Monatshefte für Chemie 129, 481–486 (1998)

Monatshefte für Chemie
Chemical Monthly

On the Tautomers of Hypericin

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Summary. Hypericin has 16 theoretically conceivable tautomers (10 with and 6 without *Kekulé* structural formulas). AM1 calculations show that two factors determine the relative stability of the tautomers. The dominant factor is the mode of conjugation of the π-electrons. A less pronounced, yet significant, factor is the steric repulsion of the carbonyl and/or hydroxy groups in positions 3 and 4. Among tautomers with equal π-electron conjugation, the least stable are those having carbonyl groups in positions 3 and/or 4. The tautomer *Q*(7, 14), with carbonyl groups in positions 7 and 14, is the far most stable one; the next two tautomers – *Q*(1, 7) and *Q*(7, 13) – have by some 40 kJ/mol higher energies, followed by *Q*(3, 7), *Q*(8, 13), and *Q*(1, 6) with energies 60, 70 and 70 kJ/mol above the energy of *Q*(7, 14), *etc*. The energy values of the hypothetical tautomers without *Kekulé* structures lie 150–200 kJ/mol above *Q*(7, 14); therefore, these are of no practical relevance.

Keywords. Hypericin; Tautomerism.

Zur Tautomerie von Hypericin

Zusammenfassung. Hypericin kann theoretisch 16 Tautomere bilden (10 mit und 6 ohne *Kekulé*-Strukturen). AM1-Rechnungen zeigen, daß die relative Stabilität der Tautomeren von zwei Faktoren bestimmt wird, wobei die Art der Konjugation der π-Elektronen dominiert. Weniger ausgeprägt, aber dennoch signifikant ist die räumliche Abstoßung der Carbonyl- und/oder Hydroxygruppen in den Positionen 3 und 4. Unter den Tautomeren mit gleicher π-Elektronen-Konjugation sind jene mit Carbonylgruppen in den Positionen 3 und/order 4 am instabilsten. Das Tautomere *Q*(7, 14) – mit Carbonylgruppen in den Positionen 7 und 14 – ist bei weitem am stabilsten; die beiden nächsten Tautomeren – *Q*(1, 7) und *Q*(7, 13) – liegen energetisch um *ca*. 40 kJ/mol höher. Sie werden gefolgt von *Q*(3, 7), *Q*(8, 13) und *Q*(1, 6) mit Energien, die jeweils 60, 70 und 70 kJ/mol über der Energie von *Q*(7, 14) liegen. Die Energiewerte der hypothetischen Tautomeren ohne *Kekulé*-Strukturen liegen 150–200 kJ/mol über *Q*(7, 14); diese sind daher ohne praktische Bedeutung.

Introduction

Hypericin (**1**) is a physiologically active constituent of *St. John's* wort (*Hypericum perforatum L.*) and related plants, causing photosensitivity of sheep. Interest in this compound has recently much increased due to its anti-viral and anti-retroviral properties.

The chemical structure of hypericin has been determined long time ago [1]. Thanks to the systematic research done by *Heinz Falk* and his coworkers, the

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chemistry of hypericin [2–8] and of related photodynamically active compounds [9–11] is nowadays well elaborated. The basic physico-chemical properties of these substances have been established [12–16], among them acid/base equilibria [12, 16], association in solution [13], hydrogen bonding [16], and absorption spectra [14,15]. Various aspects of the stereochemistry and conformational equilibria of hypericin [17, 18] and related compounds [19–21] have been investigated, and in connection with this also the problem of tautomerism was examined in due detail [17, 19–21].

All studies unequivocally point at the fact that the naturally occurring hypericin is the tautomer $Q(7, 14)$ (for details see below) which happens to be the most stable tautomer. Recently [22] another tautomer of hypericin ($Q(1, 6)$) has been isolated. This is somewhat surprising, because $Q(1, 6)$ was found [17] to be an energetically unfavorable tautomer, certainly not the second-most stable one.

Another motivation for the study of the tautomers of hypericin is the drastic difference in their conjugation modes. This feature is discussed in more detail in the subsequent section.

The relative stability of the 10 possible *Kekuléan* tautomers of hypericin has been calculated by *Etzlstorfer*, *Falk et al.* [17] by means of an MM2-type force field method. We have now re-examined the same problem by using a somewhat more sophisticated molecular-orbital approach (AM1 with full geometry optimization). For the sake of completeness, we have included in our study also the 6 imaginable non-*Kekuléan* tautomers.

Materials and Methods

The chemical structure of hypericin (or more precisely: of its 7,14-dioxo tautomer **1**) and the way in which its carbon atoms are numbered is shown in Fig. 1.

Following Refs. [17, 22], the tautomer of hypericin in which the carbonyl groups are at positions x and y will be denoted by $Q(x,y)$. In view of the symmetry of the molecule, there are 10 distinct tautomers possessing *Kekulé* structures and 6 additional non-*Kekuléan* tautomers. Their structure is shown in Fig. 2.

Hypericin is a derivative of the octacyclic benzenoid hydrocarbon phenanthro[1,10,9,8-*o,p,q,r,a,*]perylene. Its tautomer $Q(7, 14)$ possesses a conjugated π-electron system for which 24 *Kekulé* formulas can be drawn, *e.g.* k_1, k_2, k_3,...; see Fig. 1 (formula **1** represents the quantum-chemically most significant [23] *Kekulé* structure of hypericin). Because of the two carbonyl groups in positions 7 and 14, the delocalized π-electron system embraces only six hexagons, namely those corresponding to a tetrabenzo-naphthalene (*i.e.*, dibenzo[*g, p*]chrysene) fragment, indicated by the shaded area in diagram **1a** (Fig. 1). In other words, the mode of π-electron conjugation in $Q(7, 14)$ is tantamount to the conjugation in tetrabenzo-naphthalene.

Other tautomers of hypericin have different modes of π-electron conjugation (Fig. 2) pertaining to a variety of benzenoid systems. These tautomers provide thus a convenient, somewhat unorthodox, possibility to test theories describing the stability of benzenoid hydrocarbons [24].

Various theoretical approaches [24] agree in predicting that the stability decreases in the order: tetrabenzo-naphthalene (occurring in $Q(7, 14)$ having 24 *Kekulé* structures, $K = 24$), dibenzo[*a, l*]-pyrene (occurring in Q(1, 7), $Q(7, 13)$, and $Q(3, 7)$ with $K = 16$), benzo[*g,h,i*]perylene (occurring in $Q(8, 13)$, $Q(1, 6)$, $Q(1, 4)$, and $Q(3, 4)$ with $K = 14$), and anthranthrene (occurring in $Q(1, 8)$ and $Q(3, 8)$ with $K = 10$), followed by the non-*Kekuléan* systems occurring in $Q(3, 14)$, $Q(7, 8)$, $Q(3, 13)$, $Q(1, 14)$, $Q(1, 13)$, and $Q(1, 3)$.

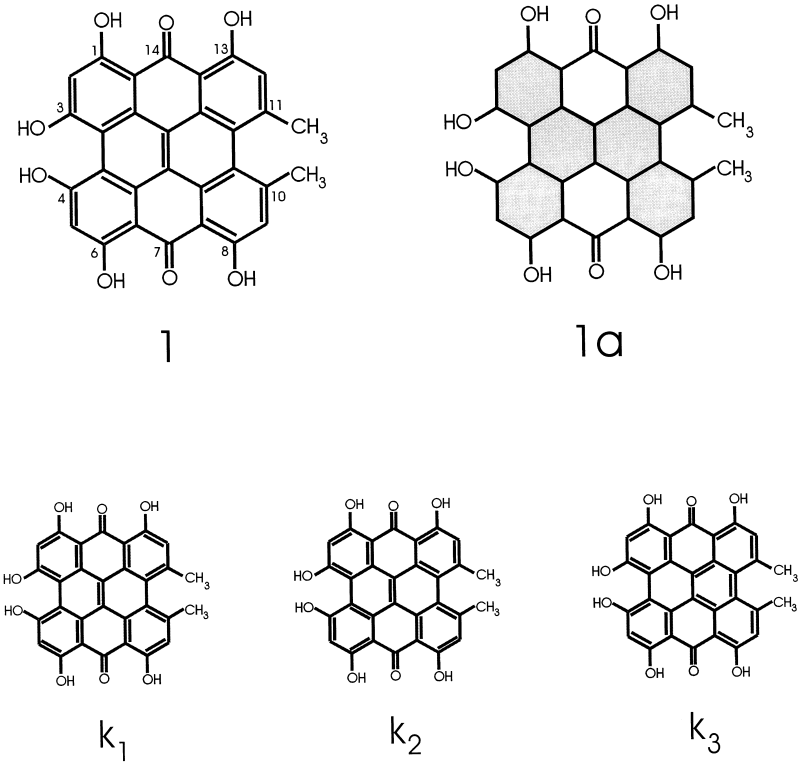
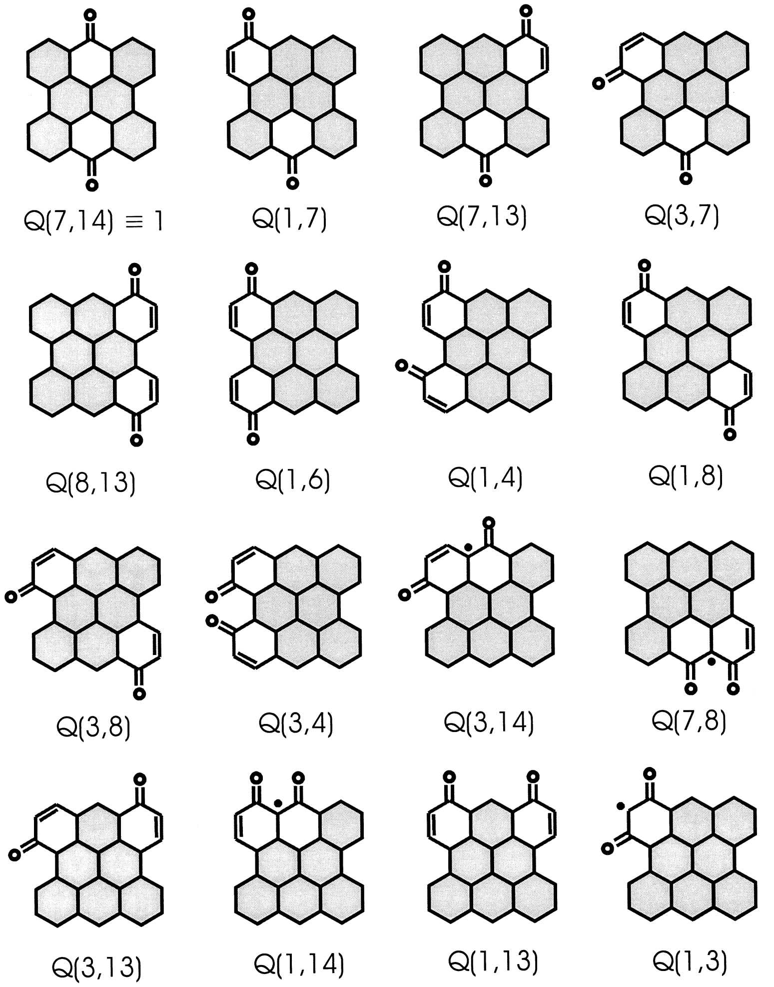


Fig. 1. Hypericin (**1**) and some of its 24 *Kekulé* structures (k_1, k_2, k_3); shading in formula **1a** indicates the dominant mode of π-electron conjugation

It has been noted [17] that the relative stabilities of the various tautomers result mainly from the differences in their π-electron energies, which, on the other hand, can be assessed by a number of simple topological methods [24] based upon the consideration of the respective benzenoid system. Our calculations confirm this conclusion, but also point at another factor, namely that-irrespective of the conjugation mode – a noticeable increase in energy (by some 15–30 kJ/mol) occurs with tautomers with carbonyl groups in positions 3 and/or 4.

In the present study, AM1 (Austin Model 1) semiempirical molecular orbital calculations have been performed at the restricted *Hartree-Fock* (RHF) level [25]. A MOPAC package has been used [26, 27]. The geometries have been fully optimized, anticipating in all cases a *propeller* type conformation [17]. We did not check if for any particular tautomer the *propeller* or the *double butterfly* conformation [17, 18] is preferred, knowing that the former one is predominant in the case of $Q(7, 14)$ [17] and $Q(1, 6)$ [22].)

RHF calculations were performed also in the case of the non-*Kekuléan* species $Q(3, 14)$, $Q(7, 8)$, $Q(3, 13)$, $Q(1, 14)$, $Q(1, 13)$, and $Q(1, 3)$ for which the existence of a closed-shell electronic configuration is by no means guaranteed [24]. Therefore, the results obtained for the non-*Kekuléan* tautomers should be taken with some caution. They certainly are less accurate than those for *Kekuléan* systems. Nevertheless, we deem that the much higher energies calculated for non-*Kekuléan* tautomers are plausible and match reality.



Q(7,14) ≡ 1 Q(1,7) Q(7,13) Q(3,7)

Q(8,13) Q(1,6) Q(1,4) Q(1,8)

Q(3,8) Q(3,4) Q(3,14) Q(7,8)

Q(3,13) Q(1,14) Q(1,13) Q(1,3)

Fig. 2. The tautomers of hypericin and their conjugation modes (indicated by shading); only the position of the two carbonyl groups is shown, whereas the remaining six hydroxy and two methyl groups are omitted (cf. Fig. 1); the molecules are ordered according to increasing energy

Results and Discussion

The following results were obtained:

Tautomer	Energy (kJ/mol)	Tautomer	Energy (kJ/mol)
Q(7, 14)	0	*Q*(3, 8)	110.2
Q(1, 7)	41.5	*Q*(3, 4)	118.3
Q(7, 13)	43.9	*Q*(3, 14)	149.8
Q(3, 7)	60.1	*Q*(7, 8)	176.4
Q(8, 13)	70.0	*Q*(3, 13)	176.5
Q(1, 6)	70.6	*Q*(1, 14)	198.4
Q(1, 4)	84.3	*Q*(1, 13)	201.0
Q(1, 8)	92.3	*Q*(1, 3)	202.3

First of all, the above results are in harmony with the previously established fact that $Q(7, 14)$ is the far most stable tautomer of hypericin. The energies of the next most stable tautomers ($Q(1, 7)$ and $Q(7, 13)$) are by more than 40 kJ/mol above $Q(7, 14)$.

Bearing in mind the formulas depicted in Fig. 2, we now see that the stability order of the tautomers of hypericin follows that anticipated for their conjugated benzenoid domains. The only exception of this is $Q(3, 4)$ for which a reasoning based solely on conjugation arguments would predict values somewhere between $Q(1, 4)$ and $Q(1, 8)$

According to our calculations, the non-*Kekuléan* tautomers lie by 150–200 kJ/mol higher than $Q(7, 14)$ and are thus chemically insignificant and do not deserve further attention.

However, factors other than conjugation are important, too. This is clearly seen when comparing the members of the triplet $Q(1, 7)$, $Q(7, 13)$ and $Q(3, 7)$ as well as of the quartet $Q(8, 13)$, $Q(1, 6)$, $Q(1, 4)$ and $Q(3, 4)$ and of the doublet $Q(1,8)$ and $Q(3, 8)$. Within each of these groups the conjugation mode is the same; yet the energies differ considerably. It is easy to realize that whenever a carbonyl group is at position 3 or 4, the energy of the respective tautomer is noticeably higher than that of its isoconjugated counterparts. If a single carbonyl group is at position 3 or 4, the increase in energy is about 15–30 kJ/mol. In the case of the only tautomer with carbonyl groups at both positions 3 and 4, *i.e.* $Q(3, 4)$, this increase is almost 50 kJ/mol, resulting in the anomalous position of $Q(3, 4)$ in the stability order.

In order to understand why a carbonyl group in position 3 or 4 is energetically unfavorable, we must recall that the hypericin molecule (*i.e.* its benzenoid carbon atom skeleton) significantly deviates from planarity [17, 18]. This is caused by the steric crowdedness of the two methyl groups (in positions 10 & 11) and of the hydroxy and/or carbonyl groups (in positions 3 & 4). As far as the relative stability of the tautomers is concerned, the effect of the methyl groups can be disregarded since it is the same in all tautomers.

Because of steric reasons, the two carbon–oxygen bonds at positions 3 and 4 will have to be deformed. As a measure of such a deformation we may take the angle α between the carbon–oxygen bond and the plane determined by the three carbon atoms nearest to the oxygen atom. In the absence of steric effects, $\alpha = 180^\circ$.

It is easier to extend or bend a single carbon–oxygen bond (as in the hydroxy group) than a double carbon–oxygen bond (as in the carbonyl group). Therefore, one would expect that the hydroxy group in position 3 or 4 is more deformed than the carbonyl group. This, however, was not found by our calculation. In all (*Kekuléan*) tautomers, the deformations of the C–O bonds are quite similar. For instance, in $Q(7, 14)$ (which has hydroxy groups both in position 3 and 4) we found $\alpha = 176^\circ$ and $\alpha = 177^\circ$ in $Q(3, 4)$ (which has carbonyl groups both in position 3 and 4) one has $\alpha = 176^\circ$ and $\alpha = 175^\circ$; in $Q(3, 7)$, $\alpha = 175^\circ$ for the hydroxy group and $\alpha = 178^\circ$ for the carbonyl group.

Because deformations of the carbon–oxygen bonds are roughly the same, and because the deformation of a carbonyl bond requires much more energy than an equal deformation of a hydroxy bond, it follows that the energy content of tautomers with carbonyl groups at positions 3 and/or 4 must be higher.

Various tautomers differ also in the type and strength of the (intramolecular) hydrogen bonds. This effect should also influence their relative stabilities. We, however, estimate that changes in hydrogen bonding cause energy differences of the order of (at most) a few kJ/mol. Such energy effects are too small for the accuracy of the calculation method employed in this work, and also too small to account for the calculated stability differences between isoconjugated tautomers.

Acknowledgements

The authors are indebted to Prof. *Heinz Falk* (Linz, Austria) for valuable comments and information on the tautomers of hypericin, and to Prof. *Zeno Simon* (Timisoara, Romania) for helpful discussions concerning steric effects.

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Received July 28, 1997. *Accepted August 4*, *1997*