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A Structural Proof for the Hypericin 1,6-Dioxo Tautomer

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Summary. Using 2D ROESY ^{1}H NMR spectroscopy it could be unequivocally shown from nuclear *Overhauser* effects and intramolecular exchange correlations of strategic signals that hypericin as well as its 3-hypericinate ion are present as the tautomers with the carbonyl groups located in positions 7 and 14 in polar solvents like dimethylsulfoxide. In apolar solvents like tetrahydrofuran hypericin prevails as the 1,6-dioxo tautomer.

Keywords. Hypericin; Tautomers; ^{1}H NMR 2D ROESY; Nuclear *Overhauser* effect.

Ein Nachweis für die Struktur des 1,6-Dioxo-Tautomeren des Hypericin

Zusammenfassung. Mit Hilfe der 2D-ROESY-^{1}H-NMR-Spektroskopie konnte über Kern-*Overhauser*-Effekte und Austauschkorrelationen strategischer Signale zweifelsfrei nachgewiesen werden, daß Hypericin und sein 3-Hypericination in polaren Lösungsmitteln wie Dimethylsulfoxid als Tautomere mit den Carbonylgruppen in Positionen 7 und 14 vorliegen. In unpolaren Lösungsmitteln wie Tetrahydrofuran dominiert das 1,6-Tautomere.

Introduction

Interest in hypericin (**1**), a natural photosensitizing polycyclic quinone, has become elicited in the past decade due to its virucidal activity as well as to its antiproliferative and cytotoxic effects on tumor cells [1]. Although investigations on **1** have started early this century, its molecular structural aspects are still not understood in every detail. Thus, according to *Brockmann's* classical work **1** has been assigned the tautomer with the carbonyl groups in positions 7 and 14 (designated as the tautomer $\mathbf{Q}^{7,14}$ in Scheme 1) [2]. This tautomeric situation has been proven for the 3-hypericinate ion by X-ray structural analyses of two crystal forms of its pyridinium derivative [3, 4]. Fundamental analyses hold that ten *Kekulé* and six non-*Kekulé* structured tautomers of **1** are possible in principle [3, 5–7]. Among all these tautomers, the $\mathbf{Q}^{7,14}$ tautomer has been found to be the most stable one by application of a variety of semiempirical quantum chemical methods up to *ab initio* calculations, regardless of its state of ionization [3, 6, 8, 9]. By

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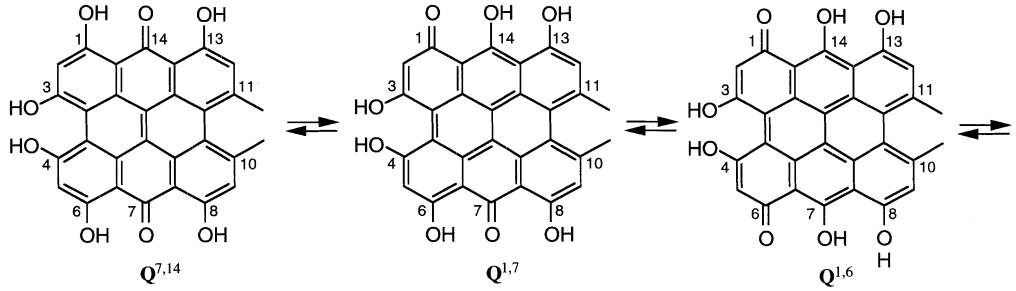

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Scheme 1

means of the latter method, the $\mathbf{Q}^{1,7}$ and $\mathbf{Q}^{1,6}$ tautomers have been found to be less stable than the $\mathbf{Q}^{7,14}$ tautomer by 45 and 83 $kJ \cdot mol^{-1}$, comprising the next most stable tautomers [8].

From this point of view it is highly intriguing that in low dielectric solvents like tetrahydrofuran **1** has been found to exist in a long-lived metastable state which very probably possesses the $\mathbf{Q}^{1,6}$ tautomeric constitution [5, 10]. In diluted solutions it experiences transformation into the most stable $\mathbf{Q}^{7,14}$ tautomer, which is the predominating species in polar solvents and in 3-hypericinates [11]. The present communication deals with an experiment that allows a definitive and unequivocal assignment of the tautomeric structure to this metastable form of **1**.

Results and Discussion

Experiments to decide about the tautomeric structure of **1** could be designed in principle either in a way to freeze the tautomeric state of the molecule or to obtain structural information by spectroscopic means *in situ*. For the first way derivatization, in peculiar O-methylation, has been advanced recently [11]. However, this method gave significant results for the $\mathbf{Q}^{7,14}$ tautomer only. For the second way application of a variety of methods including vibrational spectroscopy [10] and ^1H or ^{13}C NMR spectroscopy [5, 11] resulted in indications that the metastable form of **1** may be assigned the $\mathbf{Q}^{1,6}$ tautomeric structure; however, definitive evidence is still lacking.

Given the constitution of **1**, such definitive evidence for a certain tautomeric state can be derived in principle by ^1H NMR correlation spectroscopy and corresponding symmetry arguments. Thus, by means of a simple count of the proton signals of an unsymmetric species like the $\mathbf{Q}^{1,7}$ tautomer, it can be clearly differentiated from the C_2 symmetric ones like the $\mathbf{Q}^{7,14}$ or $\mathbf{Q}^{1,6}$. Because the tautomers of **1** are characterized by a specific positioning of the various protons at the molecular perimeter, appropriate correlation spectroscopy could become the key to solve this problem. Accordingly, on the one hand the $\mathbf{Q}^{7,14}$ tautomer should display a ^1H NMR spectrum displaying one methyl, two aromatic protons, and three hydroxyl proton signals which should be correlated by nuclear *Overhauser* effects as CH_3-10,11↔CH-9,12↔OH-8,13 and OH-1,6↔CH-2,5↔OH-3,4 with a possible additional correlation between OH-1,6 and OH-8,13. Since the hydroxyl

groups in positions 3 and 4 are strongly acidic [12–14], they are involved in a fast exchange process and therefore commonly not detected in ^{1}H NMR spectra of **1**. However, upon dissociation a signal corresponding to one proton shifted to about 18 ppm may be detected. The second correlation path will then follow OH-1,6↔ CH-2,5. On the other hand, for the $\mathbf{Q}^{1,6}$ tautomer the number of proton signals is the same as for the $\mathbf{Q}^{7,14}$ tautomer; however, in this case the correlation path should be different becoming CH_3-10,11↔CH-9,12↔OH-8,13↔OH-7,14, and in addition a noncorrelated CH-2,5 signal has to be present. For $\mathbf{Q}^{1,7}$ the number of proton signals would be twice that of the number of the $\mathbf{Q}^{7,14}$ tautomer, and correlation paths corresponding to the unique proton distribution of this tautomer should be observed.

In the case of an *Overhauser* correlation between hydroxyl groups, problems could be encountered. Therefore, we used a 2D H-H ROESY experiment [15] to differentiate between nuclear *Overhauser* correlations and short distance exchange phenomena between adjacent acidic hydroxyl groups. Figure 1 displays this

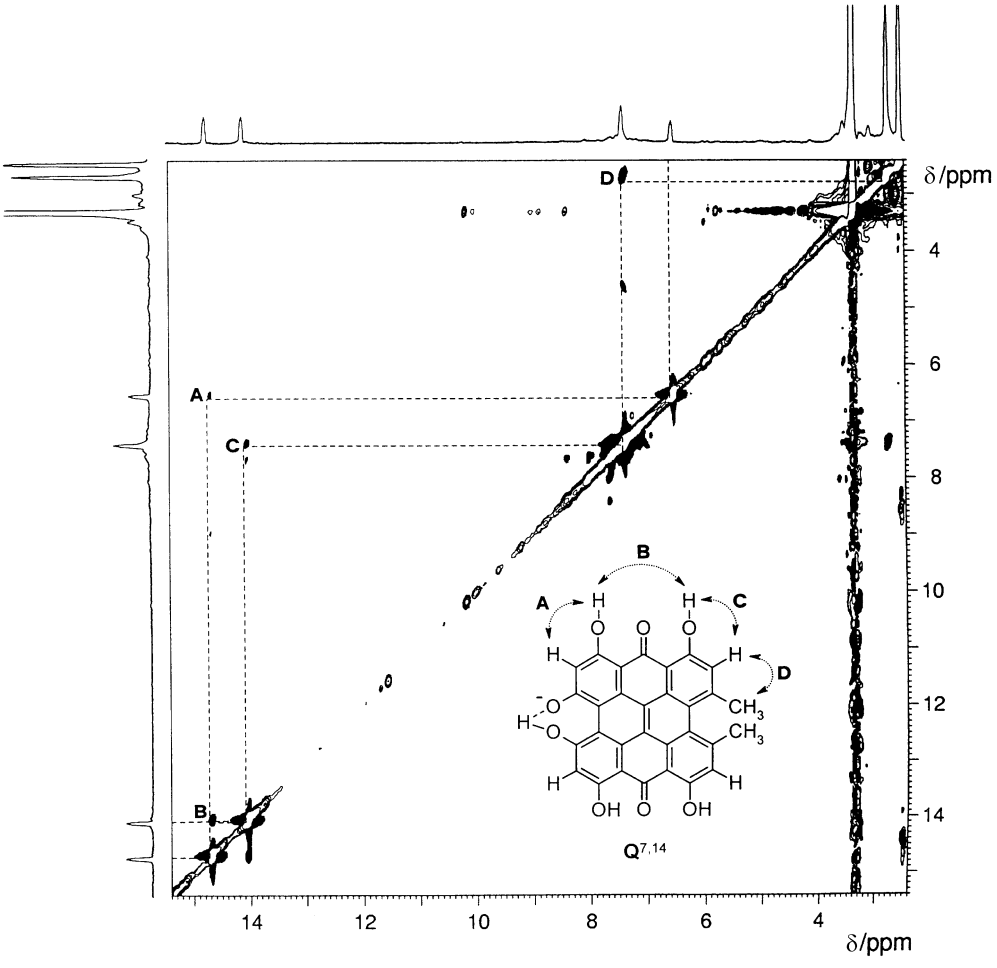

Fig. 1. 2D ROESY spectrum of **1** dissolved in *DMSO*-d_6; nuclear *Overhauser* effect correlations (A–D; negative sign) are indicated by filled contour lines

correlation in the 2D ROSEY spectrum of **1** dissolved in dimethylsulfoxide-d_6. As shown recently, the $\mathbf{Q}^{7,14}$ tautomer of the 3-hypericinate ion will be the predominating species in this case [11, 16, 17]. In addition to the *bay*-hydroxyl protons in positions 3,4 at about 18 ppm (not shown in Fig. 1), five proton signals are obtained. Only ROE correlations (negative sign signals) are observed, which on the one hand comprise CH_3-10,11↔CH-9,12↔OH-8,13 (C, D) and on the other hand OH-1,6↔CH-2,5 (A). In addition, a weaker nuclear *Overhauser* effect correlation (OH-8,13↔OH-1,6, B) is observed. The strongly acidic hydrogen bonded bay proton displays no intramolecular correlation but is involved in exchange with the trace amount of water present in the sample. According to these results, the presence of the $\mathbf{Q}^{7,14}$ tautomer of 3-hypericinate ion in dimethylsulfoxide solution is nicely corroborated. Moreover, this case also supplies a proper argumentation base for the second one.

In Fig. 2, the correlations in the 2D ROSEY spectrum of **1** dissolved in tetrahydrofuran-d_8 are displayed. As discussed recently [11], **1** is thought to be

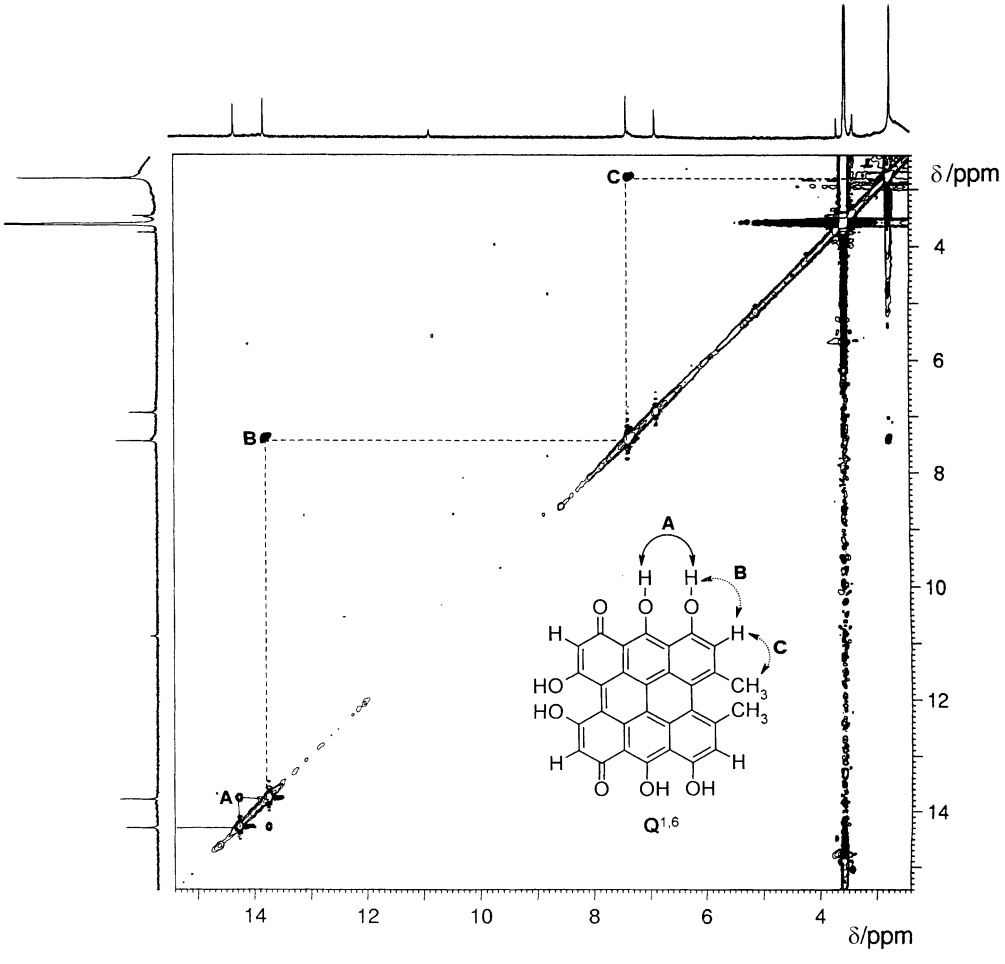

Fig. 2. 2D ROESY spectrum of **1** dissolved in *THF*-d_8; nuclear *Overhauser* effect correlations (B, C; negative sign) are indicated by filled contour lines, exchange correlations (A; positive sign) by an open contour line

present in this solvent in a metastable state which is characterized by unique UV/Vis, IR, ^{1}H, and ^{13}C NMR spectra that are significantly different from those observed for the $\mathbf{Q}^{7,14}$ tautomeric species. A nuclear *Overhauser* correlation path CH_3-10,11↔CH-9,12↔OH-8,13 (B, C) is found (negative signal signs), which is further extended by an exchange correlation (positive sign) OH-8,13↔OH-7,14 (A). Moreover, most important, the remaining aromatic protons CH-2,5 at about 7 ppm were not found to be correlated to any other proton. These features now allow for the unequivocal assignment of the $\mathbf{Q}^{1,6}$ tautomer structure to this metastable species of **1**, which is present if it is dissolved in *e.g.* tetrahydrofuran. The observed correlations are true intramolecular correlations as could be inferred from a comparison of the two experiments and the absence of correlations for the aromatic proton pair in positions 2 and 5.

Experimental

Hypericin (**1**) was prepared and purified according to Refs. [18, 11]. NMR spectra were acquired by means of a Bruker DRX 500 spectrometer. The 2D ROESY experiments on argon purged solutions of **1** ($c \approx 10^{-4}$ mol · dm^{-3}) in *DMSO*-d_6 and *THF*-d_8 were executed using the spectrometer's standard setup. The intensity of the decoupling field was 2500 Hz. Spectra with varied mixing times (400 and 800 ms) were acquired to avoid possible problems due to balanced nuclear *Overhauser* effects and exchange signal intensities.

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