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On the Tautomerism of Hypericin: The 1,6-Dioxo Tautomer

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Summary. The 1,6-dioxo-tautomer of hypericin was obtained by basic and BF_3 catalyzed tautomerization of the natural and most stable 7,14-dioxo-tautomer. The isolation of this tautomer was aided by its insolubility in methanol. It was identified and characterized by spectroscopic methods, and its detailed structure was derived by means of force field calculations.

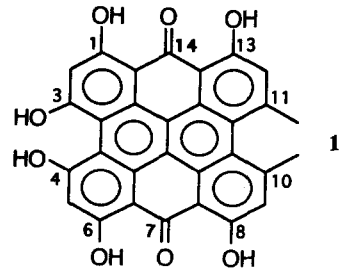
Keywords. Hypericin; 7,14-Dioxo-tautomer; 1,6-Dioxo-tautomer; NMR; UV-Vis; Fluorescence; Force field calculations.

Zur Tautomerie des Hypericins: Das 1,6-Dioxo-Tautomere

Zusammenfassung. Das 1,6-Dioxo-Tautomere des Hypericins wurde durch basen- und BF_3-katalysierte Tautomerisierung des natürlichen, stabilen 7,14-Dioxo-Tautomers erhalten. Dessen Isolierung wurde durch die Schwerlöslichkeit in Methanol ermöglicht. Es wurde durch spektroskopische Methoden identifiziert und charakterisiert, und seine detaillierte Struktur wurde aus Kraftfeld-Rechnungen abgeleitet.

Introduction

Hypericin (**1**) is the photodynamic pigment of *Hypericum* species [1]. It serves also as the photosensory pigment of the photophobic response in certain algae [2]. Recent interest in this type of compounds has been triggered by observation of its anti-viral, anti-retroviral, and photodynamic properties [3].



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The structural details of **1** have been a target of several investigations. Whereas its constitution has been elucidated mainly by means of synthesis [4], its stereochemical aspects and its tautomeric system (**1** occurs as the $\mathbf{Q}^{7,14}$ tautomer as shown in its formula; $\mathbf{Q}^{m,n}$ denote the tautomers with their two oxo substituents in positions *m* and *n*) have been solved only recently by means of spectroscopic measurements [5], X-ray crystallography [6], and force field calculations [6]. According to a fundamental analysis [6] ten tautomers of **1** are possible in principle. As illustrated in Fig. 1 by means of an enthalpy scaled interconversion graph [6] the tautomers of **1** are interconnected by one-proton transfer modes.

The present investigation was aimed at obtaining and possibly characterizing any further tautomers of **1**.

Results and Discussion

During our studies of the spectroscopic properties of **1** [7], as well as on attempts to synthesize imino derivatives of **1**, it was observed that in certain solvents, like tetrahydrofuran at high concentrations, the UV-Vis spectrum of **1** changed. Shoulders at the low wavelength slopes of its absorption bands developed. It turned out that the best conditions to obtain the material which gave rise to those shoulders, were to use tetrahydrofuran as the solvent, catalyze the isomerization by means of trifluoroboron diethyletherate, and use the low solubility of the resulting material in methanol to enrich it (see Experimental Part).

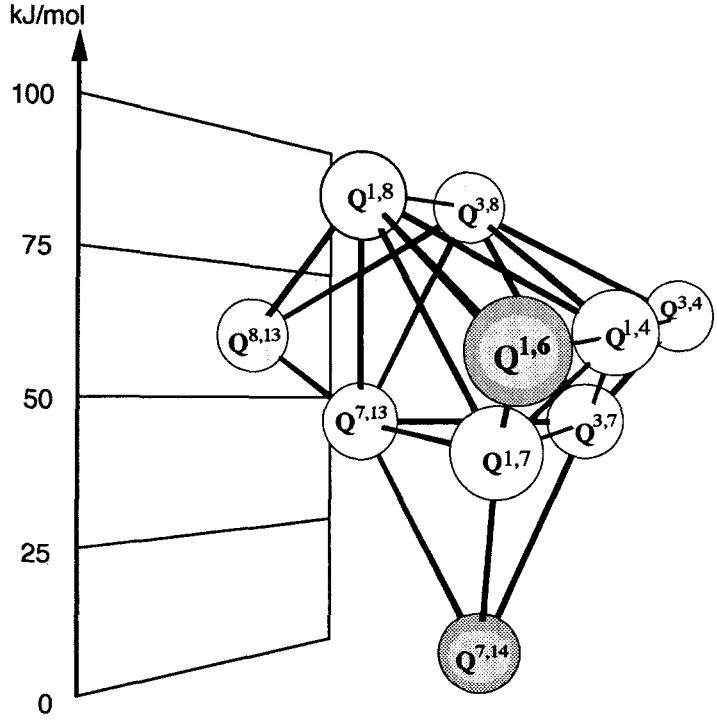


Fig. 1. Enthalpy scaled interconversion graph for the tautomers of **1** according to force field calculations [6]; $\mathbf{Q}^{m,n}$ denote tautomers with the carbonyl groups in positions *m* and *n*. The most stable $\mathbf{Q}^{7,14}$ tautomer and the one which was characterized in the present study ($\mathbf{Q}^{1,6}$) were shaded

First of all, it turned out that the spectroscopic properties of this new isomer were typical of a tautomer. Thus, the numbers of signals in its ^{1}H- and ^{13}C-NMR spectra were the same as in the corresponding spectra of the naturally occuring $\mathbf{Q}^{7,14}$ tautomer of **1**. The second fact in favour of its tautomeric structure was its thermal reversion to the educt. It took two days of heating at 80 °C in dimethyl sulphoxide until all of the isomer was converted back into its educt with less than 0.1% left. This result allowed an estimation of a lower limit of $\Delta G°$ of about 20 kJ/mol for the equilibrium between the two tautomers. The tautomerization was found to be acid catalyzed, however, bases like amines did not change the tautomerization velocity.

To investigate the structural details of this new hypericin tautomer, the NMR spectra were analyzed. As the number of proton and carbon-13 signals remained unchanged upon isomerization the new tautomer had to be one with C_{2v} symmetric substitution pattern. The $\mathbf{Q}^{7,14}$, $\mathbf{Q}^{1,6}$, $\mathbf{Q}^{8,13}$ and $\mathbf{Q}^{3,4}$ tautomers belong to that symmetry group. From NOE measurements of **1** and its tautomer **taut-1** (Fig. 2), the 10,11-methyl signal was unequivocally correlated with the 9,12-proton signal. The latter one displayed a small, but distinct NOE to the neighboring 8,13-hydroxyl group signal. Accordingly, the new tautomer was assigned the $\mathbf{Q}^{1,6}$ structure.

This assignment was corroborated by the relative chemical shifts observed in the proton signals. Whereas the 2,5-proton signal was shifted by about 0.3 ppm, the 10,11-methyl and the 9,12-proton signals were not shifted to that extent (Fig. 2). The ^{13}C-NMR spectra of **1**-$\mathbf{Q}^{7,14}$ and the $\mathbf{Q}^{1,6}$ tautomer shown in Fig. 3 were also in agreement with the structural assignment advanced above. In contrast to the minor shifts observed for the carbon signals of the C7–C14 peripheral region of the molecule, those in the C1–C6 peripheral region were up to more than ten ppm.

As shown in Fig. 4 the prominent absorption bands of the $\mathbf{Q}^{1,6}$ tautomer of **1** were hypsochromically shifted compared to the $\mathbf{Q}^{7,14}$ tautomer by about 10 nm.

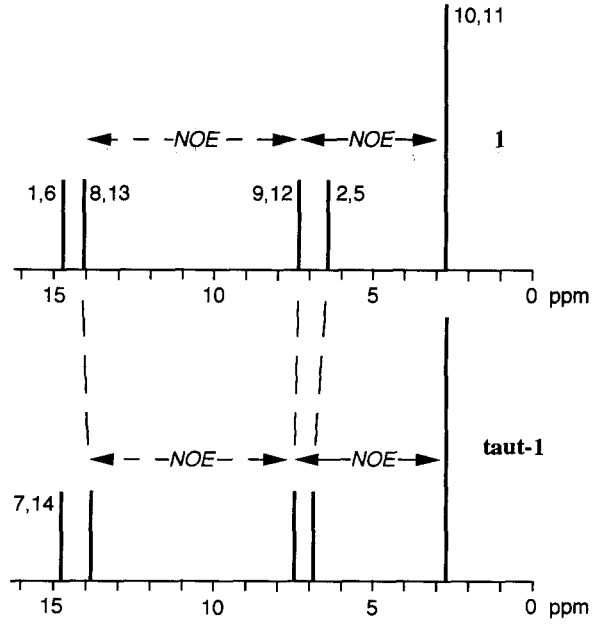


Fig. 2. Schematic ^{1}H-NMR spectra of the diethylammonium salts of **1** and of its tautomer **taut-1** (*DMSO-d_6*). The signals of the cation were omitted for sake of clarity

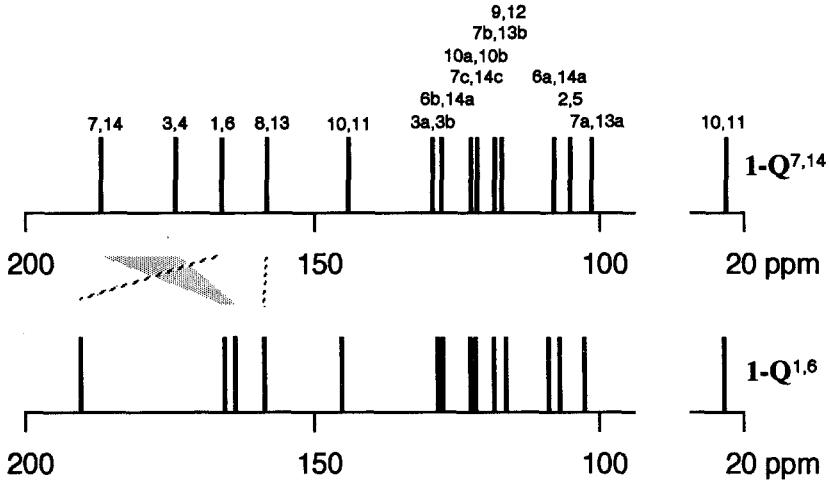


Fig. 3. Schematic ^{13}C-NMR spectra of **1-Q**^{7,14} and its tautomer **1-Q**^{1,6} (*DMSO-d*$_6$) as their diethylammonium salts. The signals of the cation were omitted for sake of clarity

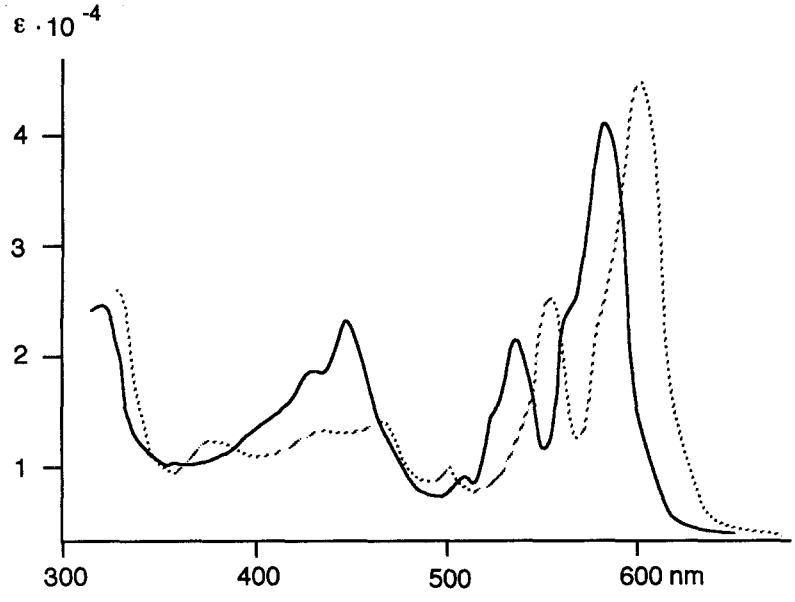
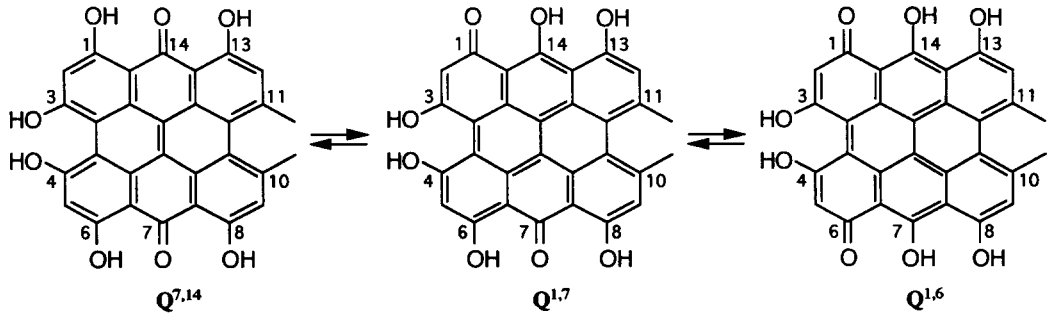


Fig. 4. UV-Vis spectra (ethanol) of **1** (dashed) and its tautomer **Q**^{1,6}

Whereas in the thermodynamically less stable tautomer the long wavelength band intensity was found to be decreased, the intensity of the band system between 400 and 500 nm was enhanced. Judged from the model of the electron in a box, this effect could point to a system in which the "dimensions" of the two main orthogonal transitions changed. With regard to the observed hypsochromic shift, the aromaticity index [8] of the **Q**^{1,6} tautomer has been derived to be 2.0, whereas it was 4.0 in the case of the **Q**^{7,14} tautomer [6]. Accordingly, the conjugation was decreased in the



Scheme 1

$Q^{1,6}$ tautomer, and hence the spectrum should be shifted hypsochromically as seen in Fig. 4.

The fluorescence spectrum of the $Q^{1,6}$ tautomer displayed a rather small Stokes shift of 4 nm, and the fluorescence quantum yield was measured to be 0.3. Both data are similar to those found for the $Q^{7,14}$ tautomer of **1** [7, 9].

With respect to the mechanistic details of the tautomerization process we were only able to speculate. However, the $Q^{1,7}$ tautomer (Scheme 1) has to be an intermediate according to the one proton transfer step interconversion graph given in Fig. 1. Of course, the formation of the $Q^{1,6}$ tautomer should not take place as judged from the standpoint of thermodynamics. Presumably, the tautomerization process leading to the $Q^{1,6}$ tautomer resulted from consecutive chelation and hydrolysis steps to overcome the restrictions of the thermodynamic situation. This process is reminescent of the chelation-hydrolysis procedure which has been used to prepare a lactim tautomer of a bilindione [10]. Interestingly enough, irradiation of both tautomers in ethanol solution did not result in changes of the absorption spectra. Thus, the tautomers of **1** are not interconvertible by means of a photo-tautomerization process.

Detailed force field calculations of the $Q^{1,6}$ tautomer revealed a "propeller" conformer of the skeleton which was stabilized compared to the "double-butterfly"

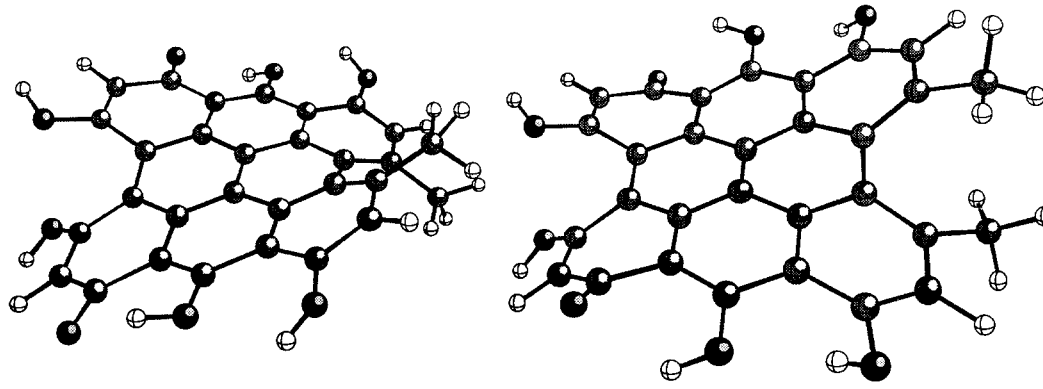


Fig. 5. Ball & Stick models [14] of the "propeller" and "double-butterfly" conformers of the $Q^{1,6}$ tautomer of **1** as derived from force field calculations

conformer by 2.1 kJ/mol. This value was similar to the one derived for the corresponding conformers of the $\mathbf{Q}^{7,14}$ tautomer [6]. The geometry of the "propeller" conformer was characterized by dihedral angles at the two biaryl sites of $\Theta_{3,3a,3b,4} = 29.6°$ and $\Theta_{10,10a,10b,11} = 40.6°$. Comparison with the corresponding torsional angles in the $\mathbf{Q}^{7,14}$ tautomer, which have been found to amount to $\Theta_{3,3a,3b,4} = 33.3°$ and $\Theta_{10,10a,10b,11} = 38.7°$ [6] showed that the overall torsional deformation of the "propeller" conformers in the two tautomers was quite similar. The "double-butterfly" conformer of the $\mathbf{Q}^{1,6}$ tautomer was found to be characterized by the dehedral angles $\Theta_{3,3a,3b,4} = 27.9°$ and $\Theta_{10,10a,10b,11} = -40.9°$. For the $\mathbf{Q}^{7,14}$ tautomer these angles have been found to amount to $\Theta_{3,3a,3b,4} = 31.8°$ and $\Theta_{10,10a,10b,11} = -39.0°$ [6]. Again, these deformation parameters are similar in both tautomers. The two conformers of the $\mathbf{Q}^{1,6}$ tautomer are shown as Ball & Stick models in Fig. 5.

The upper limit interconversion barriers between the two enantiomeric "propeller" conformers *via* a transition state with a planar "right side" biaryl fragment, the "double-butterfly" conformer and another transition state with a planar "left side" biaryl fragment were found to amount to 30.1 kJ/mol and 119.9 kJ/mol. These values should be compared with the corresponding upper limit interconversion barriers of the $\mathbf{Q}^{7,14}$ tautomer which have been derived to amount to 28.3 kJ/mol and 113.4 kJ/mol [6]. The $\mathbf{Q}^{7,14}$ tautomer was calculated to be more stable by 68.9 kJ/mol than the $\mathbf{Q}^{1,6}$ tautomer. This high destabilization of the $\mathbf{Q}^{1,6}$ tautomer was found to be mainly due to the difference in electronic structure as judged from the difference in their aromaticity indices mentioned above and the smaller stabilization energy in the MO part of the force field calculations. Different hydrogen bonding systems and steric interactions played only a minor part.

Experimental Part

The melting point was taken by means of a Kofler hot stage microscope (Reichert, Vienna). ^{1}H-, ^{13}C-, IR-, UV-VIS-, and fluorescence-spectra were recorded by means of the Bruker-WM-360-, and AC-200-, Biorad-FT-IR-45-, Hitachi-U-3210, and F-4010-instruments. For fluorescence spectroscopy ethanol, 95% of "für die Fluoreszenzspektroskopie" grade (Merck), was used as the solvent. Rhodamine B served as the fluorescence standard. **1** was prepared according to [5, 11].

For the force field calculations the MM2+ program [12] was used; its parameter adaption was described earlier [6, 13].

Preparation of the $\mathbf{Q}^{1,6}$ *Tautomer of* **1**

0.1 mmol of the potassium salt of **1** [9] was dissolved in 40 ml tetrahydrofuran and 0.1 ml of freshly distilled $BF_3 \cdot Et_2O$ was added. After stirring for 15 min under an argon atmosphere, 2 ml diethylamine was added and the mixture kept for additional 14 h at room temperature. The mixture was poured into 15 ml methanol and filtered over a short column of Sephadex LH-20. After vaccum evaporation of the solvent the residue was triturated with methanol to free it from methanol soluble material. The remaining solid (10% yield) was soluble in dimethyl sulfoxide, or to a lesser extent in acetone, and already consisted of the pure tautomer. A small contamination with **1** (<2%) could not be removed completely.

The same product could also be obtained using acetonitrile as the solvent and ethyl-diisopropylamine as the base, but the yield was low. As well, the $BF_3 \cdot Et_2O$ catalyst could be omitted, however, yields dropped dramatically.

M.p.: not until 330 °C. To provide sufficient solubility to record the NMR spectra the diethylamine salt was prepared *in situ* by adding appropriate amounts of the base. ^{1}H-NMR (*DMSO-d*$_6$, δ, 360 MHz):14.16(s, OH-7, 14), 13.73(s, OH-8,13), 7.41(s, H-9,12), 6.78(s, H-1,6), 2.70(s, CH_3-10,11) ppm; the corresponding signals of the diethylammonium ion appeared at 1.13, 2.88, and 8.17 ppm. Under these conditions the corresponding signals of the $\mathbf{Q}^{7,14}$ tautomer of **1** were observed at 14.64, 14.01, 7.29, 6.41, and 2.61 ppm. ^{13}C-NMR (*DMSO-d*$_6$, δ, 90 MHz): 186.35, 166.87, 165.92, 162.30, 145.72, 126.06, 125.85, 121.30, 120.70, 116.87, 109.67, 108.35, 104.40, 119.32, 23.95 ppm (for partial assignments see Fig. 3 and for those of the stable tautomer compare [9]). UV-Vis(ethanol): $\lambda_{max.} = 579(32\,500)$, 569(sh, 18 200), 538(15 900), 529(sh, 10 200), 503(5 400), 455(16 000), 431(sh, 11 000), 326(21 300), 280(32 500), 264(sh, 28 300) nm(ε). Fluorescence(ethanol): $\lambda_{excit.} = 538$ nm, $\lambda_{fluor.} = 583$, 630(sh), rel. intensity 4:1, $\Phi_f = 0.3$. IR(KBr): $\nu = 1624$, 1590, 1470 cm^{-1}.

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