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Concerning the Association of Hypericin Tautomers and their Hypericinate Ions

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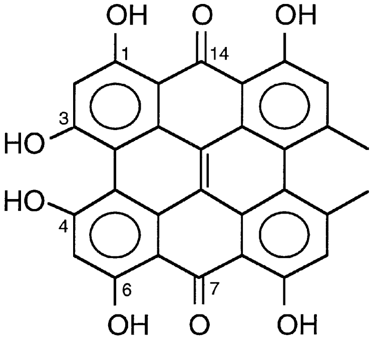
Summary. The association of the *like* and *unlike* configured propeller and butterfly conformers of unionized and *bay*-ionized 7,14-, 1,6-, and 1,7-tautomers of hypericin was investigated by means of semiempirical calculations of the MM3 type. From the various possible stack dimers it was found that the homodimer with *like* configurations of the propeller conformations of unionized hypericin was the most stable one, making them strongly stabilized over any coplanar arrangements. Oligomerization led to further stabilization of the stacked homoassociates up to five units. Adding more units did not lead to further stabilization. Accordingly, dispersion forces between the aromatic systems dominate in associate stabilization, with hydrogen bonding playing a marginal role only. These findings were compared with the rather scarce experimental evidence.

Keywords. Hypericin; Hypericinates; Tautomers; Homoassociation; Heteroassociation.

Introduction

Hypericin (**1**) is a photosensitizing polycyclic quinone which is mainly interesting for its significant virucidal activity and antiproliverative and cytotoxic effects on tumor cells [1]. Among a variety of interesting chemical features of the highly complex structure of **1**, association constitutes a main issue with respect to the resulting physical, chemical, and physiological properties of the system [1b].



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A few types of homoassociates of **1** or its *bay*-hypericinate ion have been described so far. In particular, at low concentrations of $^{(-3)}\mathbf{1}$ in aqueous solvents homodimers are formed which, according to exciton theory, are oriented with parallel mean ring planes and approximately orthogonal dipole axes [2]. At higher concentrations, oligomeric homo-H-aggregates are formed which, according to nuclear *Overhauser* effect and spin lattice relaxation time measurements, are stacked with respect to their mean ring planes, their C_2 axes being rotated against each other by about 180° [3]. This kind of homoassociation leads to a dramatic change in the absorption and fluorescence characteristics of the pigment [2,3]. Moreover, tautomeric changes observed recently have also been blamed *inter alia* to the influence of homoassociation [4].

Since a dependence of tautomerism, dissociation, and torsional isomerism upon homoassociation within the structural complexity of **1** [5] is hardly to be advanced in more detail by experiments, an investigation of this feature by means of a semiempirical model was undertaken and is reported therein.

Results and Discussion

Calculations concerning structural problems of **1** from the semiempirical up to the *ab initio* level have been very successful so far [6–15]. Structural and energetic details of the tautomeric equilibria of **1** and $^{(-3)}\mathbf{1}$ have been advanced thereby. In those parts of the equilibria network accessible by experiment, theoretical results – in particular those of advanced force field calculations – have been in very good agreement with experimental data. For a screening of the various possibilities of associates of **1** and $^{(-3)}\mathbf{1}$ and their tautomers, *ab initio* calculations or even all valence electron methods would have been difficult to execute due to the high CPU times necessary. Therefore, a semiempirical method comprising a force field associated with a semiemprical quantum chemical treatment of the π-electrons should be appropriate to achieve this goal. With respect to these prerequisites the MM3 model [16] was chosen.

Dimerization

As a first step, the dimerization of the propeller conformation of the 7,14-tautomer of hypericin (**1**, precisely to be labeled now as $\mathbf{1}^{7,14}$) was analyzed. To account for the stabilization gained from dimerization, the heats of formation of the isolated species **a** and **b** ($^{\mathbf{a}}H_{\mathrm{f}}, ^{\mathbf{b}}H_{\mathrm{f}}$), and the heat of formation of the dimer $\mathbf{a}\cdot\mathbf{b}$ ($^{\mathbf{ab}}H_{\mathrm{f}}$) were calculated, in turn affording the heat of stabilization H_{s} from $H_{\mathrm{s}} = (^{\mathbf{ab}}H_{\mathrm{f}} - ^{\mathbf{a}}H_{\mathrm{f}} - ^{\mathbf{b}}H_{\mathrm{f}})/2$. Of the various relative orientations of the two units $\mathbf{1}^{7,14}$ to yield the homoassociate, all more or less coplanar orientations in which hydrogen bonding between carbonyl and hydroxyl groups could be envisaged were found to be strongly destabilized (>100 kJ · mol^{-1}) with respect to cofacial orientations in which both the aromatic systems and the functional groups (the latter forming weak out-of-plane hydrogen bonds) could interact. In these cofacial arrangements a pronounced dependence of H_{s} upon the 'dihedral' angle ϕ between the two C_2 axes of the molecules was found. As illustrated in Fig. 1, a slightly

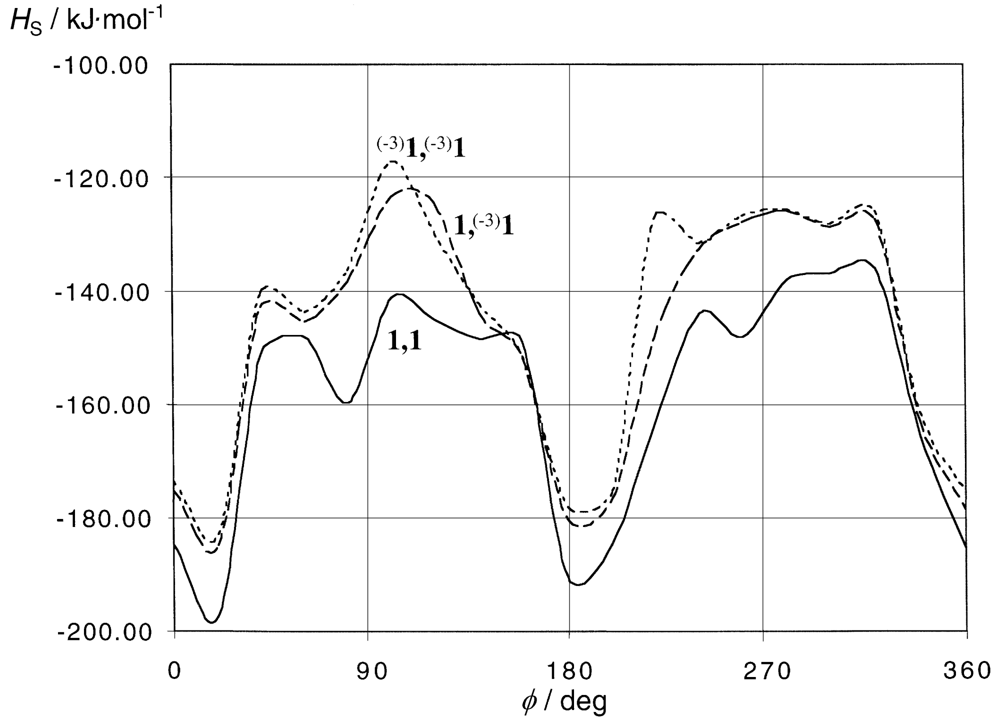

Fig. 1. Heats of stabilization (H_s) of the cofacial dimers of the *like* configured propeller conformation of the 7,14-tautomer of hypericin ($^{P}\mathbf{1}^{7,14}$, $^{P}\mathbf{1}^{7,14}$), is 3-hypericinate ion ($^{P(-3)}\mathbf{1}^{7,14}$, $^{P(-3)}\mathbf{1}^{7,14}$), and their mixed dimer ($^{P}\mathbf{1}^{7,14}$, $^{P(-3)}\mathbf{1}^{7,14}$) in dependence of their mutual orientations given by the dihedral angle ϕ between their C_2 axes (cf. Fig. 2); the angle was constrained in the calculation, and the various other geometrical parameters of the system were optimized with respect to the heat of formation of the system; the dielectric constant ε was set to 1.5

twisted ($\phi = 14°$; cf. Fig. 2) parallel orientation was found to be stabilized over an antiparallel orientation by about $10\,\mathrm{kJ \cdot mol^{-1}}$. All other orientations were found to be strongly destabilized compared with these two states. Thus, the 'orthogonal' orientation ($\phi \approx 80°$) was found to be destabilized by about $40\,\mathrm{kJ \cdot mol^{-1}}$ against the most stable orientation.

As is also shown in Fig. 1, the homoassociated *bay*-3-hypericinate ion dimer ($^{(-3)}\mathbf{1}^{7,14}$,$^{(-3)}\mathbf{1}^{7,14}$) was found to be destabilized significantly against the undissociated hypericin dimer ($\mathbf{1}^{7,14}$,$\mathbf{1}^{7,14}$). In this case a slightly twisted ($\phi = 13°$) parallel orientation of the two phenolate ions was derived to be more stable by about $7\,\mathrm{kJ \cdot mol^{-1}}$ than the system with an antiparallel orientation of their C_2 axes. However, this difference was too small to be significant for deriving a preponderance of the parallel orientation. Accordingly, solvent effects or the like could actually lead to the antiparallel orientation observed experimentally [3].

The heteroassociated dimer of undissociated and dissociated hypericin ($\mathbf{1}^{7,14}$,$^{(-3)}\mathbf{1}^{7,14}$) was found to be stabilized in its twisted parallel orientation ($\phi = 15°$) over the hypericinate dimer; however, it was still destabilized by about $15\,\mathrm{kJ \cdot mol^{-1}}$ against the hypericin homodimer.

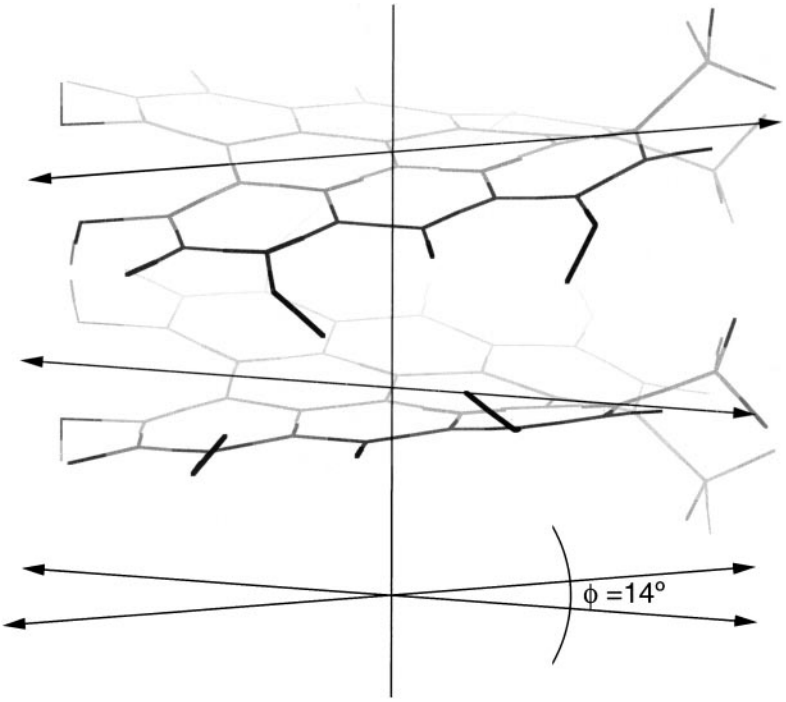

Fig. 2. Ball&Stick wireframe model [17] of the optimum geometry of the propeller conformation of the 7,14-tautomer dimer of hypericin ($^{P}\mathbf{1}^{7,14}$, $^{P}\mathbf{1}^{7,14}$) with a definition of the 'dihedral' stack twist angle angle ϕ

To probe for the sensitivity of the arrangement of the dimers upon the stereochemistry of its constituents, the various conformers of the 7,14-tautomer of hypericin $\mathbf{1}^{7,14}$ were investigated. As had been derived recently, $\mathbf{1}^{7,14}$ or its *bay*-phenolate $^{(-3)}\mathbf{1}^{7,14}$ may exist either in a propeller or a double-butterfly conformation ($^{P}\mathbf{1}^{7,14}$ or $^{B}\mathbf{1}^{7,14}$) which, due to their torsional deformation, may either adopt the (*M*) or the (*P*) configuration: $(M)-^{P}\mathbf{1}^{7,14}$, $(P)-^{P}\mathbf{1}^{7,14}$, *etc.* [7]. The propeller conformation has been found to be favored over the double-butterfly conformation by a few $\mathrm{kJ\cdot mol^{-1}}$, and a significant inversion barrier of about $80\,\mathrm{kJ\cdot mol^{-1}}$ separates the two enantiomeric forms [6,7]. Upon calculating the heats of stabilization of the possible combinations into dimers it turned out that the most stable dimer was the stereochemical homodimer $(P)-^{P}\mathbf{1}^{7,14}$, $(P)-^{P}\mathbf{1}^{7,14}$, or $(M)-^{P}\mathbf{1}^{7,14}$, $(M)-^{P}\mathbf{1}^{7,14}$ (Fig. 2) which was found to be stabilized by $H_{\mathrm{s}}=-201.6\,\mathrm{kJ\cdot mol^{-1}}$ ($\phi=14°$) as compared to the monomers. Dimers of the *like* configured butterfly species $(P)-^{B}\mathbf{1}^{7,14}$,$(P)-^{B}\mathbf{1}^{7,14}$ or $(M)-^{B}\mathbf{1}^{7,14}$, $(M)-^{B}\mathbf{1}^{7,14}$ came next in stability ($H_{\mathrm{s}}=-191.7\,\mathrm{kJ\cdot mol^{-1}}$, $\phi=14°$) and were found to be followed by the dimer of *like* configured propeller and butterfly conformers $(P)-^{P}\mathbf{1}^{7,14}$, $(P)-^{B}\mathbf{1}^{7,14}$ or $(M)-^{P}\mathbf{1}^{7,14}$, $(M)-^{B}\mathbf{1}^{7,14}$ ($H_{\mathrm{s}}=-188.4\,\mathrm{kJ\cdot mol^{-1}}$, $\phi=12°$), the dimer of the *unlike* configured butterfly and propeller conformers $(M)-^{P}\mathbf{1}^{7,14}$, $(P)-^{B}\mathbf{1}^{7,14}$ or $(P)-^{P}\mathbf{1}^{7,14}$, $(M)-^{B}\mathbf{1}^{7,14}$ ($H_{\mathrm{s}}=180.3\,\mathrm{kJ\cdot mol^{-1}}$, $\phi=15°$), the dimer consisting of *unlike* configured

Table 1. Heats of stabilization (H_s/kJ $\cdot$ mol^{-1}) for the dimers of the *like* configured propeller conformers of the neutral and *bay*-ionized hypericin 7,14-, 1,7-, and 1,6-tautomers $^{P}\mathbf{1}^{7,14}$, $^{P(-3)}\mathbf{1}^{7,14}$, $^{P}\mathbf{1}^{1,7}$, $^{P(-3)}\mathbf{1}^{1,7}$, $^{P(-4)}\mathbf{1}^{1,7}$, $^{P}\mathbf{1}^{1,6}$, and $^{P(-3)}\mathbf{1}^{1,6}$

	$^{P}\mathbf{1}^{7,14}$	$^{P(-3)}\mathbf{1}^{7,14}$	$^{P}\mathbf{1}^{1,7}$	$^{P(-3)}\mathbf{1}^{1,7}$	$^{P(-4)}\mathbf{1}^{1,7}$	$^{P}\mathbf{1}^{1,6}$	$^{P(-3)}\mathbf{1}^{1,6}$
$^{P}\mathbf{1}^{7,14}$	−202						
$^{P(-3)}\mathbf{1}^{7,14}$	−199	−188					
$^{P}\mathbf{1}^{1,7}$	−185	−189	−196				
$^{P(-3)}\mathbf{1}^{1,7}$	−174	−181	−183	−196			
$^{P(-4)}\mathbf{1}^{1,7}$	−184	−182	−187	−169	−167		
$^{P}\mathbf{1}^{1,6}$	−168	−188	−144	−151	−153	−173	
$^{P(-3)}\mathbf{1}^{1,6}$	−169	−171	−153	−150	−156	−182	−161

propeller conformers $(M)-^{P}\mathbf{1}^{7,14}$, $(P)-^{P}\mathbf{1}^{7,14}$ ($H_s = -177.0\,\mathrm{kJ}\cdot\mathrm{mol}^{-1}$, $\phi = 180°$), and finally the dimer of *unlike* configured butterfly conformers $(M)-^{B}\mathbf{1}^{7,14}$, $(P)-^{B}\mathbf{1}^{7,14}$ ($H_s = -168.5\,\mathrm{kJ}\cdot\mathrm{mol}^{-1}$, $\phi = 2°$). Interestingly enough, stereochemical homoassociation of the most stable conformational state was thus found to be favored by up to $30\,\mathrm{kJ}\cdot\mathrm{mol}^{-1}$ over all other possible combinations. Calculations of the various stereoisomers of *bay*-phenolate dimers resulted in a similar picture.

Based on these results, the following investigations of dimeric heteroassociates involving ionization and tautomers were restricted to the propeller conformers of the *like* configuration. Bearing in mind that the next metastable tautomers of hypericin were found to be the 1,7- and, in peculiar, the 1,6-tautomers [4,18], only these two were taken into account. Accordingly, the results of calculations concerning the various dimers resulting from such a combination of the *like* propeller conformers of undissociated and *bay*-ionized species of the 7,14-, 1,7-, and 1,6-tautomers of hypericin are compared in Table 1. These calculations revealed several significant issues which might contribute to the stabilization of certain tautomers under certain experimental conditions. First, the homodimer of the 7,14-tautomer ($^{P}\mathbf{1}^{7,14}$, ($^{P}\mathbf{1}^{7,14}$) is the most stable one as compared to the dimers of the 1,7- and 1,6-tautomers, with the latter destabilized by nearly $30\,\mathrm{kJ}\cdot\mathrm{mol}^{-1}$. Second, the homodimers of neutral and *bay*-ionized tautomers were found to be in most cases more stable than the heterodimers – the exceptions were the heterodimers made up of the *bay*-ionized 7,14-tautomer and the neutral 1,7-tautomer, $^{P(-3)}\mathbf{1}^{7,14}$, $^{P}\mathbf{1}^{1,7}$, stabilized by about $1\,\mathrm{kJ}\cdot\mathrm{mol}^{-1}$ over the homodimer of the *bay*-ionized 7,14-tautomer ($^{P(-3)}\mathbf{1}^{7,14}$, $^{P(-3)}\mathbf{1}^{7,14}$), and of the ionized and neutral 1,6-tautomer, $^{P(-3)}\mathbf{1}^{1,6}$, $^{P}\mathbf{1}^{1,6}$, stabilized by about $9\,\mathrm{kJ}\cdot\mathrm{mol}^{-1}$ over the homodimer of the 1,6-tautomer $^{P}\mathbf{1}^{1,6}$, $^{P}\mathbf{1}^{1,6}$. Third, the dimers made up of the neutral and the *bay*-ionized forms, as *e.g.* $^{P}\mathbf{1}^{7,14}$, $^{P(-3)}\mathbf{1}^{7,14}$, were also found to be more stabilized than other combinations. Finally, it should be mentioned that homodimers of the 1,6-tautomer with a 'planar' arrangement to allow for hydrogen bridge formation as discussed recently [4] were also found to be destabilized strongly against the corresponding stacked associates, mostly due to steric interferences between the ring protons.

Oligomers

After having probed the formation of dimers, additional units were attached to form oligomeric stacks. The heat of stabilization $H_{\mathrm{s}}(n)$ of a stack of n molecules to form a homoassociate was calculated from $H_{\mathrm{s}}(n) = H_{\mathrm{f}}(n)/n - H_{\mathrm{f}}(1)$. The results of these calculations are displayed in Fig. 3 for the *like* configured propeller conformers of the 7,14 tautomer ${}^{P}\mathbf{1}^{7,14}$ and for the propeller conformers of the *bay*-ionized 7,14-tautomer ${}^{P(-3)}\mathbf{1}^{7,14}$. First, at this point it should be stressed that varying the dielectric constant in the calculations from 1.5 to 81 did not result in significant changes neither for the dimers nor for the oligomers. This finding together with the highly instable coplanar arrangements alluded to above pointed to mainly dispersion forces between the aromatic moieties as the source of associate stabilization. Obviously, hydrogen bonding and polar interactions play a minor role. Second, as can be seen from the results displayed in Fig. 3, addition of further units to the dimer resulted in a further stabilization of the system, but it did not lead to a further gain in stabilization enthalpy above an oligomer made up of five monomers. Third, the difference in homoassociation behavior between the neutral and the ionized forms of hypericin is rather small with a little less stabilized phenolate associates, and it was found that variation of the stack twist angle ϕ (cf. Fig. 2) from 0–15° did produce but small changes in the stabilization enthalpy. Accordingly, what could be anticipated from these calculations is that at rather low concentrations in certain solvents, hypericin or its hypericinate ion will tend to dimerize, and with larger concentrations will form homoassociates of only a few

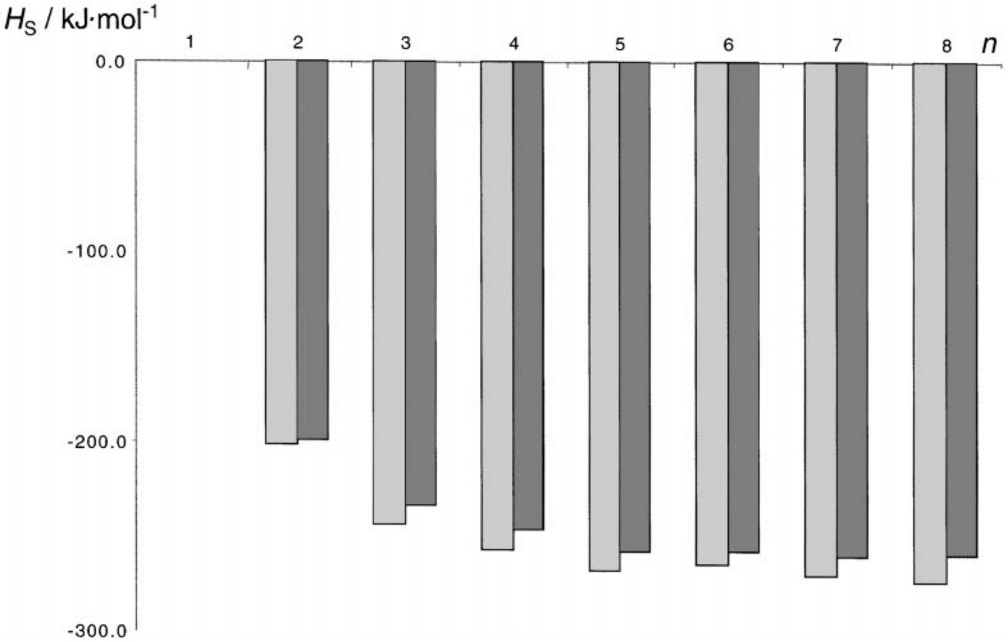

Fig. 3. Heats of stabilization $(H_{\mathrm{s}}(n))$ of stacks $(n = 1\text{–}8)$ of the propeller conformation of the 7,14-tautomer of hypericin $({}^{P}\mathbf{1}^{7,14})_n$ (light bars) and of the *bay*-ionized 7,14-tautomer of hypericin ${}^{P(-3)}\mathbf{1}^{7,14})_n$ (dark bars)

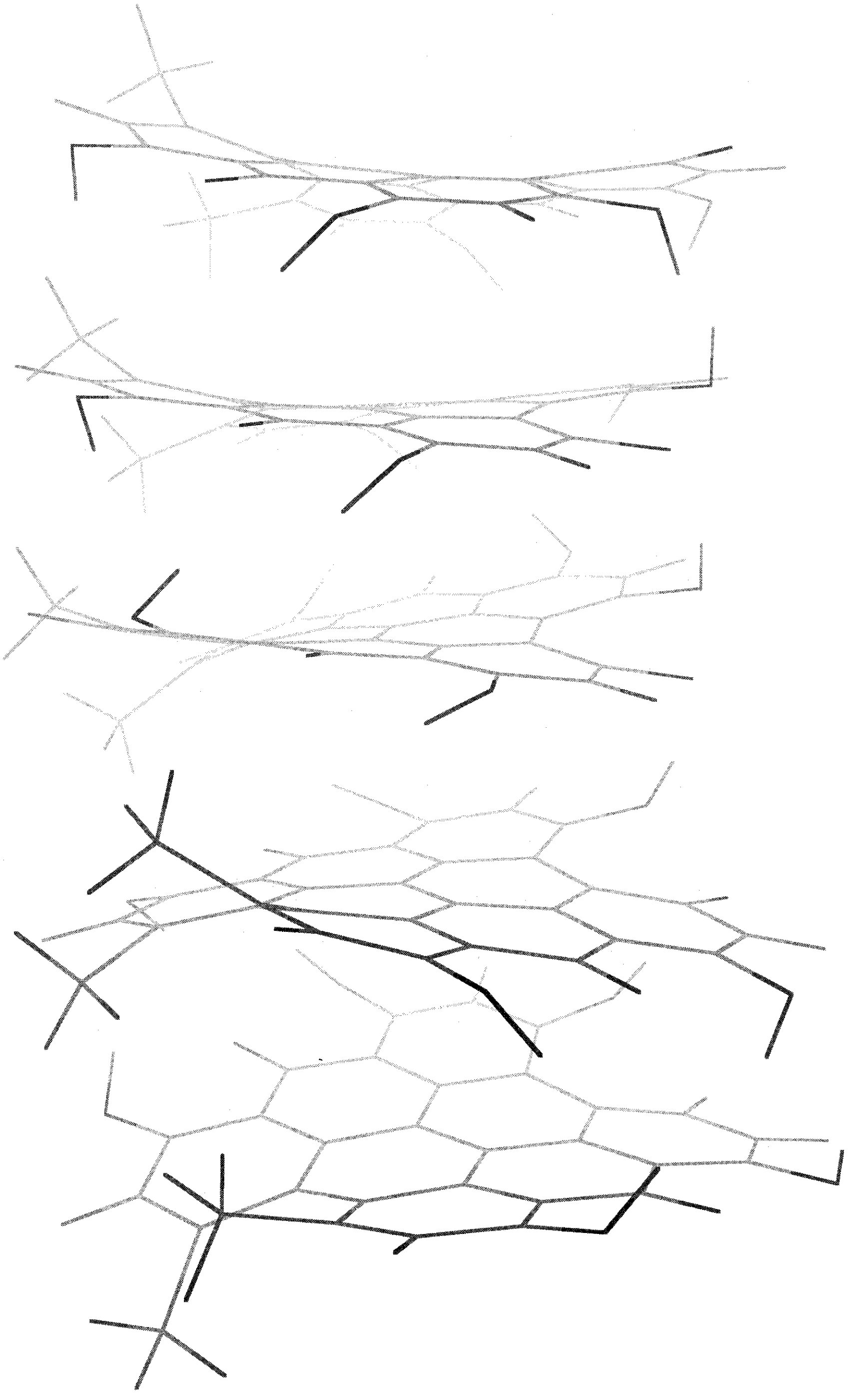

Fig. 4. Optimum geometry of the hypericinate homoassociate pentamer $(^{P(-3)}\mathbf{1}^{7,14})_5$

units. Homoassociates of more than five hypericinate monomers in *DMSO*/H_2O as the solvent have been found experimentally [3]. To illustrate the optimum geometry of a typical stack, that of the hypericinate ion pentamer $(^{P(-3)}\mathbf{1}^{7,14})_5$ is shown in Fig. 4.

In conclusion, the findings presented above are *grosso modo* compatible with the scarce experimental facts accumulated so far. For aqueous ethanol, dimerization of the hypericinate ion has been found at rather low concentrations [2], whereas for higher concentrations oligomers of more than four to five units of antiparallel orientation have been inferred from NMR measurements on aqueous *DMSO* solutions [3]. The 'orthogonal' orientation that has been inferred experimentally for the dimers [2] is instable as judged form the calculations (cf. Fig. 2); however, the solvent could play an important role in the stabilization of certain geometries, and at least partial dissociation of **1** in ethanol could also contribute. However, most important, the calculations described above did not indicate that any stabilization of tautomers or ionization states except for the 7,14-tautomer by means of homo- or heteroassociation can be expected. Therefore, other reasons for the stabilization of the metastable 1,6-tautomer under certain conditions seem to be at work and should be addressed by experimental means.

Methods

For the calculations, the MM3 model was employed throughout using the tinker package [16] on the Origin 2000 computer of the '*Linzer Zentrum für Numerische Simulation*' (LIZENS) of the Johannes Kepler University of Linz. Starting geometries were derived from MM3 calculations. Structures for stacks were built using the program Ball&Stick [17]. Optimization of all geometrical parameters was executed unless stated otherwise in the text above. The dielectric constant was set to 1.5 or 81, thereby modelling unpolar solvents or a water environment.

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