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Study of the physicochemical properties in aqueous medium and molecular modeling of tagitinin C/cyclodextrin complexes

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Abstract

The inclusion complexes of tagitinin C with β-, 2,6-di-O-methyl-β- and γ-cyclodextrin (CyD) was investigated in aqueous medium. The stoichiometric ratios and stability constants (K_f) which describe the extent of formation of the complexes have been determined by UV spectroscopy and direct current tast polarography (DC_{tast}), respectively. For each complex, a 1:1 molar ratio was formed in solution and the trend of stability constants was K_f (2,6-di-O-methyl-β-CyD) > K_f (γ-CyD) > K_f (β-CyD).

The effect of molecular encapsulation on the photochemical conversion of tagitinin C was evaluated. No significant protection efficacy was noticed with β- and γ-CyD for the complexed drug with the respect to the free one. On the other hand, the photochemical conversion rate was slowed in presence of 2,6-di-O-methyl-β-CyD.

Data from ^{1}H NMR and ROESY experiments provided a clear evidence of formation of inclusion complexes. The lactone, the ester and the unsaturated ketone parts of tagitinin C inserted into the wide rim of the CyDs torus. These experimental results were confirmed by the molecular modeling using semiempirical Austin Model 1 (AM1) method.

Keywords: Tagitinin C; Cyclodextrin; Inclusion complex; Stability constant; Photodegradation; Molecular modeling; AM1 calculations

1. Introduction

Tagitinin C is isolated from *Tithonia diversifolia*, a shrub which is native to Mexico and also grows in parts of Africa and Asia. This sesquiterpene lactone was identified as an active component against *Plasmodium* [1] and showed significant antiproliferative activity [2]. Chowdhury et al. studied the photochemical conversion of tagitinin C into tagitinin F [3] (Fig. 1). They demonstrated that tagitinin F was obtained from a solution of tagitinin C which was irradiated with a mercury lamp.

Complexation with cyclodextrins (CyDs) provides a way to increase the solubility, stability and bioavailability of drugs [4–6]. CyDs are able to form inclusion complexes with different classes of molecules, modifying their physical, chemical and biological properties. These cyclic polymers which are produced in enzymatic hydrolysis of starch are formed by glucose molecules linked through 1–4 bonds. The α-, β- and γ-CyDs contain six, seven or eight glucose units, respectively. The cone-shaped cavity of CyD allows the accommodation of the apolar part of the guest molecules such as sesquiterpene lactones. Studies involving the complexation of artemisinin with CyDs have been reported in the literature [7–10] although none of them employed tagitinin C.

The aims of the present paper are firstly to investigate the possibility of complex formation of tagitinin C with β-CyD, its 2,6-di-O-methyl derivative and γ-CyD in aqueous medium and secondly to study the influence of inclusion complexes on the photochemical conversion of tagitinin C into tagitinin F. UV

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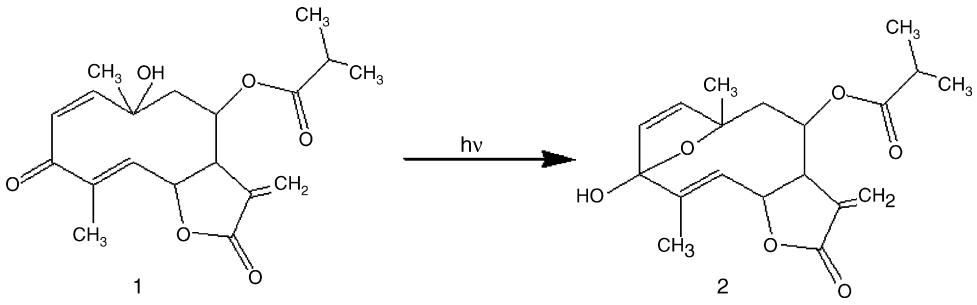

Fig. 1. Photochemical conversion of tagitinin C (1) into tagitinin F (2).

spectroscopy, polarographic techniques, ^{1}H NMR and molecular modeling were used to characterize the tagitinin C/CyD complexes.

2. Experimental

2.1. Chemicals and reagents

β-CyD (Eur. Ph. 4th Edition) was kindly provided by Roquette (Lestrem, France). 2,6-Di-O-methyl-β-CyD (>99%) and γ-CyD (>98%) were, respectively, obtained from Acros (New Jersey, USA) and Fluka (Buchs, Switzerland).

Deuterium oxide (99.97%) was purchased from Euriso-top (Saint-Aubain, France).

Analytical grade of phosphoric acid and sodium hydroxide were obtained from Merck (Darmstadt, Germany). A solution of phosphoric acid (0.10 M) was adjusted to pH 3, 5 with sodium hydroxide.

Reference tagitinin C was extracted from *T. diversifolia* powder leaves (<63 μm) by SFE [11] and purified in our laboratory as described previously by Goffin et al. [1].

2.2. Characterization of complexes

2.2.1. Job plots

The UV–vis absorption spectra were obtained with a Perkin-Elmer UV–vis double beam spectrophotometer Lambda 40 (Perkin-Elmer Limited, Shelton, USA). Rectangular quartz cells (Hellma, Müllheim, Germany), which have a pathlength of 1 cm, were employed in the spectral range of 190–350 nm. The scanning speed and the slit were fixed at 60 nm min^{-1} and at 2 nm, respectively.

A stock solution of tagitinin C was prepared at 1.00×10^{-4} M in water. The complex TC/CyD was formed at a constant volume by adding various concentration of each CyD also dissolved in water at several molar ratios (r), which varies from 0.1 to 0.9, to reach a final total molarity of 1.00×10^{-4} M. The maximum absorbance of the solution of tagitinin C was carried out using the peak around 248 nm. The absorbance difference $\Delta A = A_0 - A$ was determined by measuring the absorbance of tagitinin C with (A) and without (A_0) CyD. The product $\Delta A \times$ [tagitinin C] versus r was then plotted to determine the stoichiometry of the complex which was 1:1 when $\Delta A \times$ [tagitinin C] reached its maximum for $r = 0.5$.

2.2.2. Electrochemical determination

The polarograms were obtained with a Metrohm 746 VA processor (Metrohm AG, Herisau, Switzerland). A Metrohm 694 VA stand was used with a mercury drop electrode. The three electrodes system was completed by means of a Ag/AgCl (3 M KCl) reference electrode and a Pt auxillary electrode.

The current titrations were carried out by keeping constant the concentration of tagitinin C while the concentration of CyDs varies.

2.2.2.1. Differential pulse polarography. The working electrode was operated in the dropping mercury electrode (DME) mode. A PTFE polarographic cell was used to shield the solution from the light.

Ten milliliters of tagitinin C solution (1.90×10^{-5} M), previously acidified with 0.1 M phosphoric acid (pH 3.5) was transferred into the polarographic cell. The solution was de-aerated by bubbling nitrogen for 5 min. The differential pulse polarograms were obtained between −650 and −1000 mV for β- and γ-CyD and between −700 and −1100 mV for 2,6-di-O-methyl-β-CyD. The pulse amplitude and the scan rate were −50 mV and 10 mV/s, respectively.

2.2.2.2. Direct current tast polarography (DC_{tast}). The working electrode was operated in the static mercury drop electrode (SMDE) mode.

Ten milliliters of tagitinin C solution (5.41×10^{-5} M), previously acidified with 0.1 M phosphoric acid (pH 1) was transferred into the polarographic cell. The solution was de-aerated by bubbling nitrogen for 5 min. The DC polarograms were obtained between −400 and −900 mV for β- and γ-CyD and between −400 and −1000 mV for 2,6-di-O-methyl-β-CyD. The scan rate was 7.5 mV/s. The experiments were performed at 25 ± 1 °C.

2.3. Photochemical conversion study

Photochemical conversion study were analyzed by a HPLC system composed of a Merck liquid chromatograph Model Lachrom equipped with an Alltima RP-18 column (150 mm × 4.6 mm i.d., 5 μm particle size, temperature 30 °C) coupled with a Merck Diode Array detector L-7455 detector. A sample loop of 100 μL capacity was used in which 10 μL was injected. The mobile phases were: solvent A-acetonitrile and solvent B-water. The elution was isocratic with A:B (50:50) at

a flow rate of 1.0 mL min^{-1} for 9 min. Tagitinin C was eluted at ca. 3.8 min on this system. The experimental conditions were based on the work of Goffin et al. [12].

Solutions containing tagitinin C at a concentration of 2.00×10^{-4} M with CyDs at a concentration of 2.00×10^{-3} and 2.00×10^{-2} M and without were used for the study. The solutions were transferred into rectangular quartz cells which have an internal volume of 3 mL and a pathlength of 1 cm. The samples were exposed to an artificial light during 14 days. After each proper time of exposure, 100 μL of sample was mixed with 100 μL of acetonitrile before injection into the HPLC system.

The analysis of tagitinin C was carried out using an external standard method. Working solutions containing 1.44, 14.36 and 28.73×10^{-5} M of the analyte were prepared from a stock solution in the mobile phase.

2.4. NMR study

2.4.1. ^1H NMR study

One-dimensional ^{1}H NMR spectra were recorded at 25 °C on a Bruker Avance 500 operating at a proton NMR frequency of 500.13 MHz using a 5 mm probe and a simple pulse-acquire sequence. Acquisition parameters consisted of a spectral width of 10330.6 Hz, a pulse width of 2.541 μs, an acquisition time of 3.17 s and a relaxation delay of 1 s. 128 scans were recorded. FIDs were Fourier transformed with LB = 0.3 Hz and GB = 0. The resonance at 4.700 ppm due to residual solvent (HOD) was used as internal reference.

Reference solutions were prepared by separately dissolving an appropriate amount of tagitinin C and CyDs directly in 600 μL D_2O in order to obtain a concentration of 1.50 mM. The resulting solutions were then transferred in the 5 mm RMN tubes.

Sample solutions were prepared by dissolving tagitinin C and CyDs in 600 μL D_2O in order to obtain the final concentration of 1.50 mM (complexes in a molar ratio 1:1).

^{1}H NMR chemical shifts ($\Delta\delta$) caused upon complexation were measured to confirm the inclusion of tagitinin C and calculated according to the following formula:

$$\Delta\delta = \delta_{\text{complexed state}} - \delta_{\text{free state}}.$$

2.4.2. ROESY experiment

Rotating-frame Overhauser Effect SpectroscopY (ROESY) spectra were acquired in the phase sensitive mode using the same spectrometer and Bruker standard parameters (pulse program roesyph). Each spectrum consisted of a matrix of 2 K (F2) by 256 (F1) points covering a spectral width of 5122.9 Hz. Spectra were obtained from the samples solutions prepared for the ^{1}H NMR studies, using a spin-lock mixing time of 350 ms. Thirty-two scans were recorded.

2.5. Molecular modeling

The geometry optimization was performed with the Gaussian 98 package [13] by using semiempirical AM1 (Austin Model 1) method [14]. The geometries were fully optimized without any constraint by minimization of the analytical gradient. The nature of the located critical points is determined by vibrational frequency calculation derived from the second derivative matrix. When all the eigenvalues of this Hessian matrix are positive, the energy is minimum in each direction associated to the variables. For each equilibrium structure, the thermochemistry data are derived from the analytical frequency calculation at 298.15 K and 1 atm [15].

The same procedure was used in the study of miconazole, cyproterone acetate and RO 28-2653 inclusion complexes [16–18] Starting from the optimized geometry local minimum of each complex, tagitinin C and CyD were re-optimized separately. This procedure allows the determination of consistent energetic data, as each relative energy is calculated by reference to the geometry of the partners starting from one determined in the complex.

3. Results and discussion

3.1. Stoichiometry of inclusion complexes

The stoichiometry of the inclusion complexes is carried out by the continuous variation method known as Job's plot (Fig. 2). For each complex, *r* is equal to 0.5 at the maximum which indicated that a complex with 1:1 molar ratio was formed in solution.

3.2. Measurement of association constants of inclusion complexes

Tagitinin C is enclosed in *Tithonia diversifolia* at low concentration levels, thus sensitive and less material-consuming methods for the determination of stability constant had to be selected. Several authors suggested using polarography techniques to determine the association constant of inclusion complexes at very low concentration levels (>10^{-5} M) of the interest compound [19–21]. In addition, the presence of the unsaturated ketone cycle seemed to make polarography techniques more suitable to the objective of our study. Indeed, it has been previously demonstrated that a pH reversible one electron polarographic wave is observed for dienones [22].

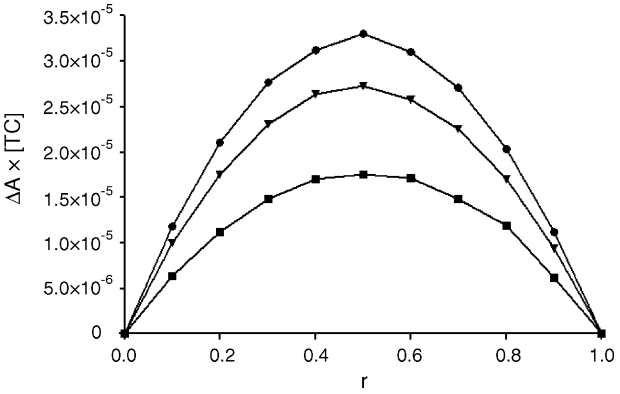

Fig. 2. Job's plot for the formation of tagitinin C complexes with β-CyD (■), 2,6-di-O-methyl-β-CyD (●) and γ-CyD (▼).

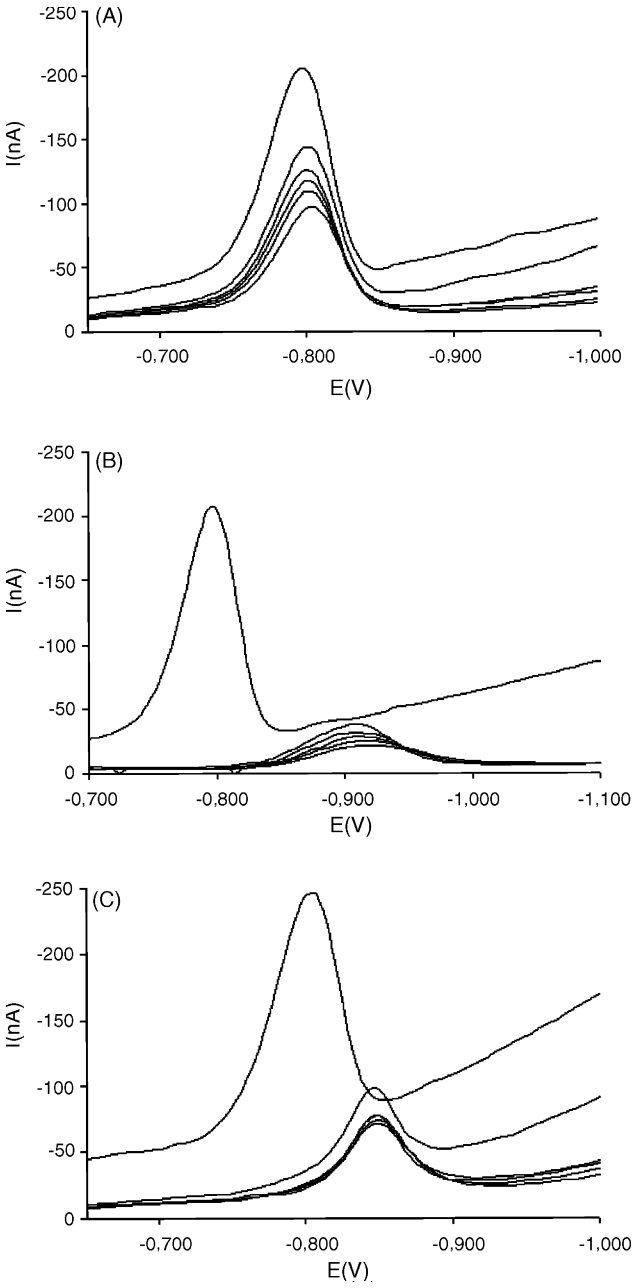

Fig. 3. Intensity of the DPP curves of tagitinin C (1.90×10^{-5} M) in absence and presence of cyclodextrins. (A) 1.130, 1.477, 1.811, 2.133 and 2.887 mM of β-CyD. (B) 1.198, 1.589, 1.976, 2.359 and 2.927 mM of 2,6-di-O-methyl-β-CyD. (C) 2.890, 3.805, 4.698, 5.568 and 6.586 mM of γ-CyD.

First, the electrochemical reduction of tagitinin C on mercury in aqueous solution (pH 3.5) has been studied by differential pulse polarography (DPP). As can be seen in Fig. 3, tagitinin C shows a well resolved polarographic peak with a peak potential at −0.8 V.

The addition of β-, 2,6-di-O-methyl-β- and γ-CyD to a solution of tagitinin C causes three main changes in the tagitinin C polarograms (Fig. 3). Firstly, the intensity of the DPP curves decreased, secondly, the cathodic peak potential shifted in a negative direction and thirdly the width at half height increased. The shift and the broadening of the cathodic peak seem to indicate that the behaviour of tagitinin C on the working electrode changed in presence of CyDs, especially with the 2,6-di-O-methyl-β-CyD.

In order to calculate the complex formation constants, the following titration equation was applied:

$$\frac{1}{[\mathrm{CyD}]} = K_\mathrm{f}\frac{1-A}{1-(I/I_0)} - K_\mathrm{f}$$

where K_f (M^{-1}) is the apparent complex formation constant, I_0 and I the peak current in the absence and presence of CyD, respectively, [CyD] the molar concentration of CyD and A is the proportional constant. The conditions of using this equation are that the inclusion complexes with 1:1 molar ratio are formed in solution and that the concentration of cyclodextrins is much larger than the total concentration of the drug. Moreover, the behaviour of the complexed drug on the working electrode must be the same than that of the free drug. This last condition is critical when DPP is used to determine the apparent complex formation constant. Indeed, the diffusion current is dramatically dependent on the reaction mechanism of the drug. According to the shift and the broadening of the cathodic peaks indicating that the behaviour of tagitinin C in presence of CyDs changed, the current titration method cannot be applied.

Second, the current titration method was carried out using $\mathrm{DC_{tast}}$. This polarographic method allows to avoid the dependence of the diffusion current on the reaction mechanism of the drug. $\mathrm{DC_{tast}}$ polarograms of tagitinin C are presented in Fig. 4. The half-wave potential is around −0.6 V and a second order maximum appears at the wave end. The maximum disturbs the determination of the diffusion current of free tagitinin C leading to decrease the precision on the determination of I_0 and thus the complex formation constant. In order to reduce the influence of the maximum on the measurements and also to obtain a defined plateau both sides of the wave, the pH of the solution was fixed at 1. The addition of CyDs causes main changes in polarograms (Fig. 4): a decrease of the height of the wave, a shift of the half-wave potential in a negative direction, a flattening of the wave and a suppression of the maximum phenomenon. The decrease of the height of the wave can be ascribed as a diminution of the diffusion current due to a diminution of the apparent diffusion coefficient of the tagitinin C included in the cyclodextrin when compared with its apparent coefficient diffusion.

The equations of straight lines obtained by the least squares linear regression ((1/[CyD]) versus $(1/(1-I/I_0))$) were used to calculate the formation constants (K_f) from the ordinate intercepts which are equal to 165 and 107 M^{-1} for the 2,6-di-O-methyl-β-CyD and γ-CyD, respectively (Table 1). Unfortunately, the determination with β-CyD was impractical taking into account the limited solubility of CyD in water. Indeed no significant change of the height of the wave was noticed until a concentration of 18 mM of CyD. At a higher temperature (60 °C), decreases of the current intensity were observed with the addition of 18 and 50 mM indicating the inclusion of tagitinin C in the β-cyclodextrin cavity. The lack of precision in the measurements of the currents at the both sides of the polarographic waves and the limited number of evaluation points (18 and 50 mM) do not allow to determine a correct K_f value, which

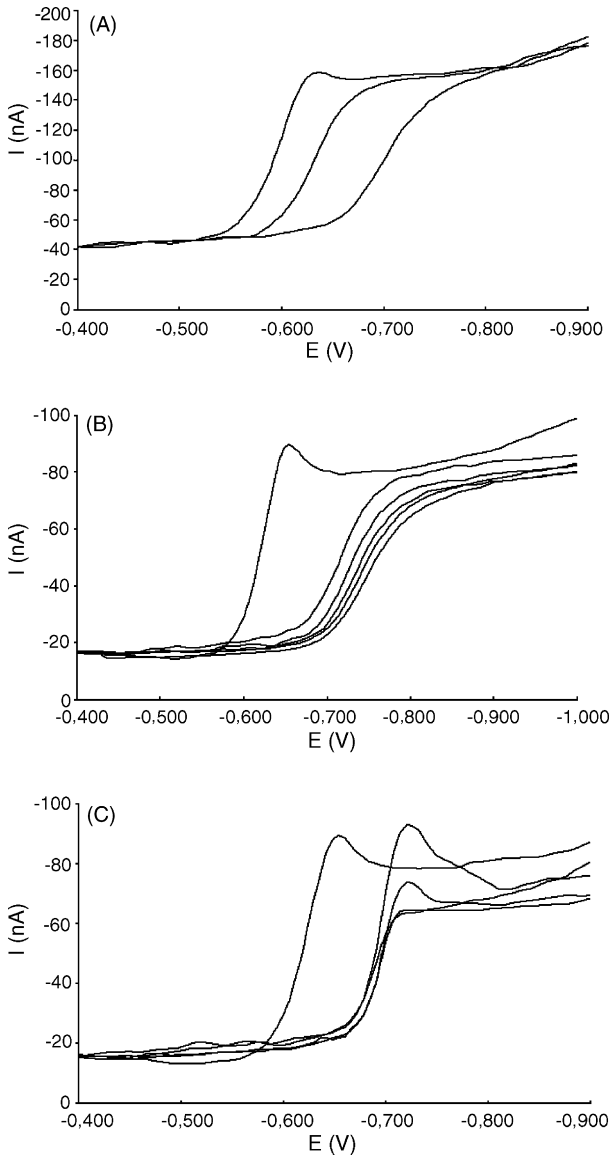

Fig. 4. DC_{tast} polarograms of tagitinin C (5.41×10^{-5} M) in absence and presence of cyclodextrins. (A) 18 and 50 mM of β-CyD. (B) 3.28, 6.55, 9.83, 13.11 and 16.38 mM of 2,6-di-O-methyl-β-CyD. (C) 3.03, 6.06, 9.09 and 12.12 mM of γ-CyD.

is estimated very close to zero. The affinity of this cyclodextrin for tagitinin is therefore very weak.

DC_{tast} indicated that 2,6-di-O-methyl-β-CyD is the most powerful complexing agent for the tagitinin C and that the trend

Table 1
Equations obtained by the least squares linear regression ((1/[CyD]) vs. $(1/(1 - I/I_0))$) from the current titration method using DC_{tast}

CyD	DC_{tast}		
	Slope	Intercept K_f (M^{-1})	r^2
β-CyD[a]	N.D.	N.D.	N.D.
2,6-Di-O-methyl-β-CyD	56	−165	0.9981
γ-CyD	77	−107	0.9951

[a] Determination was impractical due to the limited solubility of β-CyD in water.

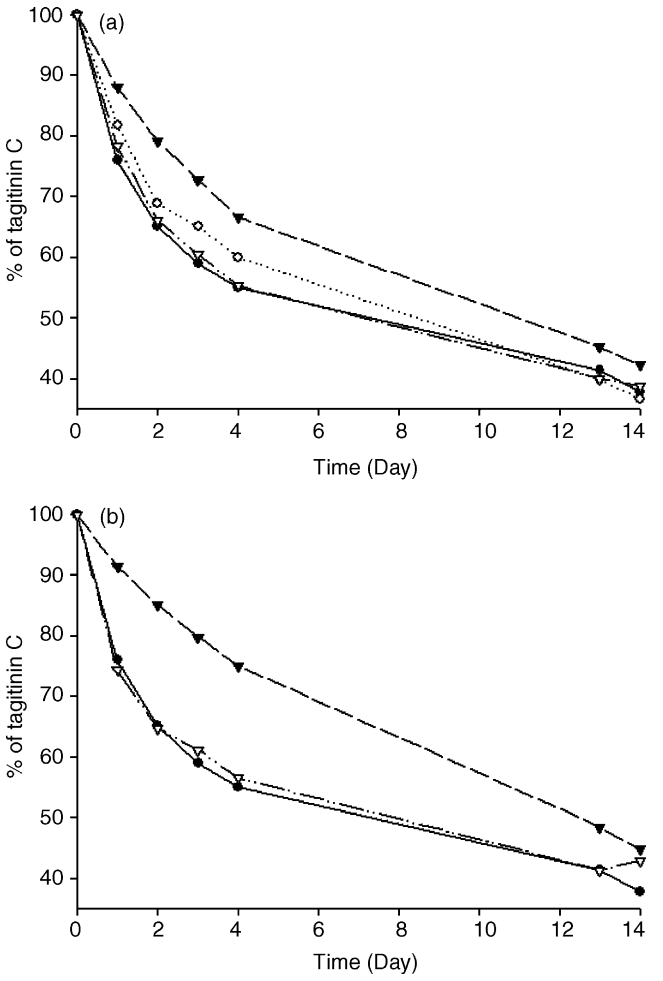

Fig. 5. Photochemical conversion profiles of tagitinin C solutions (2×10^{-4} M) in absence (●). (a) In presence of β-CyD (◯), 2,6-di-O-methyl-β-CyD (▼) and γ-CyD (▽) (2×10^{-3} M). (b) In presence of 2,6-di-O-methyl-β-CyD (▼) and γ-CyD (▽) (2×10^{-2} M). Experiments were carried out in duplicate.

of K_f values (ordinate intercept) was K_f (2,6-di-O-methyl-β-CyD) > K_f (γ-CyD) > K_f (β-CyD).

3.3. Photochemical conversion of tagitinin C in aqueous medium

Aqueous solutions containing tagitinin C used as reference solution and aqueous solutions containing the inclusion complexes which were characterized by various molar ratios (1:10 and 1:100), all at the same concentration of tagitinin C, were simultaneous exposed to artificial light. Unfortunately, the tagitininC/β-CyD complex with a 1:100 molar ratio was not carried out according to the solubility of β-CyD in water. At a selected time, aliquots of irradiated solutions were diluted with ACN before the injection into a HPLC and the tagitinin C peak was determined. The results are illustrated in Fig. 5.

As can be seen on Fig. 5, the β- and γ-CyD complexations did not modify significantly the photochemical conversion rate of tagitinin C into tagitinin F with both molar ratios. In the other hand, the addition of 2,6-di-O-methyl-β-CyD slowed the photochemical conversion rate of tagitinin C. The dependence of

Table 2
The half-life degradation time of tagitinin C solutions (2.00×10^{-4} M) in presence of β-, 2,6-di-O-methyl-β (2.00×10^{-3} and 2.00×10^{-2} M) and γ-CyD (2.00×10^{-3} and 2.00×10^{-2} M)

[CyD] (M)	$t_{0.5}$ (Day)			
	Tagitinin C	β-CyD	2,6-Di-O-methyl-β-CyD	γ-CyD
2.00×10^{-3}	8.4	8.6	10.7	8.5
2.00×10^{-2}		N.D.	12.1	8.3

the photostability on the molar ratio can be explained by the fact that the increase of CyD concentration brought about the decrease of the free drug concentration. The half-life degradation time ($t_{0.5}$) of taginitin C and its inclusion complexes was determined by plotting ln % of tagitinin C versus time. Table 2 shows that the complexation with 2,6-di-O-methyl-β-CyD can slightly increase $t_{0.5}$ of tagitinin C.

3.4. NMR study

Fig. 6 shows a typical ^{1}H NMR spectrum of tagitinin C in D_2O.

Direct evidence for the formation of inclusion complex and identification of parts of the guest which are inside the CyD cavity can be obtained from ^{1}H NMR shifts [23]. The significant displacements of chemical shifts (upfield) of tagitinin C protons confirmed that the inclusion complexes were formed in aqueous medium. The values of chemical shifts for different protons of tagitinin C are listed in Table 3 except H7 proton because the presence of CyD protons interferes with its determination.

Contrary to the complex obtained with β-CyD, a downfield shift is observed for the H2 and H8 protons of tagitinin C. It can be suggested that a part of the unsaturated ketone cycle is close to the rim of the 2,6-di-O-methyl-β- and γ-CyD.

The structural study of tagitinin C/CyD complexes was carried out with ROESY experiments. Tagitinin C/β-CD complex (Fig. 7) showed correlations between the internal H3 and H5 protons of the CyD and the protons of tagitinin C. The internal H3 proton is correlated with the protons of the lactone part and the unsaturated ketone cycle while the internal H5 proton is correlated with the protons of the ester part, which could suggest that the tagitinin C is deeply inserted into the cavity by the larger rim of the truncated cone, where secondary alcohol groups of the oligosaccharide are present, with the ester and lactone parts oriented towards the primary alcohol groups of the CyD.

Tagitinin C/2,6-di-O-methyl-β-CyD complex (Fig. 8) showed the same correlations between the internal H3 and H5 protons than these obtained with the β-CyD suggesting that the tagitinin C is deeply inserted in the cavity with the ester and lactone parts oriented towards the primary alcohol groups of the CyD. In addition, the 2-OCH_3 and 6-OCH_3 groups of CyD were also correlated with the protons of the ester part and the methyl groups of the unsaturated ketone cycle, respectively. The interactions between the 2-OCH_3 of CyD and methyl groups of the

Table 3
Variation of ^{1}H NMR chemical shifts (ppm) of tagitinin C in the presence of β-, 2,6-di-O-methyl-β- and γ-CyD (1.5 mM, 1:1 molar ratio)

Protons	Δδ (β-CyD)	Δδ (2,6-di-O-methyl-β-CyD)	Δδ (γ-CyD)
3′&4′	−0.040	0.006	−0.062
14	−0.035	0.027	0.023
15	−0.056	−0.004	0.025
9	−0.067	−0.047	−0.073
9′	−0.041	−0.002	−0.014
2′	−0.049	−0.070	−0.031
7[a]	N.D.	N.D.	N.D.
8	−0.020	0.033	0.041
6	−0.082	−0.123	−0.097
13′	−0.047	−0.030	−0.014
5	−0.052	0.016	−0.067
13	−0.038	−0.028	−0.006
1	−0.037	−0.021	−0.007
2	−0.037	0.023	0.046

$\Delta\delta = \delta_{\text{complexed state}} - \delta_{\text{free state}}$.
[a] Presence of CyD protons interferes with the determination.

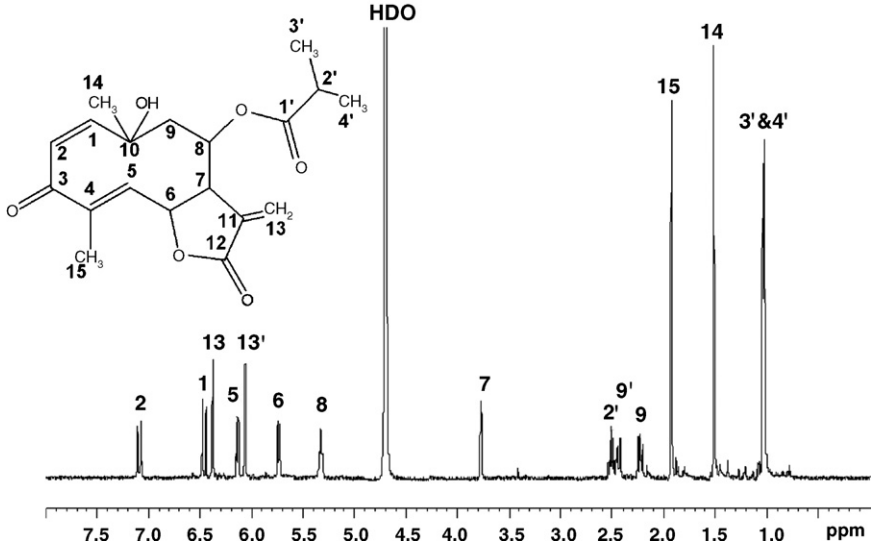

Fig. 6. Mono-dimensional ^{1}H NMR spectrum of tagitinin C in D_2O (1.5 mM).

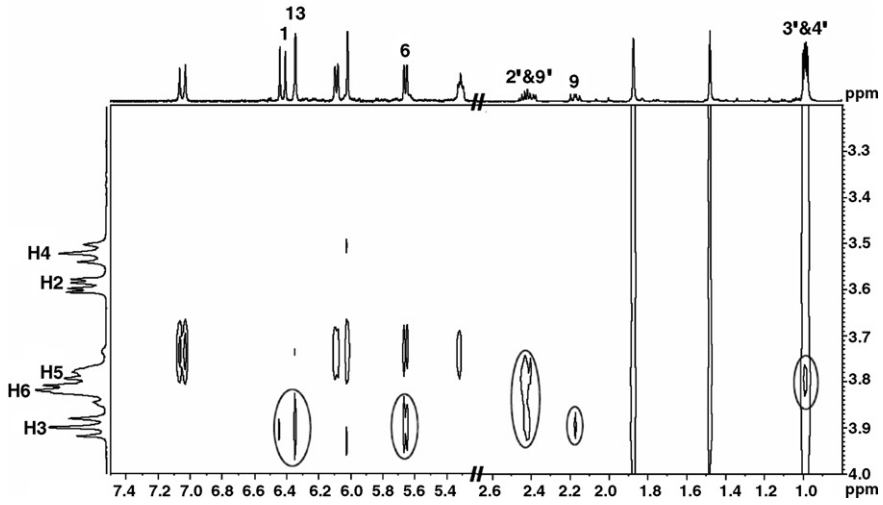

Fig. 7. Partial contour plot of the two-dimensional ROESY spectrum of tagitinin C in presence of β-CyD complex in D_2O (1.5 mM, 1:1 molar ratio).

unsaturated ketone cycle could explain the results obtained with the electrochemical determination and with the photochemical conversion study as a consequence of a steric effect of 2-OCH_3 groups of the CyD on the unsaturated ketone cycle.

Finally, the tagitinin C/γ-CyD complex (Fig. 9) showed intense correlations between the internal H3 and H5 protons of the CyD which also suggest that tagitinin C is deeply inserted in the cavity of CyD. Contrary to the complexes with β-CyD and its 2,6-di-O-methyl derivative, the two orientations of tagitinin C inside the cavity of γ-CyD seems to be possible. Indeed, its cavity is larger than the cavity the β-CyD and thus it could allow the insertion of the ester and lactone parts towards the primary alcohol or the secondary alcohol groups of the CyD.

3.5. Molecular modeling

The results shown in Table 4 are presented as energetic outcome expressed as complexation, deformation and interaction energies. The complexation energy ($E_{complexation}$) is the difference between the energy of the complex and the sum of both partners (tagitinin C and CyD) in their respective equilibrium geometry. The deformation energy ($E_{deformation}$) is determined by the difference between the energy of the partners of the complex at their respective equilibrium geometry and their energy at the complex geometry. The interaction energy ($E_{interaction}$) is defined as the difference between the energy of the complex and the sum of the energies of both partners at their complex geometry. Entropy and enthalpy are also presented in these tables. Two inclusion modes were considered depending on the orientation of ester-lactone parts towards the primary alcohol groups (pag) or towards the secondary alcohol groups (sag) of the CyD. These complexes are noted Ester_Lactone/pag and Ester_lactone/sag, respectively.

As observed in Table 4, the difference of free energy (ΔG) of complexes are positive in all cases indicating that a contribution of energy is necessary to form the complexes. In addition the energy of deformation of β-CyD is very important and varies dramatically according to the inclusion orientation of tagitinin C inside its cavity. These results confirm the high capability of β-CyD to adopt very different geometries leading to more or less stable complexes with a guest molecule as previously demonstrated with miconazole by Piel et al. [16].

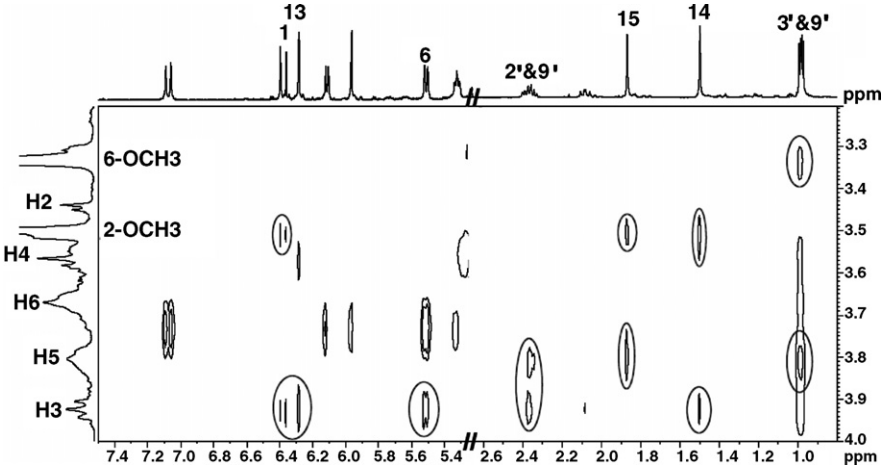

Fig. 8. Partial contour plot of the two-dimensional ROESY spectrum of tagitinin C in presence of 2,6-di-O-methyl-β-CyD in D_2O (1.5 mM, 1:1 molar ratio).

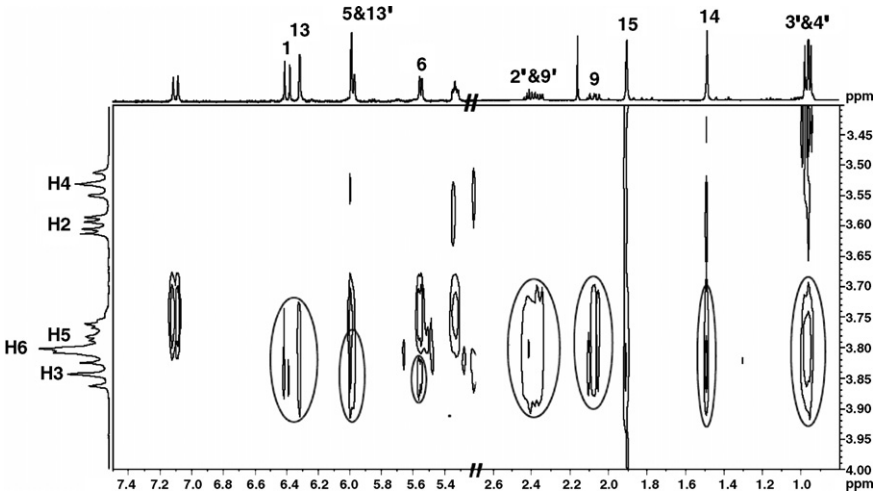

Fig. 9. Partial contour plot of the two-dimensional ROESY spectrum of tagitinin C in presence of γ-CyD in D_2O (1.5 mM, 1:1 molar ratio).

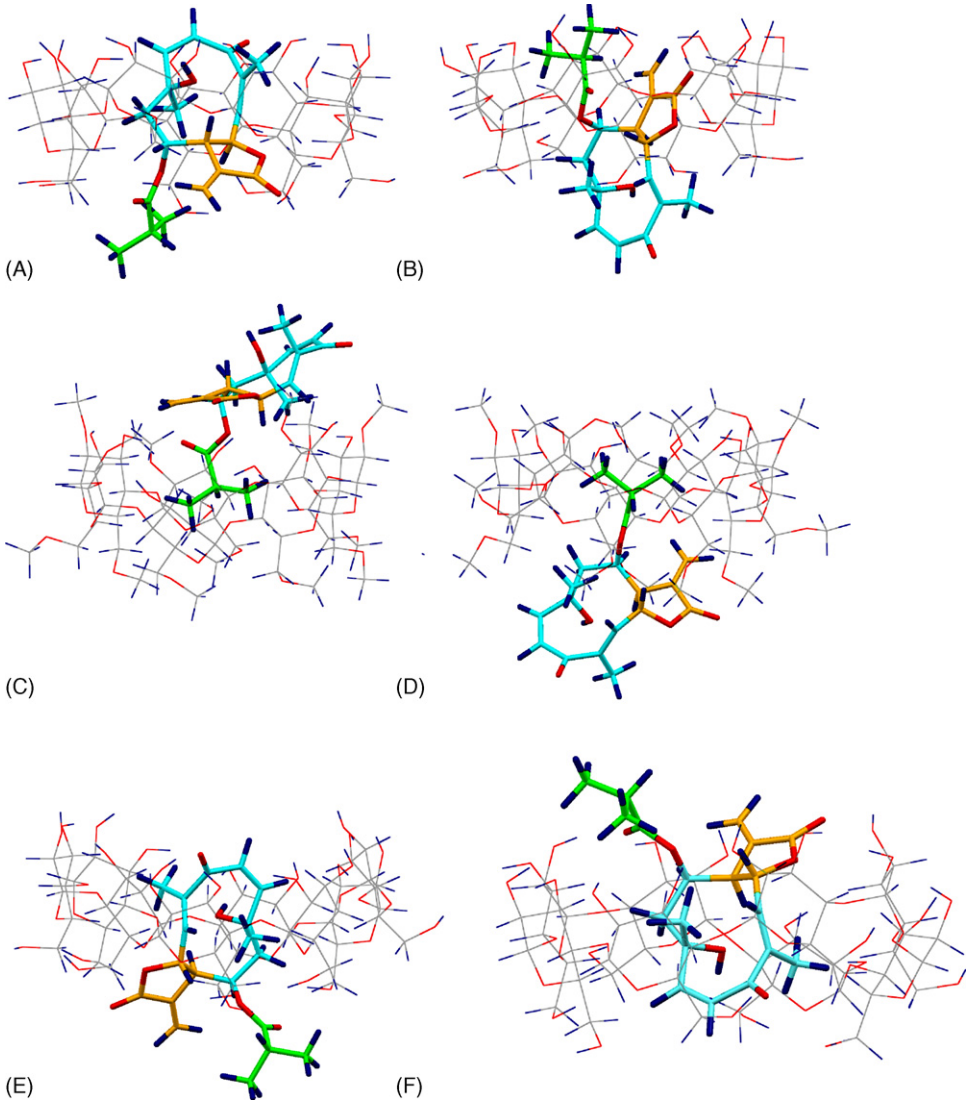

Fig. 10. Optimized structures of tagitinin C/CyD complexes. β-CyD: oriented Ester_Lactone parts towards the primary alcohol groups (A) and towards the secondary alcohol groups (B). 2,6-Di-O-methyl-β-CyD: oriented Ester_Lactone parts towards the primary alcohol groups (C) and towards the secondary alcohol groups (D). γ-CyD: oriented Ester_Lactone parts towards the primary alcohol groups (E) and towards the secondary alcohol groups (F). Ester, lactone and unsaturated ketone parts of tagitinin C are coloured in green, orange and cyan, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

Table 4
Complexation, deformation and interaction energies in kcal/mol (ΔS in cal mol^{-1} K^{-1}) with the reference to both reoptimized β-CyD and tagitinin C, 2,6-di-O-methyl-β-CyD and tagitinin C and γ-CyD and tagitinin C

Model	Ester_Lactone/pag	Ester_Lactone/sag
$E_{complexation}$		
ΔE	0.138	11.753
ΔH	3.050	13.377
ΔS	−73.411	−71.077
ΔG	24.937	34.568
$E_{deformation}$		
Tagitinin C	−2.683	−5.588
β-CD	−10.054	−6.344
$E_{interaction}$	−12.600	−0.179
$E_{complexation}$		
ΔE	−5.020	−1.465
ΔH	−3.643	0.289
ΔS	−58.698	−63.129
ΔG	13.858	19,111
$E_{deformation}$		
Tagitinin C	−1.088	−0.704
Dimethyl-β-CD	−2.171	−4.907
$E_{interaction}$	−8.279	−7.077
$E_{complexation}$		
ΔE	−10.093	−14.807
ΔH	−8.380	−12.873
ΔS	−64.697	−67.961
ΔG	10.910	7.390
$E_{deformation}$		
Tagitinin C	−0.652	−1.272
γ-CD	−3.986	−4.990
$E_{interaction}$	−14.731	−21.069

$E_{interaction}$: energy of the complex − sum of the energy of each partner at the complex geometry. $E_{deformation}$: energy of the partner − energy of the partner in the complex. $E_{complexation}$: energy of the complex − sum energy of each part in their respective equilibrium geometry. ΔG is calculated at 298.15 K.

For tagitinin C/β-CyD, the inclusion of Ester_Lactone/pag is more favourable than the inclusion of Ester_Lactone/sag taking into account that ΔE is close to 0. This inclusion mode has also a high $E_{interaction}$ equal to −12,600 kcal mol^{-1}. The optimized structure of both complexes is depicted in Fig. 10A and B: ester, lactone and unsaturated ketone parts of tagitinin C are coloured in green, orange and cyan, respectively. As can be seen in Fig. 10A, the optimized structure fits in with the experimental results obtained with the ROESY experiments.

The two inclusion modes of tagitinin C/2,6-di-O-methyl-β-CyD, shown in Fig. 10C and D, give stable complexes ($\Delta E < 0$). As in the tagitinin C/β-CyD, the inclusion of Ester_Lactone/pag is more favourable than the inclusion of Ester_Lactone/sag as expressed ΔE as $E_{interaction}$. These results also agree with those obtained with the ROESY experiments. In addition, the possibility of interactions between CyD 2-OCH_3 groups and the methyl groups of the unsaturated ketone cycle is confirmed by the molecular modeling.

Finally, stable complexes are obtained with both inclusion modes of tagitinin C with γ-CyD. Contrary to the results obtained with the β- and 2,6-di-O-methyl-β-CyD, the inclusion of Ester_Lactone/sag (Fig. 10F) is more favourable than the inclusion of Ester_Lactone/pag (Fig. 10E). This result can be explained by the fact that the cavity of γ-CyD is large enough to include the unsaturated ketone cycle towards the secondary alcohols allowing interactions between Ester_Lactone and sag which stabilize the complex. Although this optimized structure is favourable, both are in accordance with the experimental results obtained by the ROESY experiments.

4. Conclusion

This study demonstrated that tagitinin C formed 1:1 inclusion complexes with β-, 2,6-di-O-methyl-β- and γ-CyD in aqueous medium and that 2,6-di-O-methyl-β-CyD is the most powerful complexing agent tested for the tagitinin C using DC_{tast}. The present work also showed that DPP was not a suitable method for the determination of the stability constant of inclusion complexes.

The photochemical conversion rate of tagitinin C into tagitinin F was slowed in presence of 2,6-di-O-methyl-β-CyD while no significant effect was observed in presence of β- and γ-CyD. The interactions between 2-OCH_3 groups of CyD and CH_3 groups of the unsaturated cycle of tagitinin C can explain the increase of the tagitinin C resistance to the photochemical conversion in its complexed form.

Finally, the concomitant use of ^{1}H NMR and molecular modeling allowed to investigate thoroughly the geometry of TC/CyD complexes.

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