Concerning the Deprotonation of the Photooxidized 3-Hypericinate Ion

Christoph Etzlstorfer[1]**, Ivan Gutman**[2]**,** and **Heinz Falk**[1,*]

[1] Institut für Chemie, Johannes Kepler Universität, A-4040 Linz, Austria
[2] Faculty of Science, University of Kragujevac, YU-34000 Kragujevac, Yugoslavia

Summary. By means of AM1 calculations it was established that the radical produced by photooxidation of the 3-hypericinate anion ($^{(3)}\mathbf{1}^{\bullet}$) possesses strong acidic properties and is at least as acidic as hypericin itself. Dissociation of $^{(3)}\mathbf{1}^{\bullet}$ yields a proton which stems from the 6-*peri*- and/or 4-*bay*-hydroxyl group (which seem to have comparable acidities). The *peri*-hydroxyl groups of $^{(3)}\mathbf{1}^{\bullet}$ at positions 1,8, are 13 are significantly less capable of deprotonation. This result sheds new light on the interpretation of a recent experimental finding involving intermolecular proton transfer following excitation of hypericinate and several of its alkyl derivatives. On the basis of observing a gradual change of proton transfer from these derivatives one could conclude that the proton might stem from the *bay*- and/or the *peri*-region hydroxyl groups.

Keywords. Hypericin radical; AM1 calculations; Acidity; Radical anions.

Zur Deprotonierung des photooxidierten 3-Hypericinations

Zusammenfassung. Mit Hilfe von AM1 Rechnungen wurde abgeleitet, daß das Radikal aus der Photooxidation des 3-Hypericinat-Anions ($^{(3)}\mathbf{1}^{\bullet}$) stark sauer ist – wenigstens so sauer wie das Hypericin selbst. Die Dissoziation von $^{(3)}\mathbf{1}^{\bullet}$ ergibt ein Proton, welches von der 6-*peri*- und/oder 4-*bay*-Hydroxylgruppe herrührt (diese dürften etwa gleiche Acidität aufweisen). Die *peri*-Hydroxylgruppen von $^{(3)}\mathbf{1}^{\bullet}$ in den Positionen 1, 8 und 13 unterliegen einer Deprotonierung weniger leicht. Dieses Ergebnis wirft neues Licht auf die Interpretation von kürzlich mitgeteilten experimentellen Ergebnissen über den intermolekularen Protonentransfer, der der Anregung von Hypericinat und einigen seiner Alkylderivate folgt. Auf der Basis des beobachteten graduellen Wechsels des Protonentransfers bei diesen Derivaten kann geschlossen werden, daß der Protonentransfer von den *bay*- und/oder *peri*-Hydroxylgruppen stammt.

Introduction

Hypericin (**1**), or strictly speaking its *bay*-3-hypericinate anion ($^{(3)}\mathbf{1}^{-}$)[a], which is the predominating hydroxyphenanthroperylene quinone species under physiological conditions, displays virucidal and antiproliferative activity and has cytotoxic

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* Corresponding author

[a] We denote the position from which a proton was abstracted in superscript parentheses on the left side of the formula number and its state (charge: $+,-$; radical: $^{\bullet}$) as superscript on the right side

effects on tumor cells under the influence of light [1]. From the mechanistic point of view, photodynamic therapy involving $^{(3)}\mathbf{1}^-$ could on the one hand be due to sensitized singlet oxygen or superoxide radical generation [2,3], or on the other hand to proton expulsion following the photoexcitation of the pigment [4]. The latter phenomenon could also play a substantial role in the early stages of the photosensory signal generation in protozoa involving the hydroxyphenanthroperylene quinones stentorin or blepharismin [1].
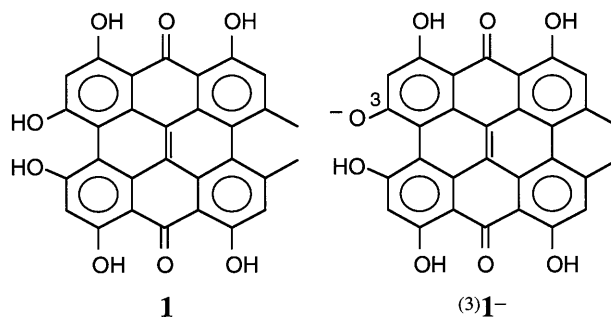
An intermolecular proton transfer as a consequence of photoexcitation of $^{(3)}\mathbf{1}^-$ has been proven experimentally [4,5]. It has been recently hypothesized [6] that in close analogy to the case of the tyrosin radical cation [7] this intermolecular proton transfer could be due to the enhanced acidity displayed by the radical cation formed by electron transfer from an excited state. However, there is obviously an inconsistency in this reasoning. Since $^{(3)}\mathbf{1}^-$ is already deprotonated upon loss of an electron, it does not become a radical cation as in the case of tyrosin, but an uncharged radical. To ameliorate this problem it has been reasoned [5] that the strong hydrogen bonding in $^{(3)}\mathbf{1}^-$ [8] leads to such a strong stabilization that removal of an electron from the system would not severely interfere with the *bay*-part of the molecule, and the radical cation formed would be localized at another part of the molecule leading to a kind of a zwitterionic radical species. Because this issue could not be addressed so far by experimental methods it was studied by means of quantum-chemical semiempirical calculations. The results of this investigation are presented in this paper.

Results and Discussion

The semiempirical AM1 method [9] has proven to yield reliable results within the energetic and geometric realms of the phenanthroperylene quinones [10–12] which also nicely compare with *ab initio* calculation results [11]. Therefore, an AM1 approach was chosen to study the structure of the species derived from $^{(3)}\mathbf{1}^-$ by electron abstraction and the relative acidities of its various remaining bydroxyl groups.

A reasonable starting point for this investigation was to calculate the heats of formation of $\mathbf{1}$, $^{(3)}\mathbf{1}^-$, $^{(1)}\mathbf{1}^-$, and $^{(8)}\mathbf{1}^-$, which resulted in ΔH_f values of -889.4, -1113.3, -1059.1, and $-1057.5\,\mathrm{kJ\cdot mol^{-1}}$. As encountered also recently [8], the significantly different acidities of the *bay*- ($pK_a = 1.8$ [13]; $\Delta\Delta H_f = -223.9\,\mathrm{kJ\cdot mol^{-1}}$) and *peri*-hydroxyl groups ($pK = 9.2$ [13], $\Delta\Delta H_f \approx -169\,\mathrm{kJ\cdot mol^{-1}}$) of $\mathbf{1}$
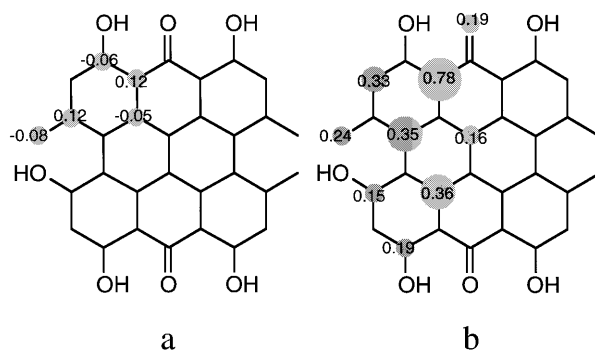
Fig. 1. (a) Differences between the charges (as calculated by means of the *Mulliken* population analysis) of $^{(3)}\mathbf{1}^{\bullet}$ and **1**; b) absolute values of the coefficients of the half-filled molecular orbital of $^{(3)}\mathbf{1}^{\bullet}$ (only the coefficients whose values exceed 0.15 are drawn – these all pertain to p_z orbitals)

were nicely reproduced by the enthalpy difference between the two deprotonation regions of about $55 \, \text{kJ} \cdot \text{mol}^{-1}$. Thus, one could expect the method to describe relative acidities correctly. Calculation of the ΔH_f values for a second deprotonation step of $^{(3)}\mathbf{1}^{-}$ yielded an acidity ranking of $^{(3,1)}\mathbf{1}^{2-}$ ($-970.7 \, \text{kJ} \cdot \text{mol}^{-1}$), $^{(3,13)}\mathbf{1}^{2-}$ ($-1039.8 \, \text{kJ} \cdot \text{mol}^{-1}$), $^{(3,6)}\mathbf{1}^{2-}$ ($-1056.5 \, \text{kJ} \cdot \text{mol}^{-1}$), $^{(3,8)}\mathbf{1}^{2-}$ ($-1067.7 \cdot \text{kJ} \cdot \text{mol}^{-1}$), and $^{(3,4)}\mathbf{1}^{2-}$ ($-1078.8 \, \text{kJ} \cdot \text{mol}^{-1}$), from which the most stable species, $^{(3,4)}\mathbf{1}^{2-}$, is interestingly enough, the one in which the two charges are placed closest to each other. This can be understood assuming a symmetric charge delocalization into both carbonyl groups.

Removing one electron from $^{(3)}\mathbf{1}^{-}$ produces the radical $^{(3)}\mathbf{1}^{\bullet}$ which was found at $\Delta H_f = -716.4 \, \text{kJ} \cdot \text{mol}^{-1}$. As illustrated in Fig. 1a there is no significant charge displacement relative to the charge distribution of hypericin (**1**) itself. Figure 1b illustrates the coefficients of the half-filled molecular orbital which displays pronounced π-character. Accordingly, $^{(3)}\mathbf{1}^{\bullet}$ is an aromatic radical species with a typical electron distribution. Moreover, in contrast to recent assumptions [5] this species does not indicate significant signs of charge separation to form a zwitterionic species.

In order to analyze the acidity of the radical species $^{(3)}\mathbf{1}^{\bullet}$, the five hydroxyl group deprotonation sites 1, 4, 6, 8, and 13 had to be taken into acount. Figure 2 compares the calculated values of this set with the data obtained above. Clearly, $^{(3,6)}\mathbf{1}^{\bullet-}$ ($-963.6 \, \text{kJ} \cdot \text{mol}^{-1}$) and $^{(3,4)}\mathbf{1}^{\bullet-}$ ($-975.0 \, \text{kJ} \cdot \text{mol}^{-1}$) are significantly more stable than $^{(3,1)}\mathbf{1}^{\bullet-}$ ($-931.4 \, \text{kJ} \cdot \text{mol}^{-1}$), $^{(3,8)}\mathbf{1}^{\bullet-}$ ($-925.1 \, \text{kJ} \cdot \text{mol}^{-1}$), and $^{(3,13)}\mathbf{1}^{\bullet-}$ ($-914.4 \, \text{kJ} \cdot \text{mol}^{-1}$). Accordingly, the deprotonation of the photooxidatively produced radical $^{(3)}\mathbf{1}^{\bullet}$ is most likely to proceed from the hydroxyl groups at positions 4 or 6 – a result as in the case of the dianions and a feature not in accordance with common chemical feeling. Interestingly enough, this result of the calculation could be rationalized by considering the resonance forms of the five parent tetraoxo species in question. This is illustrated in Fig. 3 from which it can be deduced that both species, the 3,6,7,14- and the 3,4,7,14-tetraoxophenanthoperylenes with a *Kekulé* structure count of $K = 5$ [14] are much better stabilized than the 3,7,8,14-tetraoxo system with $K = 1$ and 1,3,7,14- and 3,7,13,14-tetraoxo species – the latter two have $K = 0$ and are thus biradicalic species.
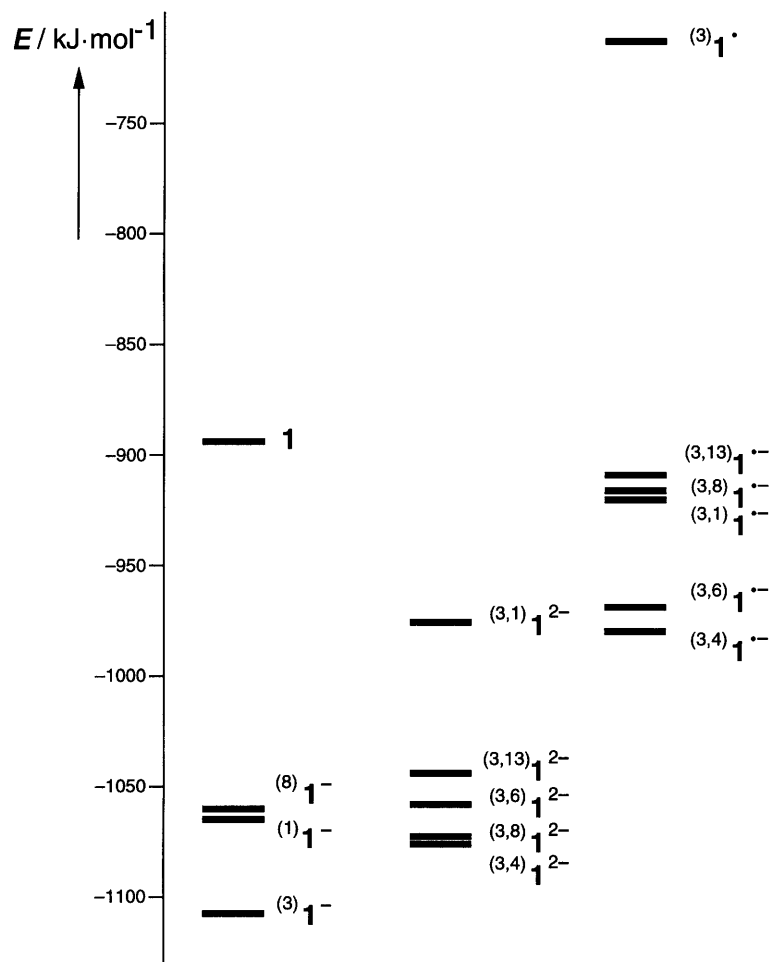
Fig. 2. Calculated heats of formation for the various species derived from hypericin (**1**)

For the five anion radicals of **1** the coefficients of the half-filled molecular orbitals are shown in Fig. 4. Only the electron distribution of $^{(3,1)}\mathbf{1}^{\cdot-}$ deviates significantly from what is expected for a 'normal' radical anion. The spin density was found to be more or less localized on the two oxygen and the carbon atoms in positions 1,2, and 3 – a result which is not yet fully understood.

Conclusion

The results of the AM1 calculations of the radical speices $^{(3)}\mathbf{1}^{\cdot}$ derived by photoxidation of the 3-hypericinate anion ($^{(3)}\mathbf{1}^{-}$) clearly indicate that it is a 'normal' radical without any sign of charge separation. However, this radical is unique due to its enhanced acidity which is even comparable to that of the parent compound hypericine (**1**). According to the calculations, the radical $^{(3)}\mathbf{1}^{\cdot}$ is most acidic at positions 4 and 6 yielding the most stable radical anions $^{(3,4)}\mathbf{1}^{\cdot}$ and $^{(3,6)}\mathbf{1}^{\cdot}$. This result demands a reinterpretation of the experimental facts obtained in a recent
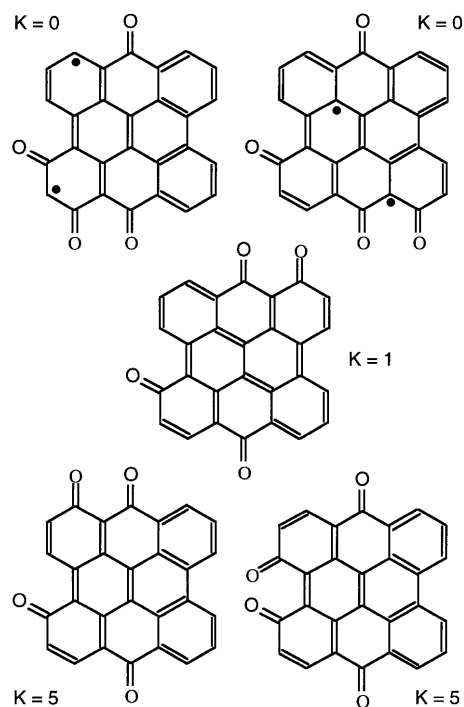
Fig. 3. Canonical structures of the parent tetraoxo species derived from **1**[·]
and their *Kekulé* structure counts *K*

investigation [5]. Using epifluorescence microscopy on lipid vesicles containing **1** or **1** alkylated at the strategic positions 3, 3,4, 1,6,8,13, and 1,3,4,6,8,13 together with an indicator it has been observed that upon excitation of the pigment a proton is transferred to the indicator. This transfer has been observed to be very efficient in the case of **1**, to a lesser extent in the next two derivatives alkylated in the *bay*-region, decreasing significantly in the *peri*-alkylated system, and virtually vanishing in the case of the completely alkylated compound. This has been interpreted in the way of a yes or no decision, leading to the conclusion that the proton is only transferred from the *peri*-hydroxyl groups. In the light of the AM1 calculations described above this conclusion has to be modified. The experiments show a gradual difference in intermolecular proton transfer following excitation, and according to the calculations, the transferred proton could either originate from the *bay*- or the *peri*-hydroxyl grouups, that in position 6 being the most probable one of the *peri*- hydroxyl groups.

Methods

The AM1 method [9] from the MOPAC package [15] was used. Calculations were performed at the SGi Origin 2000 of the LIZENS (*Linzer Zentrum für numerische Simulation*). For input geometries, the results of former calculations [11] were adopted. It should be mentioned that after optimization of the different species a propeller conformation resulted that was virtually identical for all cases studied.
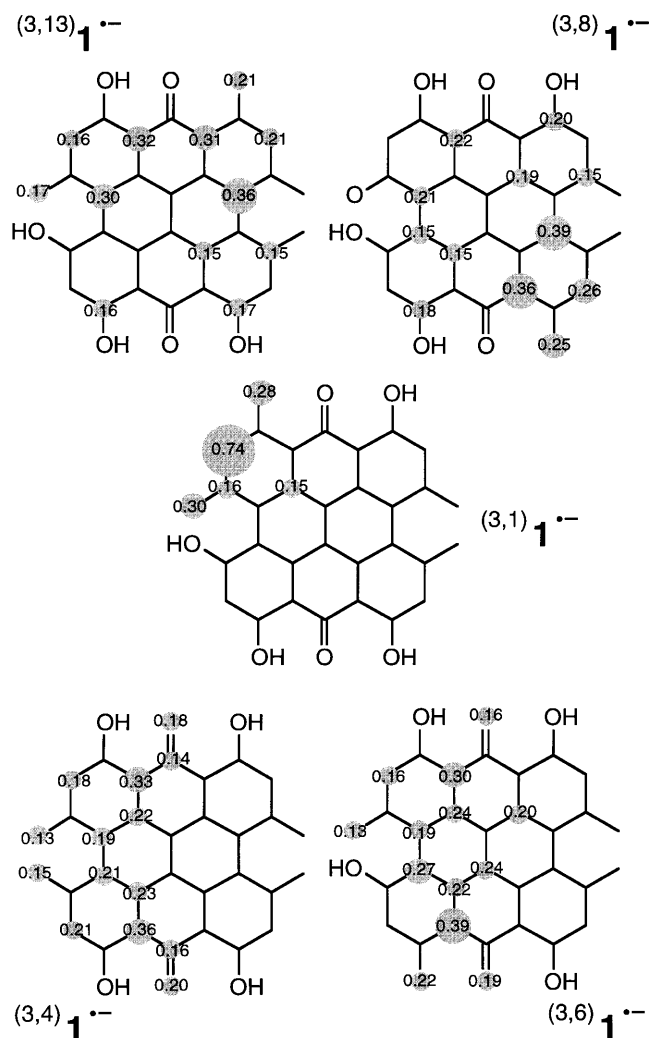
Fig. 4. The coefficients of the half-filled molecular orbitals of $^{(3,6)}\mathbf{1}^{\bullet-}$, $^{(3,4)}\mathbf{1}^{\bullet-}$, $^{(3,1)}\mathbf{1}^{\bullet-}$, $^{(3,8)}\mathbf{1}^{\bullet-}$, and $^{(3,13)}\mathbf{1}^{\bullet-}$ displayed in the same way as in Fig. 1b

Acknowledgements

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