglected, and for the free molecule **2** and the inclusion complex the values of the quantum yield of decarboxylation, *i.e.*, 0.25 and 0.43, are taken into account and the lifetime of the precursor triplet T_1, *i.e.*, 0.8 and 0.36 μs, respectively, are assumed, the ratio $k^{\mathrm{CD}}_{\mathrm{dc}}/k^{\mathrm{free}}_{\mathrm{dc}}$ is estimated by *Eqn. 3* to be *ca.* 4.7 at 298 K, corresponding to a reduction of *ca.* 3.8 kJ/mol in the energy gap $T_2 - T_1$ (*Fig. 8*). This reduction can be understood in the light of the structural features of the inclusion complex, with the thiophenecarbonyl moiety deeply embedded in the β-CD cavity, by considering that the decrease of the environmental polarity raises the energy of the lowest π,π^* triplet state and lowers that of the second n,π^* triplet.

$$k^{\mathrm{CD}}_{\mathrm{dc}}/k^{\mathrm{free}}_{\mathrm{dc}} = \exp((\Delta E^{\mathrm{free}} - \Delta E^{\mathrm{CD}})/RT) = (\Phi^{\mathrm{free}}_{\mathrm{isc}}/\Phi^{\mathrm{CD}}_{\mathrm{isc}}) \cdot (\Phi^{\mathrm{CD}}_{\mathrm{dc}}/\Phi^{\mathrm{free}}_{\mathrm{dc}}) \cdot (\tau^{\mathrm{free}}_{\mathrm{T}}/\tau^{\mathrm{CD}}_{\mathrm{T}}) \quad (3)$$

To rationalize the effect of the β-CD inclusion on the photodecarboxylation quantum yield of SPF (**3**), we have first to consider that in this molecule in homogeneous aqueous medium, a very fast self-quenching process ($k_{\mathrm{sq}} = 1.9 \cdot 10^9\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$) affects the lifetime of the lowest triplet (the precursor state), so that the photodegradation quantum yield ($\Phi_{-\mathrm{SPF}}$) strongly depends on the drug concentration. Indeed, the triplet lifetimes range from *ca.* 1–2 μs at 10^{-4} M to *ca.* 40 μs at infinite

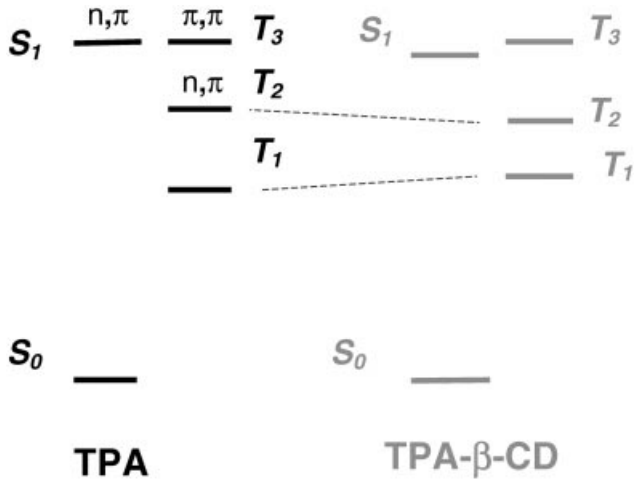


Fig. 8. *Schematic energy ordering of electronic states in free TPA* (**2**) (from [11]) *and in the TPA-β-inclusion complex*

dilution, and the corresponding values of Φ_{-SPF} range from *ca.* 0.01 to *ca.* 0.16 [7]. Inclusion in β-CD strongly affects the photobehavior of the drug: the photodegradation quantum yield becomes much less dependent on the concentration [7] and sensibly higher (*Fig. 4, b*), the triplet state is populated less efficiently (*ca.* 70 %)[3]), and its lifetime is lengthened (from 1.2 to 12 μs at $3.5 \cdot 10^{-4}$ M, *Fig. 7*). The ratio $k_{dc}^{CD}/k_{dc}^{free}$ can be calculated according to *Eqn. 3*, with the parameters relevant to the free SPF (**3**) at infinite dilution and those to the SPF inclusion complex at 298 K, *i.e.* $\Phi_{isc}^{free} = 1$, $\Phi_{isc}^{CD} = 0.7$, $\tau_T^{free} = 40$ μs, $\tau_T^{CD} = 12$ μs, $\Phi_{-SPF} = 0.16$, and $\Phi_{SPF}^{CD} = 0.22$. A value $k_{dc}^{CD}/k_{dc}^{free}$ of 6.5 is derived, corresponding (see *Eqn. 3*) to a reduction of *ca.* 4.6 kJ/mol in the $T_2 - T_1$ energy difference. The increase produced in the SPF triplet lifetime by β-CD inclusion is thus related to the role of the concomitant process of self-quenching, which is slowed in the complex by more than one order of magnitude ($k_{sq}^{CD} = 1 \cdot 10^8$ M^{-1} s^{-1}) [7]. Indeed, the efficient protection of the SPF excited state from bimolecular interactions with ground-state molecules is well accounted for by the almost complete insertion of the whole aromatic moiety in the cavity (*Fig. 3, a*) and the well-defined complex structure, indicated by the calculations.

With TPA (**2**), due to the short lifetime of the precursor triplet, the detection of a second species with lifetime 1.4 μs was possible. This species, identified as the decarboxylated biradical, has in the presence of β-CD the same lifetime as observed in buffer [12], a fact pointing to a fast exit of the guest from the cavity upon release of the CO_2 fragment. The incomplete insertion of **2** in the β-CD cavity (*Fig. 2, a*) appears to be consistent with such a fast dissociation. We had no evidence for a similar process with the deeply embedded SPF (**3**) guest. The rationale for this is twofold. On one side, the long lifetime of the complexed SPF triplet (12 μs), prevents direct detection of any other shorter-lived species (the biradical in buffer exhibits a lifetime of *ca.* 4 μs [7]), on

[3]) This decrease is reasonably due to a less favorable coupling between S_1 and T_3 due to a destabilization of T_3 by β-CD inclusion (see *Fig. 8*).

the other side, effective trapping of unstable intermediates in the cavity is consistent with the drastic reduction in the formation of decarboxylated oxidation products observed in presence of β-CD [7].

Prolonged irradiation of both TPA-β-CD and SPF-β-CD complexes indicates that a secondary photoreaction takes place, characterized by quantum yields about one order of magnitude lower than those of photodecarboxylation, and likely involving, as light absorbing species, the decarboxylated photoproducts of the drugs. These products are characterized by a higher hydrophobicity than the starting drug, so that their complexation is favored. The association constant to β-CD is indeed higher for DTPA (**7**; 5100 M^{-1}) than for TPA (**2**; 3000 M^{-1}). Since no products other than DTPA or DSPF are detected by HPLC in the photolyzed solutions, covalent binding of the decarboxylated drug to the β-CD macrocycle is believed to occur, so that the photoadducts formed may be retained by the chromatographic column together with the excess β-CD. Similar photochemistry was exhibited by β-CD inclusion complexes of several benzophenone derivatives [22][23] and ketoprofen (**1**) [6], and is likely initiated by H-abstraction from β-CD by the ketone n,π^* triplet. Accordingly, laser-flash photolysis of the DTPA-β-CD complex points to the formation of the corresponding triplet state and ketyl radical (*Fig. 6*).

In summary, this study shows that the change of environment from the aqueous solvent to the apolar β-CD cavity affects the energy of the lowest excited states of TPA (**2**) and SPF (**3**) and changes the efficiency of the photodecarboxylation reaction. The photostability of the two drugs is decreased. The decarboxylated photoproducts are photoreactive in turn and give rise, likely, to adducts to the macrocycle. This reaction could be relevant to the observed decrease of the photosensitization properties of decarboxylating drugs containing a benzophenone chromophore by β-CD inclusion, since the toxic photoproducts could be sequestered by the β-CD and forced to react within the cavity, instead of attacking other biological substrates.

In general, the use of β-CD complexation appears to be of interest for modelling the influence of nonaqueous biological environments on the photochemistry of drugs.

Experimental Part

General. The 5-benzoyl-α-methylthiophene-2-acetic acid (=tiaprofenic acid; TPA; **2**) was purchased from *Roussel* (Venezuela), extracted from pills (*Torpas*) with CH_2Cl_2 and α-methyl-4-(2-thienylcarbonyl)benzene-acetic acid 2-(4-thenoyl)phenylpropanoic acid (=suprofen; SPF; **3**) was from *Sigma-Aldrich* (Milan, Italy). Both **2** and **3** were racemates. The (5-ethyl-2-thienyl)phenylmethanone (=decarboxytiaprofenic acid; DTPA; **7**) and (4-ethylphenyl)(2-thienyl)methanone (=decarboxysuprofen; DSPF) were synthesized from **2** and **3** according to [24]. β-Cyclodextrin samples from *Serva*, Heidelberg, Germany, or from *Aldrich*, Milan, Italy, were used as received. All the organic solvents were HPLC grade. H_2O was purified by passage through a *Millipore MilliQ* system or purified by triple distillation. Phosphate buffers (0.01M) at pH 7.2 or 7.4 were used. UV Spectra: *Perkin-Elmer Lambda-9* spectrophotometer. Induced circular dichroism (ICD) spectra: *Jasco J-715* spectropolarimeter. Emission spectra: *Spex Fluorog-111A* spectrofluorimeter.

Nanosecond Flash Photolysis. The transient absorption was taken by excitation with the third (355 nm) or fourth (266 nm) harmonic of a Nd-*YAG-JK* laser of 20 ns pulse duration. The experimental setup and the procedures have been described in detail in [7] and [12].

Steady-State Photolysis and HPLC Analysis. The concentration of TPA (**2**) and SPF (**3**) was $5 \cdot 10^{-4}$ M in 0.01M phosphate buffer at pH 7.2. Under these conditions, the molecule ($pK_a < 5$) was almost totally dissociated into the corresponding carboxylate anion. β-Cyclodextrin was $5 \cdot 10^{-3}$ M, to assure an extent of complexation $\geq 90\%$ with each drug on the basis of the association constants determined by ICD (see below). Solns. of the

drugs in the absence and presence of β-CD were irradiated under N_2 in a *Rayonet* photoreactor (emission between 310 and 420 nm, maximum at 350 nm), at 298 K with stirring for different periods of time (0–60 min). The irradiated samples were analyzed by HPLC (*LiChroCART 125-4*, *LiChrosphere 100RP-18*; MeOH/H_2O/AcOH 580:420: 2 (*v*/*v*), flow rate 1 ml min^{-1}, UV detection at 330 nm). Conversion of **2** was calculated with suprofen as internal standard (peak-area ratio was evaluated).

The experiments for the determination of the absolute value of Φ^{CD}_{-SPF} at low SPF concentration ($5 \cdot 10^{-5}$ M) were performed by irradiating samples under N_2 with monochromatic light obtained from a He-Cd 325 nm laser (*Liconix* series *200*, St. Clara, CA, USA). The incident photon flux was *ca.* $5 \cdot 10^{15}$ quanta s^{-1}. HPLC Analysis (linear gradient of 0–15%, MeCN in 0.01M phosphate buffer (pH 7) within 15 min, flow rate 1 ml min^{-1}; UV monitoring at 250 and 300 nm) was performed below 10% conversion of the starting compound. A quantitative evaluation of injected material in the presence of $5 \cdot 10^{-3}$ M β-CD assured that the column retained no inclusion products. The value of Φ^{CD}_{-SPF} was determined by *Eqn. 4*, where $-$ d[SPF]/d*t* is the initial rate of disappearance of SPF (**3**), *v* is the volume of the irradiated sample, $F = 1 - 10^{-A}$ is the fraction of photons absorbed by SPF at the excitation wavelength, and *I* is the light intensity in mol of photons min^{-1}.

$$\Phi^{CD}_{-SPF} = (-\,d[SPF]/dt)v/FI \tag{4}$$

Calculations. The theoretical methods and the procedures used to determine the structure of the inclusion complexes of TPA (**2**) and SPF (**3**) with β-CD are described in detail in *Sect. 2* (see above).

We acknowledge partial financial support by the *Istituto Superiore di Sanità*, Rome, in the framework of the program 'Physicochemical Properties of Drugs and their safe Use'. We are indebted to Dr. *Bernd Mayer*, University of Vienna, for part of the calculations.

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Received April 17, 2001