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Tautomerism and Stereochemistry of Hypericin: Force Field, NMR, and X-Ray Crystallographic Investigations

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Summary. Stereochemistry and tautomerism of hypericin, pseudohypericin, and several of their partial structure models were investigated using an MM2 derived force field method. Besides the "propeller" type conformer, which was also found by the X-ray crystallographic study, the complicated energy hypersurface was shown to contain a novel "double-butterfly" conformer of similar stability. The upper limit interconversion barrier between these conformers and their enantiomers was found to be in the order of 115 kJ/mol. ^{1}H-NMR experiments suggested a lower limit interconversion barrier of at least 80 kJ/mol. From the ten tautomers possible in principle, the 7,14-species was derived to be the most stable one by at least 48 kJ/mol.

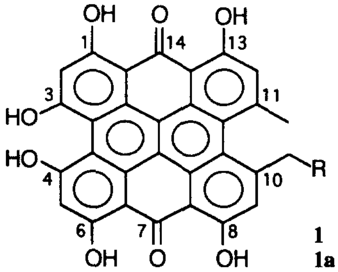
Keywords. Hypericin; Pseudohypericin; Force field calculations; X-ray crystallography; Tautomerism; Interconversion barrier.

Tautomerie und Stereochemie von Hypericin: Untersuchungen mit Hilfe der Kraftfeld-Methodik, NMR-Spektroskopie und Röntgenstrukturanalyse

Zusammenfassung. Die Stereochemie von Hypericin, Pseudohypericin und einigen seiner Partialstrukturmodelle wurde mit Hilfe einer von MM2 abgeleiteten Kraftfeldmethodik untersucht. In der komplizierten Energiehyperfläche wurde neben dem auch durch Röntgenstrukturanalyse gefundenen „Propeller"-Konformeren ein neues „Doppelschmetterling"-Konformer ähnlicher Stabilität aufgefunden. Die obere Grenze für die Interkonversionsbarrieren zwischen diesen Konformeren und ihren Enantiomeren sind in der Größenordnung von 115 kJ/mol. Aus ^{1}H-NMR-Experimenten konnte eine untere Grenze von wenigstens 80 kJ/mol abgeleitet werden. Es wurde gefunden, daß von den zehn prinzipiell möglichen Tautomeren die 7,14-Spezies die um wenigstens 48 kJ/mol stabilste ist.

Introduction

Hypericin (**1**) and pseudohypericin (**1a**) are the photodynamic agents of St. Johns wort [1]. Moreover, **1** constitutes the photopigment of phototaxis in certain algae like *Stentor* [2], and bromo derivatives of **1** were even found in maritime organisms [3]. Recent activities in the investigation of this compound were triggered by its anti-viral and anti-retroviral properties [4]. Therefore it seemed to be of interest to study its fundamental chemistry in more detail [5].



1 R=H
1a R=OH

The present study will focus on the still unanswered qestions of tautomerism and stereochemistry of the hypericin molecule. These aspects will be investigated using X-ray crystallography, NMR spectroscopy, and a semiempirical force field model. The latter has recently been shown to properly describe tautomerism and stereochemistry of condensed aromatic quinones in the case of natural substituted perylene quinones of the cercosporins [6].

Methods and Experimental Details

The force field calculations were performed using the MM2+ program which is based on the QCPE program 395 and the MM2(77) force field [7]. Minor modifications and parametrization have been described in a preceding paper [6]. Molecular models were drawn using the Ball & Stick program [8].

1 and **1a** were prepared according to Ref. [9]. A crystal (0.22 × 0.1 × 0.06 mm) was grown from a saturated pyridine solution of **1** placed in a saturated *n*-hexane atmosphere at 6 °C in the dark. All X-ray experiments were carried out at 85 K on a locally modified STOE 4-circle diffractometer equipped with a locally constructed low temperature gas stream device. CuK_α-radiation without monochromator but with a Ni-filter was used. Unit cell dimensions (obtained by refinement against the diffractometer setting angles of 47 reflections with $8° < 2\vartheta = 29°$): $a = 6.93(3)$ Å, $b = 19.39(8)$ Å, $c = 11.69(4)$ Å, $\alpha = 96.98(31)°$, $\beta = 101.57(31)°$, $\gamma = 107.38(32)°$, $V = 1440.6(6.1)$ Å^3, space group P-1.4171 reflections in the 2ϑ range from 6° to 110° were measured, 4039 of them were unique and 2043 significant [$F > 4\sigma(F)$]. The maximum deviation of the standard intensities was 6.3%.

Reflection statistics indicated a centrosymmetric space group. However, in spite of several efforts with direct methods and with Patterson search techniques, the structure could not be solved in space group P-1. Eventually, we were successful with direct methods in space group P-1, where we obtained two fragments consisting of 56 atoms. This partial structure was used to calculate the position of the center of inversion from three pairs of coordinates. Transformation of the original coordinates to space group P-1 (origin at the center of inversion) and refinement in this space group yielded all atoms except for the hydrogen atoms. The refinement with isotropic a.d.p.'s (atomic displacement parameters) converged at an *R*-factor of about 21% (including empirical absorption- and extinction corrections). Difference Fourier maps showed high peaks (≈ 3 Å^{-3}) in the vicinity of most of the atoms. Refining the non-hydrogen atoms with anisotropic temperature coefficients yielded for all atoms very large thermal ellipsoids whose main axes pointed approximately perpendicular to the plane of the molecule (Fig. 1). Refinement converged at 15.6% with 451 parameters and 2043 observables. The highest peak in the last difference Fourier map was 0.55 eÅ^{-3}, the lowest one -0.73 eÅ^{-3}. The programs used were

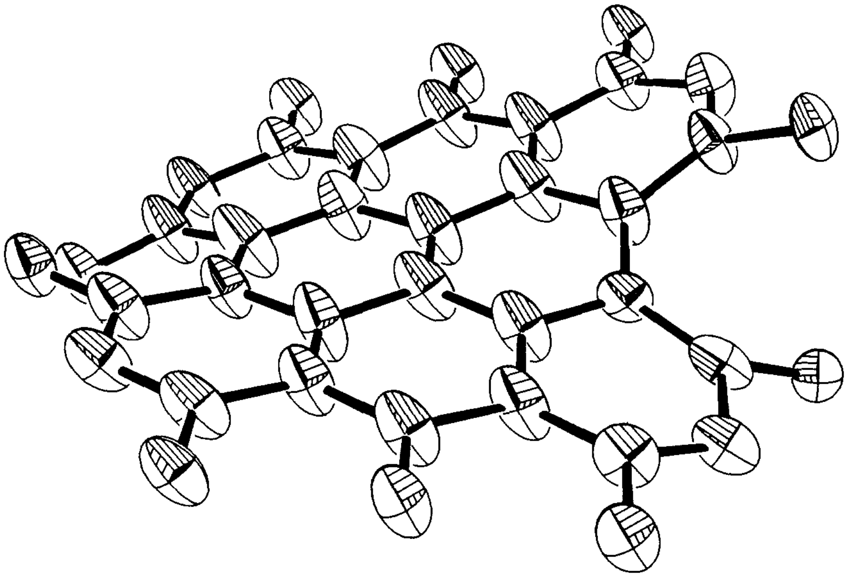


Fig. 1. ORTEP drawing of **1**; thermal ellipsoids are shown with 30% probability

SHELX-86, SHELX-76, and DIFABS [10]. Thus, the questions of tautomerism and overall stereochemistry could be answered beyond any doubts. However, it is not possible to tell whether hypericin was present in the crystal as its phenolate ion coordinated to pyridine as could be thought from its dissociation behavior [5, 9], or if it was the free phenolic system with pyridine as the crystal solvent. The latter is more probable as judged from the observed pyridine ring orientation. Moreover, the pyridine nitrogen atom showed a rather large averaged distance of 11.1 Å from the 3,4-phenolic groups.

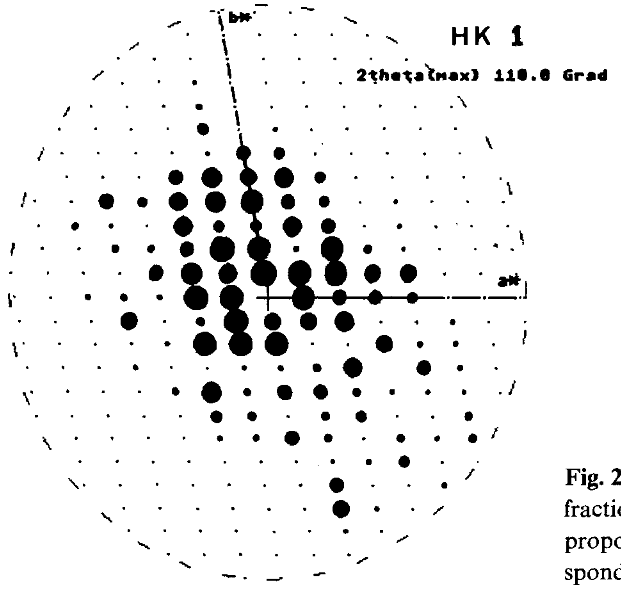


Fig. 2. Section *hkl* through the observed diffraction pattern. The size of the spots is proportional to the intensity of the corresponding peak

The large anisotropic a.d.p.'s together with the poor fit between observed and calculated structure factors ($R = 0.15$) requires some discussion since it could be the result of several effects.

(1) Large systematic errors in the intensity measurements.
(2) Large anisotropic absorption, extinction or volume effect.
(3) A wrong space group assignment (i.e. the space group P1 rather than P-1).
(4) A genuine molecular libration.

We believe that the observed a.d.p.'s are a genuine result of anisotropic molecular libration, since the data were corrected for absorption and related effects ($\mu = 0.89\,\mathrm{cm}^{-1}$, program DIFABS [10]), and we were not aware of any observations which hinted at unusually large systematic errors in the diffraction data. Figure 2 shows a section (*hk*1) through the observed diffraction pattern. A striking feature of this pattern is its very anisotropic shape with a rapid intensity drop in the [110] direction. Such an anisotropy could hardly be accounted for by a different space group assignment *alone*. We therefore conclude that the observed large overall temperature factor is the result of a static or dynamic molecular libration.

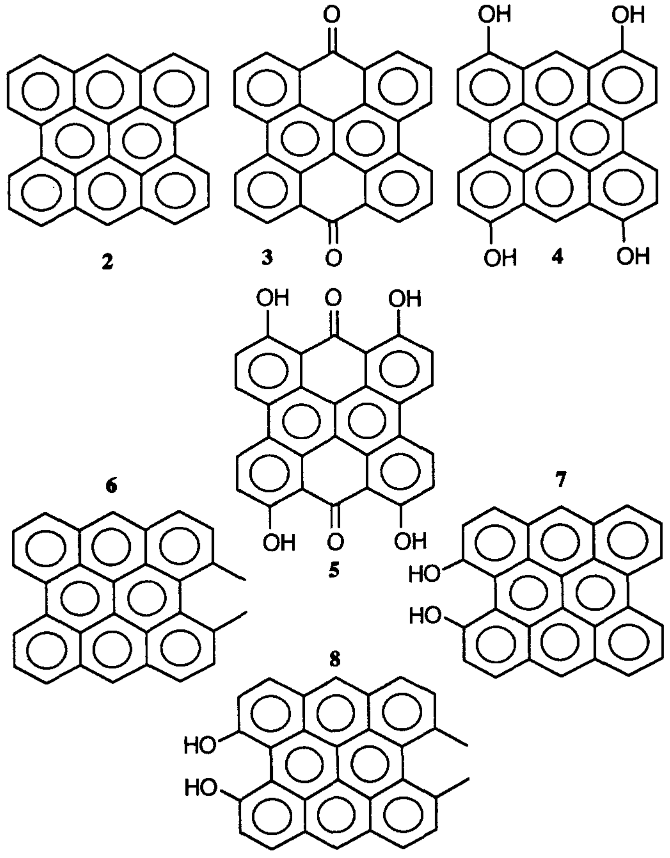
^{1}H-NMR spectra of **1** were recorded on the Bruker WM-360 instrument using $CDCl_3$ as the solvent, and adding (+)-1-phenylethylamine (Fluka) up to a 100 fold molar excess. A splitting of the hypericin signals could not be detected (<0.1 Hz) at room temperature. The temperature dependence of the ^{1}H-NMR signals of **1a** was recorded with *DMSO-d*$_6$ as the solvent. The signals were assigned using common irradiation experiments.

Results and Discussion

To probe the details of the stereochemistry of the highly substituted **1**, the approach of studying partial structures was used. This approach has been successfully used in a recent study of the hydroxyperylenequinones of the cercosporin series [6]. The force field calculation on phenanthroperylene (**2**) resulted in an essentially planar structure with deviations of the $\Theta_{3,3a,3b,4}$ and $\Theta_{10,10a,10b,11}$ dihedral angles of only 0.7°. This result is similar to the one obtained with perylene [6], an aromatic system known from X-ray crystallography to be planar [11]. Comparable results were also obtained for the quinone **3** and the tetrahydroxy derivative **4** ($\Theta_{3,3a,3b,4} = \Theta_{10,10a,10b,11} = 3°$). Upon overlay of the partial structures **3** and **4** into **5**, a small but significant dihedral distorsion of its skeleton ($\Theta_{3,3a,3b,4} = \Theta_{10,10a,10b,11} = 10°$) was obtained.

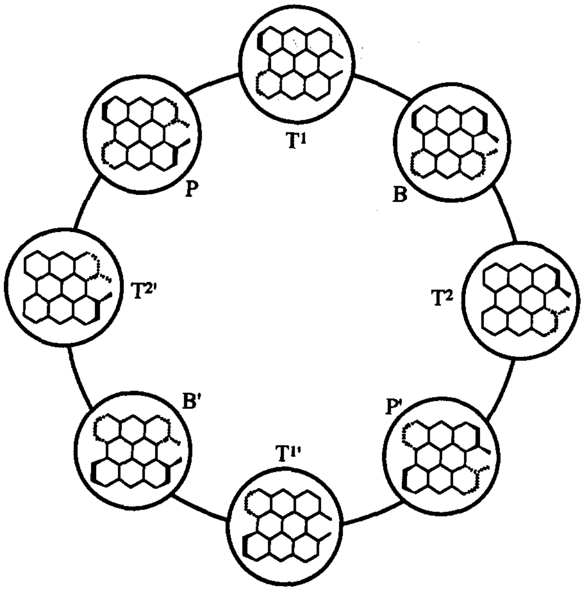
On introduction of two methyl groups in positions 10 and 11 of the phenanthroperylene **2**, the dimethyl derivative **6** was obtained. The force field calculation revealed a system which is still rather flat at the unsubstituted side of the molecule ($\Theta_{3,3a,3b,4} = 4.6°$), but is strongly twisted at the substituted one ($\Theta_{10,10a,10b,11} = 38.3°$).

The upper limit of the transition enthalpy between the enantiomeric conformers was calculated ($\Theta_{10,10a,10b,11} = 0°$) to amount 114.5 kJ/mol. With the 3,4-dihydroxyphenanthroperylene **7** the unsubstituted side of the molecule again remained more or less flat ($\Theta_{10,10a,10b,11} = 4.1°$) whereas the substituted part became strongly twisted ($\Theta_{3,3a,3b,4} = 33.6°$). The upper limit of the interconversion barrier was computed to amount 30.4 kJ/mol. Thus, the torsion angle and the interconversion



barrier involving the hydroxy groups are smaller than those involving the methyl groups. This effect has been observed also in the perylene series [6], and it is corroborated by the smaller ΔG° value of $-OH$ (3.6 J/mol) compared to the one of $-CH_3$ (7.1 kJ/mol) of the *equatorial-axial* equilibrium of substituted cyclohexanes [12].

Addition of the substitution patterns of **6** and **7** resulted in **8**. Due to the combination of two possible conformers at the two sides of the molecule, two diastereomeric conformers were obtained. These are similar to the ones which were derived for the first time in the perylene series (compare the interconversion graph of Scheme 1) and have been termed "propeller" (P and its enantiomer P′) and "double-butterfly" (B and its enantiomer B') conformers [6]. The "propeller" conformer P of **8** was characterized by the two dihedral angles $\Theta_{3,3a,3b,4} = 33.1^\circ$ and $\Theta_{10,10a,10b,11} = 38.3^\circ$. The "double-butterfly" conformer B exhibited $\Theta_{3,3a,3b,4} = 34.2^\circ$ and $\Theta_{10,10a,10b,11} = -38.8^\circ$. The "propeller" conformers P and P′ were calculated to be more stable than the "double-butterfly" P and P′ conformers by 10.7 kJ/mol. The upper limit of the transition barrier involving the hydroxyl part of the molecule (T^1 and $T^{1'}$) was estimated to amount 32.2 kJ/mol, whereas the flipping at the methyl substituted part of the molecule (T^2 and $T^{2'}$) demanded 114.7 kJ/mol. Comparison of these values with those of **6** and **7** suggested that the two biarylic subsystems of **8** behaved quite independently.



Scheme 1

To construct the natural product hypericin (**1**) the quinone partial structure **3** had to be added to system **8**. Again, the conformational situation of **1** could be described by the interconversion graph of Scheme 1. The enantiomeric "propeller" conformers P and P′ of **1** were found to be characterized by the dihedral angles $\Theta_{3,3a,3b,4} = 33.3°$ and $\Theta_{10,10a,10b,11} = 38.7°$, and $\Theta_{3,3a,3b,4} = -33.3°$ and $\Theta_{10,10a,10b,11} = -38.7°$, respectively, which did not differ from the values found for **8**. Accordingly, the geometry of **1** is exclusively determined by the steric requirements of the 3,4-dihydroxy and 10,11-dimethyl substitution pattern. The enantiomeric "double-butterfly" conformers B and B′, which were found to be less stable

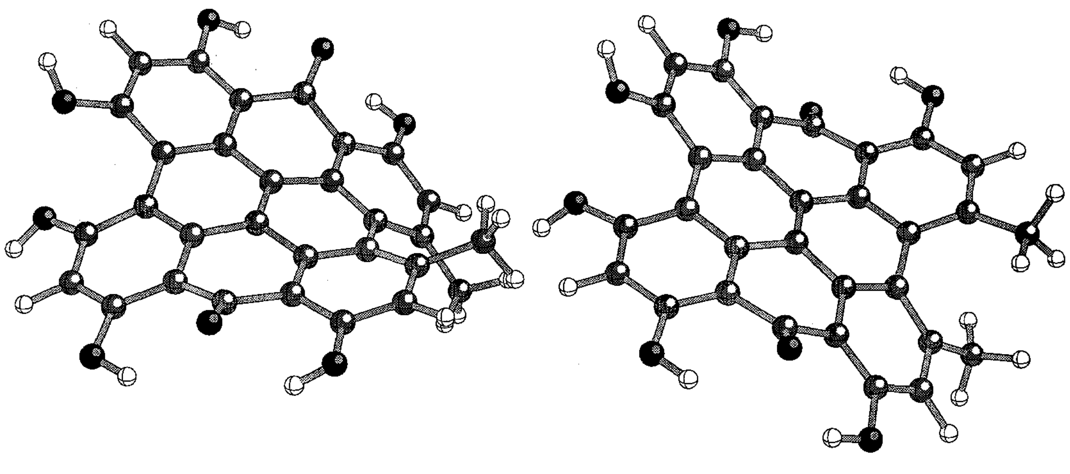


Fig. 3. "Ball & Stick" models [8] of the "propeller" (P, left side) and "double-butterfly" (B, right side) conformations of **1** as derived from force field calculations

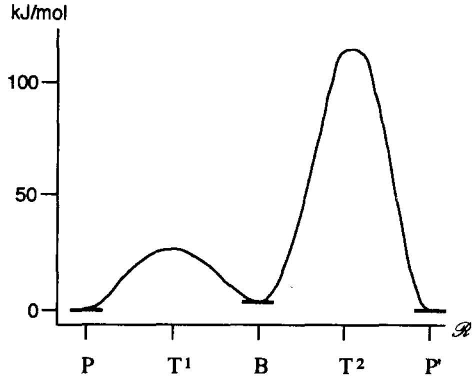


Fig. 4. Enthalpy diagram of the conformational situation of **1** as derived from force field calculations

than the "propeller" conformers by only 1.2 kJ/mol, were characterized by dihedral angles of $\Theta_{3,3a,3b,4} = 31.8°$ and $\Theta_{10,10a,10b,11} = -39.0°$, and $\Theta_{3,3a,3b,4} = 31.8°$ and $\Theta_{10,10a,10b,11} = -39.0°$, respectively. The two diastereomeric conformers P and B are illustrated in Fig. 3. The upper limit transition barriers between enantiomeric conformers were derived to amount to 28.3 kJ/mol ($\Theta_{3,3a,3b,4} = 0°$, $\Theta_{10,10a,10b,11} = 37.4°$) and 113.4 kJ/mol ($\Theta_{3,3a,3b,4} = 32.4°$, $\Theta_{10,10a,10b,11} = 0°$). Comparison with the corresponding values of **8** revealed that the hydroxyquinoid system modulated the conformational situation only marginally. An enthalpy diagram of the conformational interconversions of **1** along the reaction coordinate $\mathscr{R}$ from one "propeller" conformer P *via* the transition conformation T^1 to the corresponding "double-butterfly" conformer B, and the transition conformation T^2 into the enantiomeric "propeller" conformer P′ according to the interconversion graph of Scheme 1 is illustrated in Fig. 4. Although a propeller type isomer of **1** had been indicated occasionally in Ref. [4, 13], the rather complicated stereochemical features of this compound had escaped attention so far.

With respect to the geometrical features of **1** (or, less probable, its pyridinium salt; cf. the exp. section) X-ray crystallography clearly indicated the "propeller" conformer as the predominant one in the crystalline state. It was characterized by dihedral angles of $\Theta_{3,3a,3b,4} = 21.8°, \Theta_{10,10a,10b,11} = 28.5°$. A Ball & Stick model of **1** together with the pyridin solvent is given in Fig. 5.

Figure 6 displays an overlay of the structure of **1** as derived from X-ray crystallography with the force field structural result of its "propeller" conformer. Thus, with only minor deviations experimental and calculated results nicely agreed.

The upper limit of an inversion barrier between the enantiomeric conformers P and P′ of 113.4 kJ/mol suggested that at room temperature they should be stable enough to be detected. However, circular dichroism measurements on fresh preparations of natural **1** did not show optical activity [9]. Of course, this may imply that the natural compound is a racemic mixture of thermally stable atropisomers. Now, if **1** was present as a racemic mixture, addition of a chiral enantiomerically pure agent to produce a diastereomeric interaction should have resulted in species which could have been differentiated by spectroscopic measurements. Nevertheless, addition of (+)-1-phenylethyl amine to **1** up to a hundred-fold molar excess did not produce any splitting of ^{1}H- and ^{13}C-NMR signals at room temperature. Obviously, the diastereomeric species produced in this way did not differ sufficiently to allow

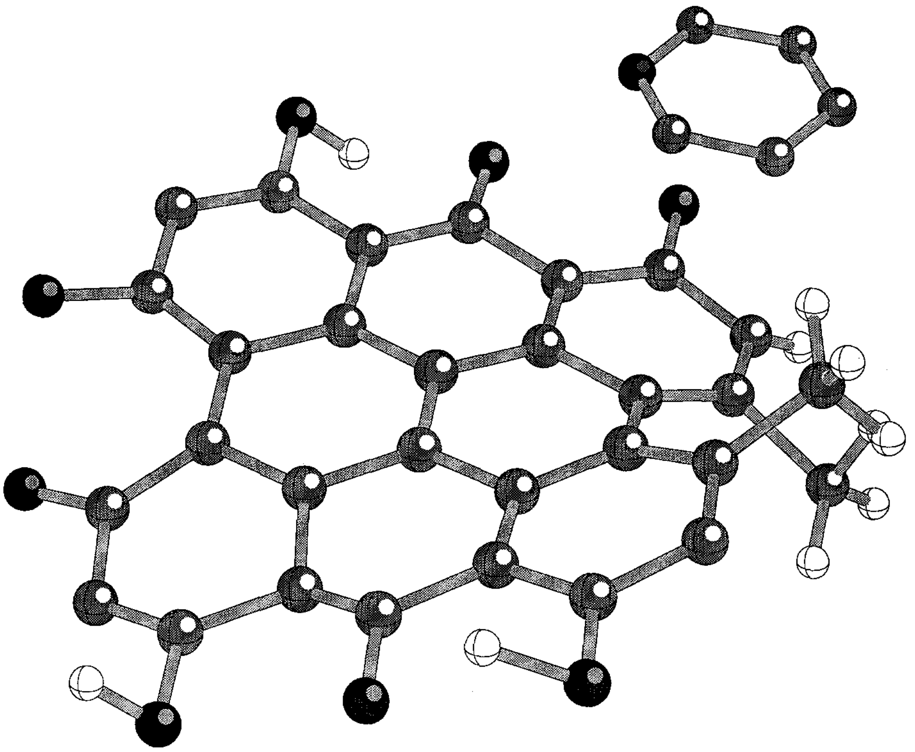


Fig. 5. "Ball & Stick" model [8] of the X-ray crystallographic analysis of **1** together with one crystal solvent pyridine molecule

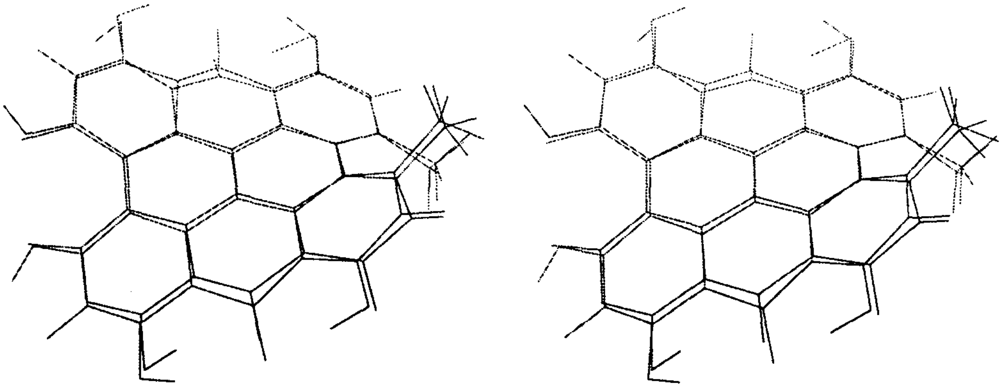


Fig. 6. "Ball & Stick" wire frame stereo model [8] overlay of the "propeller" conformer deduced from the force field calculation and the X-ray crystallographic analysis of **1**

their detection. The enthalpy difference of only 1.2 kJ/mol between the "propeller" and the "double-butterfly" conformers suggested that the latter could be present in solutions. But the rather low exchange barrier between them would prevent detection of small amounts of the latter. Indeed, no experimental indication could be found in the spectra of **1** so far.

In pseudohypericin (**1a**) dissolved in *DMSO-d*$_6$ the 10-hydroxymethyl group exhibited a ^{1}H-NMR AMX system at 300 K (for its spectrum in $CDCl_3$ solution compare [14]). It was caused by the coupling between the diastereotopic methylene protons on the one hand ($\delta_A = 4.70$ ppm, $\delta_X = 5.12$ ppm, $J_{AX} = 12$ Hz) and the hydroxyl proton on the other hand ($\delta_M = 5.32$, $J_{AM} = 5$ Hz, $J_{MX} = 7$ Hz). At 350 K the rapid exchange between CH_2OH and residual solvent H_2O lead to the coalescence of the coupling between the hydroxyl and the methylene protons. The remaining AX system persisted up to the measurement limit of 405 K. Thus, the interconversion barrier should amount to at least 80.4 kJ/mol. The force field calculations yielded "propeller" and "double butterfly" conformers characterized by the dihedral angles $\Theta_{3,3a,3b,4} = 32.7°$ and $\Theta_{10,10a,10b,11} = 41.8°$, and $\Theta_{3,3a,3b,4} = 32.5°$ and $\Theta_{10,10a,10b,11} = -39.9°$, which are also energetically close to the values calculated for **1**. An upper limit of the high interconversion barrier of 118.7 kJ/mol was calculated for **1a**. This was about 5 kJ/mol higher than the one of **1**, nicely reflecting the more space demanding hydroxymethyl compared to the methyl group. In conclusion, according to the calculations and experiments on **1** and **1a** the interconversion barrier of the system should have a height between about 80 and 117 kJ/mol. Therefore atropisomers of **1** and **1a** could be isolable in principle although their thermal stability at room temperature could be not very high.

To investigate the tautomeric situation of **1** by means of force field calculations, the total number of possible tautomers was first deduced to be ten by analyzing the various proton shifts possible according to Scheme 2. These tautomers were characterized by the position numbers of the respective carbonyl groups, e.g., $Q^{7,14}$.

Figure 7 shows interconversion graph according to Scheme 2. It also illustrates the energetic situation of the tautomers of **1** as derived from the force field calculations of their conformational global minima (in all cases it was found to be a "propeller" conformer with dihedral angles of $\Theta_{3,3a,3b,4} = 21...34°$ and

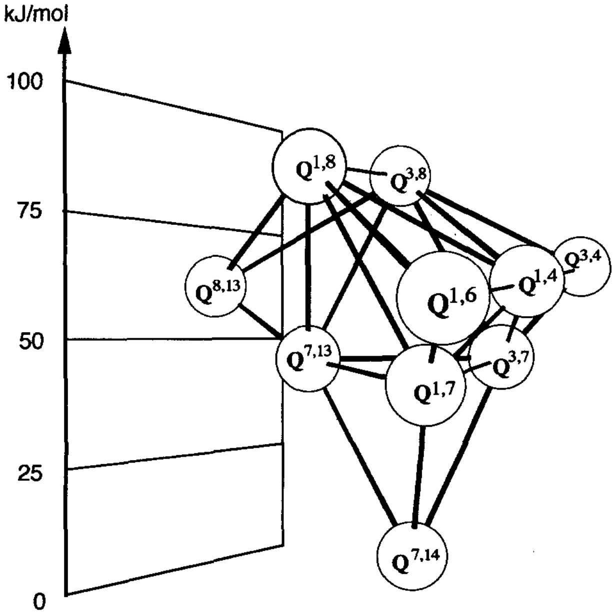
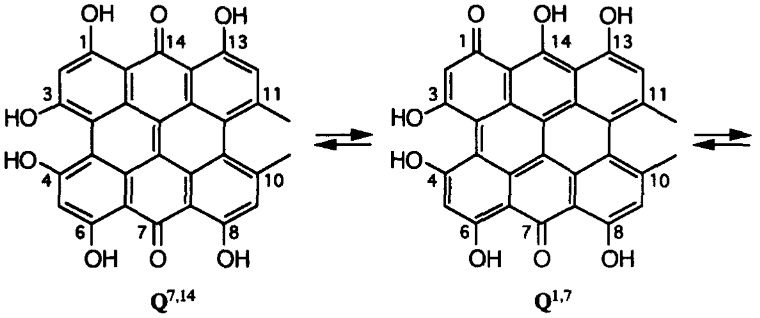


Fig. 7. Enthalpy difference scaled interconversion graph of the tautomers of **1**



Scheme 2

$\Theta_{10,10a,10b,11} = 32...41°$). The graph shows that the $Q^{7,14}$ tautomers is the most stable one by at least 47.9 kJ/mol. This tautomer had been recognized as the one present in preparations of **1** throughout Ref. [1,2,4]. It was corroborated for the crystalline state by the X-ray crystallographic results. Average bond lengths of the two carbon oxygen bonds in position 7 and 14 of 1.31 Å as compared to an average carbon oxygen distance of 1.36 Å of the other groups were observed. For solutions the $Q^{7,14}$ tautomer has been derived also as the most stable, and thus, predominant one [9].

It is interesting to note, that the stabilization of destabilization of the various tautomers resulted mainly from the differences in their SCF-π-energy. This effect, which strongly disfavors tautomers with a carbonyl group in positions 3 or 4 as well as the combinations $Q^{1,8}$ or $Q^{3,8}$, is modulated by steric interactions by only a few kJ/mol. A simple graph theoretical procedure [15] reproduced this π-effect also quite well: only with $Q^{7,14}$ the overall index of aromaticity attained its maximum value of 4.00. In all other cases this number is considerably smaller, e.g., for $Q^{1,7}$ it was calculated to amount only 2.00.

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