

Concerning the Structural Complexity of Hypericin: The Relative Stabilities of Some Neutral and Deprotonated Tautomers

Christoph Ettlstorfer and Heinz Falk*

Institut für Chemie, Johannes Kepler University, A-4040 Linz, Austria

Summary. The structural complexity of hypericin was investigated with respect to the three most relevant tautomers and their *bay*-deprotonation products using *ab initio* calculations at the 6-31G level. The 1,7- and 1,6-tautomers were found to be less stable than the 7,14-tautomer by 45.3 and 83.0 kJ/mol. Upon deprotonation, the same order of relative stabilities was found to be retained. The small differences of absolute stabilities thus obtained were judged to be too small to account for changes of the tautomeric equilibria upon deprotonation. The dipole moments were also found to be similar for these tautomers, making shifts in tautomeric equilibria induced by solvent/solute interactions and *pH* changes improbable. The geometrical features calculated for the seven species are discussed with respect to torsional deformation and hydrogen bonding.

Keywords. Hypericin; Tautomerism; *ab initio* 6-31G calculations; Heats of formation; Hydrogen bonding.

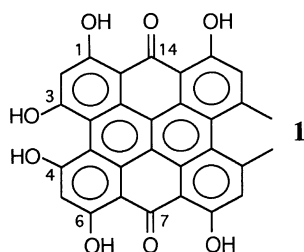
Zur strukturellen Komplexität des Hypericins: Die relative Stabilität einiger neutraler und deprotonierter Tautomeren

Zusammenfassung. Die strukturelle Komplexität des Hypericins wurde in Hinblick auf seine drei relevantesten Tautomeren und deren *bay*-Deprotonierungsprodukte mit Hilfe von *ab initio*-Rechnungen mit 6-31G Basissätzen studiert. Die 1,7- und 1,6-Tautomeren sind gegenüber dem 7,14-Tautomeren um 45.3 und 83.0 kJ/Mol instabiler. Bei Deprotonierung bleibt dieselbe Stabilitätsreihenfolge erhalten. Kleine Differenzen, die hinsichtlich der absoluten Stabilitäten erhalten wurden, reichen nicht aus, um eventuelle Änderungen im Tautomeriegleichgewicht durch Deprotonierung zu verursachen. Geringfügige Differenzen der Dipolmomente sollten ebenfalls kaum zu Verschiebungen der Gleichgewichte durch Solvens/Solut Wechselwirkungen oder *pH*-Änderungen führen. Die Geometrie, die für die sieben Spezies errechnet wurde, wird in Hinblick auf Torsionsdeformation und Wasserstoffbrückenbindungen diskutiert.

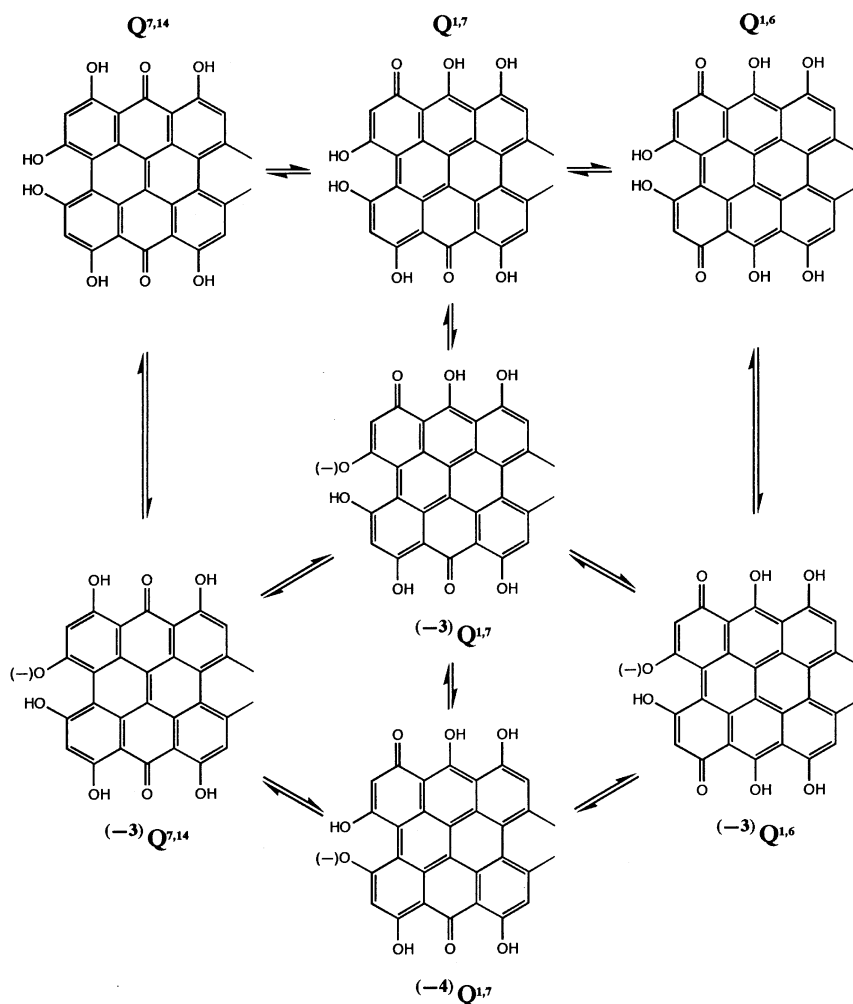
Introduction

Hypericin (**1**) has attracted renewed attention in recent years due to its interesting physiological properties [1]. However, it has been studied also for its unique

* Corresponding author



chemical and physical properties [2]. In spite of the structural complexity of **1** which results from the coupling of tautomeric, dissociative, conformational, and associative equilibria, experimental approaches have been rather limited so far. Therefore, it seemed to be important to study specific details of the problem by means of theoretical methods. Thus, semiempirical calculations have been applied to the problems of tautomerism [3], light absorption [4], chiroptical properties [5], geometry [6], and energy [7] yielding valuable information for the interpretation of experimental results.



Scheme 1

So far, *ab initio* methods have been applied only with respect to the intramolecular *bay* hydrogen bonding situation of the *bay*-phenolate $\mathbf{1}^{(-3)}$ [8]. Because the results of this study have proven to be very accurate with respect to the geometrical features as compared to X-ray data [6,9], it seemed to be of interest to study the experimentally most relevant part of the intertwined tautomerization/dissociation equilibria of $\mathbf{1}$ as given in Scheme 1 ($\mathbf{Q}^{7,14}$ denotes the tautomer of $\mathbf{1}$ with the carbonyl functionalities in positions 7 and 14, $^{(-3)}\mathbf{Q}^{7,14}$ denotes the corresponding *bay*-phenolate deprotonated in position 3, etc.; it should also be noted that equilibria connected to certain points of the Scheme – e.g. $\mathbf{Q}^{7,14} = \mathbf{Q}^{8,14}, \dots$ – were omitted for the sake of clarity). Since the *bay* region of $\mathbf{1}$ has been found to be characterized by a rather low pK_a value of about 1.8 [10], dissociation could in principle influence the tautomeric equilibrium. To contribute to answers to this problem, *ab initio* calculations of the energy situation of the species of Scheme 1, which result from one- and two-proton flips, are presented in this report.

Results and Discussion

As a first step to provide orientation and to arrive at useful initial geometries for the *ab initio* calculations, AM1 calculations of the ten tautomers of $\mathbf{1}$ were executed. They produced *grosso modo* much the same fundamental relative energetic order ($\mathbf{Q}^{7,14}$, 0; $\mathbf{Q}^{1,7}$, 44.6; $\mathbf{Q}^{7,13}$, 46.5; $\mathbf{Q}^{8,13}$, 58.5; $\mathbf{Q}^{3,7}$, 59.6; $\mathbf{Q}^{1,6}$, 71.2; $\mathbf{Q}^{1,4}$, 83.4; $\mathbf{Q}^{1,8}$, 95.9; $\mathbf{Q}^{3,4}$, 107.3; $\mathbf{Q}^{3,8}$, 108.0 kJ/mol) as previously found by means of force field calculations [3], with energies close to but somewhat lower than those reported recently by *Gutman* and also obtained by means of the AM1 method [11]. The small energy discrepancies between the two investigations were thought to result from problems to reach the global minimum due to a rather dense population of side minima around the global minimum. It should be noted that the consistency of results obtained by different calculation methods pointed mainly to π -conjugation as the main source for the relative stabilization of the various tautomers.

When the thus found initial geometries were subjected to *ab initio* calculations, they converged nicely at much the same geometrical features as the AM1 results. As has been deduced recently by means of force field and HMO calculations [3], the 7,14-tautomer of $\mathbf{1}$ ($\mathbf{Q}^{7,14}$) was found to be the most stable one (of course, these calculations pertained to the gaseous state), followed by the 1,7- and then the 1,6-tautomers $\mathbf{Q}^{1,7}$ and $\mathbf{Q}^{1,6}$ (42.7 and 72.1 kJ/mol). This result is also consistent with the heats of formation calculated by means of the AM1 method. However, it turned out that the AM1 method converged only at a geometry with the *bay*-hydroxyl groups (in 3,4-positions) non-hydrogen bonded and in an *anti* arrangement to each other! It should be mentioned that the same situation was encountered also with force field calculations [3]. Obviously, these two methods somewhat over-emphasize the loss in energy stemming from the torsional deformation of the skeleton and the H-O-C_{aromat}-C_{aromat} dihedral angle to reach optimal hydrogen bonding geometry. When an initial geometry with a hydrogen bond in the *bay* region was chosen for the *ab initio* calculations (derived from the AM1 optimized geometry by rotating one *bay*-OH into a hydrogen bonding position with its neighboring hydroxyl group), they converged upon a hydrogen bonded *bay*-hydroxyl system which was significantly better stabilized than the non-bonded

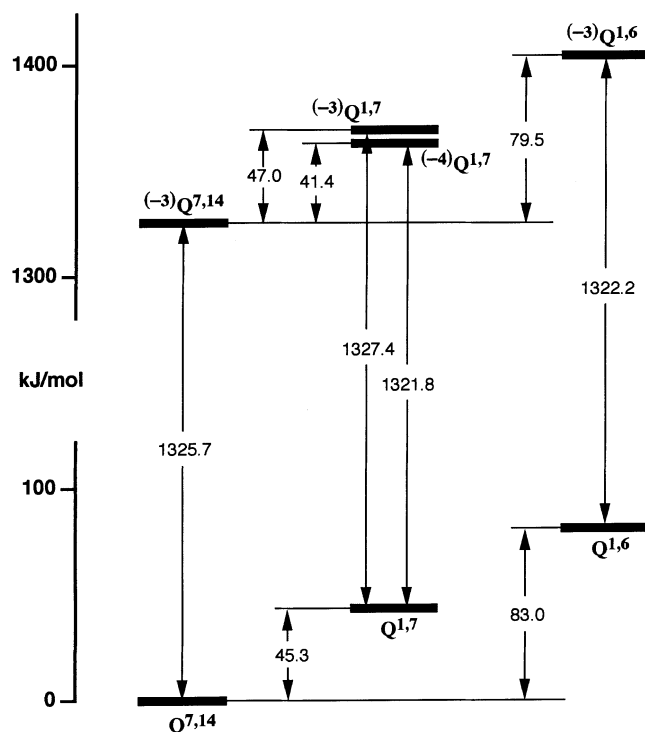
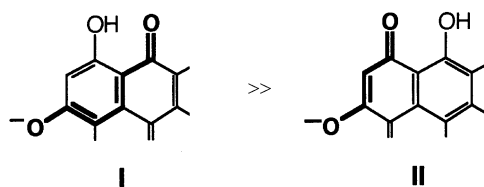


Fig. 1. *Ab initio* (6-31G) relative heat of formation values for the three tautomers $Q^{7,14}$, $Q^{1,7}$, and $Q^{1,6}$ and their respective *bay*-phenolates

system. Thus, the 3,4-hydrogen bridged $Q^{7,14}$ tautomer was found to be more stable by 24.5 kJ/mol than the 3,4-non-bridged one. The corresponding 4 \rightarrow 3- and 3 \rightarrow 4-hydrogen bonded tautomers $Q^{1,7}$ were then calculated to be less stable than this $Q^{7,14}$ tautomer by 45.3 and 50.7 kJ/mol. The 3,4-hydrogen bonded $Q^{1,6}$ tautomer was found to be less stable by 83.0 kJ/mol. The results of these calculations of the species of Scheme 1 are summarized in Fig. 1.

The same fundamental order of tautomer stabilities was also calculated for the *bay*-phenolates. The two different *bay*-phenolates $(-3)Q^{1,7}$ and $(-4)Q^{1,7}$ differ in their stability by 5.6 kJ/mol, and the more stable one of the two, $(-4)Q^{1,7}$, was found to be less stable than the deprotonated most stable tautomer $(-3)Q^{7,14}$ by 41.4 kJ/mol. Thus, in the deprotonated system the 1,7-tautomer was only more stable by 3.9 kJ/mol as compared to the undissociated system. The destabilization of the 1,6-*bay*-phenolate $(-3)Q^{1,6}$ (79.5 kJ/mol) was found to be smaller by 3.5 kJ/mol than that of the undissociated $Q^{1,6}$ tautomer. These data imply also that the tendency to dissociate one proton (the *bay*-phenolate acidities) are at about the same region for the 1,6-tautomer $Q^{1,6}$ and for the 1,7-tautomer $Q^{1,7}$ as compared to the 7,14 tautomer $Q^{7,14}$. Thus, the only slightly different acidities of the tautomers could hardly constitute a main reason for shifts in the tautomerization equilibrium induced by solvent or *pH* changes.

It is also interesting to note that this stabilization of the 4-phenolate $(-4)Q^{1,7}$ – as given by **I** in Scheme 2 – over the 1,7-tautomer-3-phenolate $(-3)Q^{1,7}$ – as given



Scheme 2

by **II** in Scheme 2 – implies that the stabilization increases with increasing conjugation path of the vinylogous acid as indicated in Scheme 2.

The results of the *ab initio* calculations are also interesting with respect to the geometrical features of the three tautomers and their *bay*-phenolates. Accordingly, as can be seen in Figs. 2–4, the hydrogen bonding pattern of the *peri*-hydroxyl groups are characterized by their simultaneous bonding to the 14- and 7-carbonyl groups in the $Q^{7,14}$ and $Q^{1,7}$ tautomers as well as in their corresponding *bay*-phenolates $(-4)Q^{1,7}$ and $(-3)Q^{1,7}$. The 1- and 6-carbonyl groups of the $Q^{1,6}$ and $Q^{1,7}$ tautomers as well as those of their *bay*-phenolates $(-3)Q^{1,6}$, $(-4)Q^{1,7}$, and $(-3)Q^{1,7}$ are hydrogen bonded to the adjacent *peri* hydroxyl group, which in turn is bonded to its adjacent *peri*-hydroxyl group. The O-H-bond distances in the first type of hydrogen bonding were found to measure about 0.98 Å, the hydrogen bonding distances of the type CO···HO are in the order of 1.70 Å. In the latter case, O-H distances of 0.99 Å were calculated, and the HO···HO distances were found to vary between 1.5 and 1.8 Å.

The hydrogen bonds in the non-dissociated *bay*-region were found to be characterized by O-H bond lengths in the order of 0.96 Å and HO···HO hydrogen bond lengths of about 1.7 Å. Whereas the *peri* hydrogen bonds constitute mostly planar local arrangements with torsional deformations in their vicinity of only a few degrees, the *bay* hydrogen bonds are significantly skewed as can be seen from Fig. 5. Accordingly, the geometry in this region is characterized by a torsional

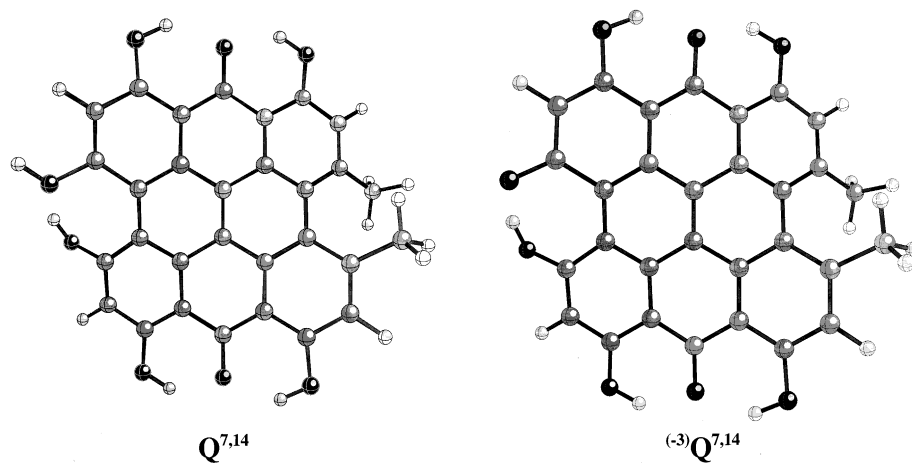


Fig. 2. Ball and stick representations of the tautomer $Q^{7,14}$ and its *bay*-phenolate $(-3)Q^{7,14}$ as derived from *ab initio* (6-31G) calculations

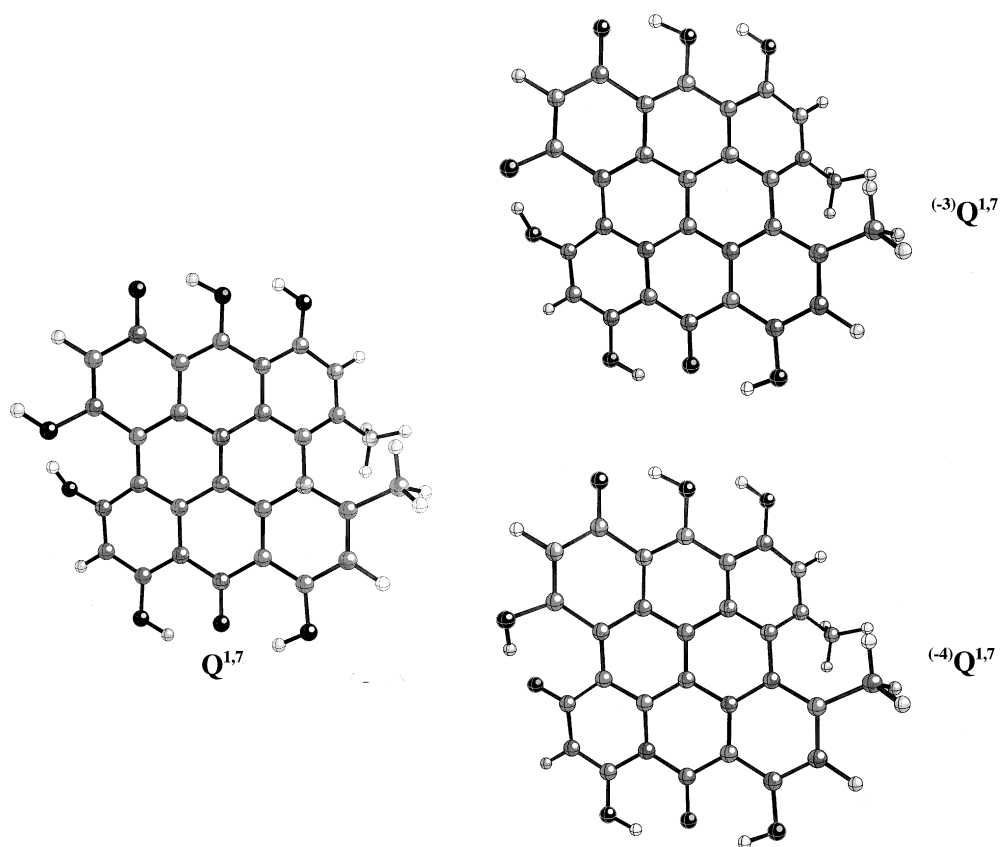


Fig. 3. Ball and stick representations of the most stable *bay*-4 \rightarrow 3-hydrogen bonded tautomer $Q^{1,7}$ and its *bay*-phenolates $(-3)Q^{1,7}$ and $(-4)Q^{1,7}$ as derived from *ab initio* (6-31G) calculations

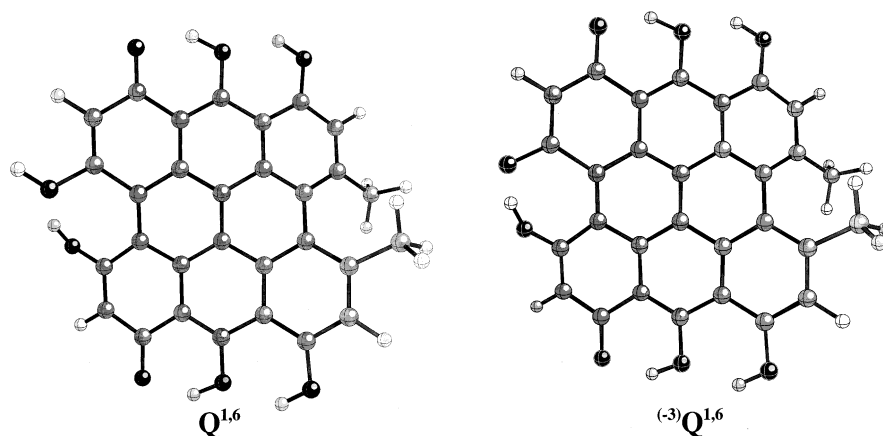


Fig. 4. Ball and stick representations of the tautomer $Q^{1,6}$ and its *bay*-phenolate $(-3)Q^{1,6}$ as derived from *ab initio* (6-31G) calculations

deformation of the backbone with $\theta_{3,3a,3b,4} = 25.2^\circ$, 23.7° , and 22.4° for $Q^{7,14}$, $Q^{1,7}$, and $Q^{1,6}$. The corresponding *bay*-phenolates $(-3)Q^{7,14}$, $(-3)Q^{1,6}$, $(-4)Q^{1,7}$, and $(-3)Q^{1,7}$ display a strong but unsymmetrical hydrogen bonding in the *bay*-region

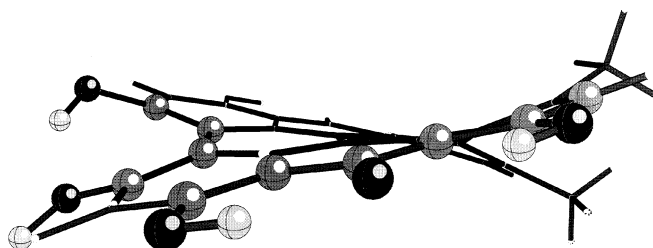


Fig. 5. Ball and stick representation of $Q^{7,14}$ viewed along the quinoid carbonyl axis

which lowers its torsional deformation to $\theta_{3,3a,3b,4} = 19.7^\circ, 18.0^\circ, 18.9^\circ,$ and 18.1° . For the *bay*-phenolates, the O-H bonds measured in the order of 1.0 Å, and the corresponding hydrogen bonding distance $O^{(-)} \cdots HO$ was found to amount to about 1.45 Å, corroborating a very strong unsymmetrical hydrogen bonding [8].

The torsional deformation in the methyl substituted *bay*-region (cf. Fig. 5) proved to be more or less independent of the tautomeric and ionization states. Thus, for the tautomers $Q^{7,14}$, $Q^{1,6}$, and $Q^{1,7}$ and their *bay*-phenolates $(^{-3})Q^{7,14}$, $(^{-3})Q^{1,6}$, $(^{-4})Q^{1,7}$, and $(^{-3})Q^{1,7}$ the corresponding torsional angles ($\theta_{10,10a,10b,11}$) were found to amount to 33.2, 34.3, 33.7, 34.5, 34.9, 34.7, and 34.5° .

The present *ab initio* calculations of the three tautomers and their *bay*-deprotonation products provided also a set of interesting electronic details. An inspection of the charge densities at the various atoms of the seven species given in Fig. 6, which correspond to those of Scheme 1, revealed that the main features of charge distribution are more or less independent of tautomerization. This was also clearly found for the dipole moments of the tautomers $Q^{7,14}$, $Q^{1,6}$, and $Q^{1,7}$ which were calculated to amount 4.00, 3.95, and 4.41 D. Their corresponding *bay*-phenolates $(^{-3})Q^{7,14}$, $(^{-3})Q^{1,6}$, $(^{-4})Q^{1,7}$, and $(^{-3})Q^{1,7}$ were characterized by 12.49, 13.93, 12.55, and 13.51 D. According to the rather small differences of the ground state polarities between the three tautomers and also between their *bay*-deprotonated species, stabilization of one against the other by means of solvent-solute interactions or *pH* changes seemed to be rather improbable.

Conclusions

In conclusion, the 7,14-tautomer $Q^{7,14}$ was found to be stabilized in the 6-31 G *ab initio* calculations against the most relevant one- and two-proton flip tautomers $Q^{1,7}$ and $Q^{1,6}$. The same order of stabilization was found to be retained upon dissociation of one of the respective *bay*-hydroxyl groups. It should be stressed that the geometrical features of the non-ionized tautomers calculated by means of the *ab initio* method deviate significantly from those found by means of AM1 or force field calculations with respect to the hydrogen bonding situation of the *bay*-region. The energy difference found between the non-ionized and ionized states of one and the same tautomer are too small to account for significant changes in the tautomeric equilibria upon deprotonation. Moreover, significant differences in their dipole moments could not be found, and thus it could be expected that shifts in tautomeric equilibria induced by solvent/solute interactions or *pH* variation should also be negligible. The intramolecular hydrogen bonding pattern was found to be

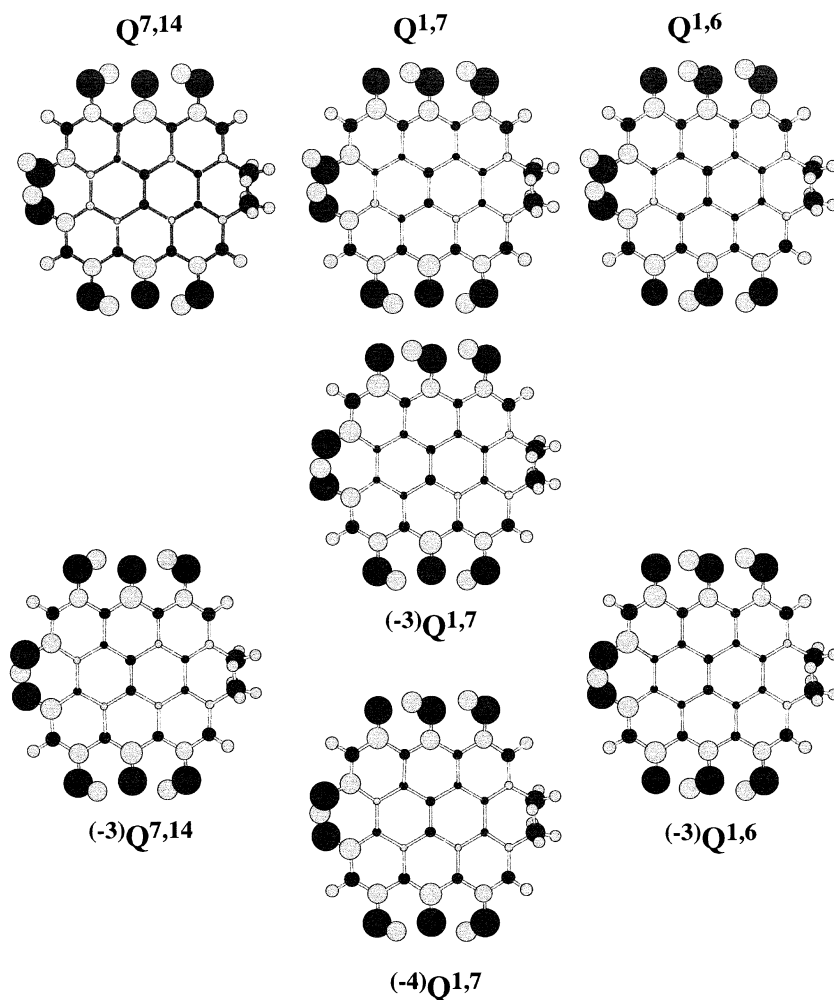


Fig. 6. Mulliken charge densities of the species of Scheme 1; circle diameters at the various atoms represent density values, dark and light shading indicates negative and positive signs

optimized as well as for the *bay*- as for the *peri*-hydroxyl groups in all the species studied. The very strong hydrogen bond in the *bay*-region of the *bay*-phenolates was found to exhibit an unsymmetrical hydrogen bond potential.

Methods

The *ab initio* calculations with a 6-31G basis set were performed using the Gaussian 94 program [12] on the DEC Alpha Server 2100 4/275 of the Vienna University Computer Center. Convergence at the same geometry and energy was reached starting from different initial geometries of one and the same structural configuration. Thus, one could be somewhat confident that the global minimum at least within a certain structural configuration was really attained. Initial geometries to which to apply the *ab initio* procedure were calculated using the AM1 method [13] from the MOPAC package. These calculations were performed at the Convex C3440 of the LIZENS (*Linzer Zentrum für Numerische*

Simulation). For their input geometries, the results of MM2+ calculations or X-ray structural data [6, 9] were used. Molecular models were drawn using the program Ball & Stick [14].

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