

Structural Aspects and Electronic Absorption of the Hydroxyphenanthroperylene Quinones Fringelit D, Hypericin, and Stentorin

C. Etzlstorfer, H. Falk*, N. Müller, and T. N. H. Tran

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

Summary. PPP type semiempirical quantum chemical calculations of absorption spectra were performed for hypericin, fringelit D, stentorin, and their respective conformers, tautomers, and deprotonated species. The results agree with the experimental absorption spectra of hypericin, stentorin, and fringelit D, their deprotonated species, and the polarized absorption spectra of an ω, ω' -long chain appended hypericin derivative embedded in stretched polyethylene.

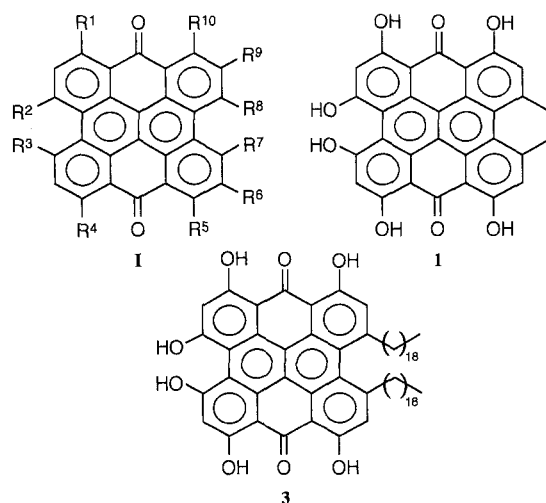
Keywords. Fringelite; Stentorin; Hypericin derivative; Absorption spectra; PPP-calculations; Polarization spectra; Deprotonation.

Strukturelle Aspekte und elektronische Absorptionseigenschaften der Hydroxyphenanthroperylenchinone Fringelit D, Hypericin und Stentorin

Zusammenfassung. Semiempirische quantenchemische Rechnungen (PPP-Typ) der Absorptionsspektren von Hypericin, Fringelit D, Stentorin und ihrer Konformeren, Tautomeren und deren Deprotonierungsprodukten wurden durchgeführt. Die Resultate stehen in Einklang mit den experimentellen Absorptionsspektren von Hypericin, Fringelit D, Stentorin, ihrer Deprotonierungsprodukte und dem polarisierten Absorptionsspektrum eines in gestrecktem Polyethylen eingebetteten ω, ω' -substituierten Hypericinderivates.

Introduction

Hydroxyphenanthroperylene quinones (**1**), among them hypericin (**1**) [1], the fringelites (*e.g.* fringelit D (**2**): $R^6 = R^9 = \text{H}$, $R^1 - R^5 = R^7 = R^8 = R^{10} = \text{OH}$) [2], stentorin ($R^6 = R^9 = \textit{iso}$ -propyl; $R^1 - R^5 = R^7 = R^8 = R^{10} = \text{OH}$) [3], or blepharismine ($R^1 = R^3 - R^5 = R^7 = R^8 = R^{10} = \text{OH}$, $R^6 = \text{H}$, $R^2 = \text{O-4-ethylbenzoic ester}$, $R^9 = \text{CH}_2\text{OH}$ [4]), play important roles in photophysiology and medicine. Their chemistry has been recently the subject of investigations from several laboratories [5–13]. Despite the various studies with regard to synthesis [9], tautomerism [10], photochemistry [5, 7, 11], photophysics [12], and protonation behavior [13], the absorption properties of this class of compounds are less understood. Thus, only the absorption spectrum of **1** has been assigned on the basis of a fictitiously planar molecule and uncalibrated PPP calculations together with a polarized emission spectrum [14]. Therefore, it was thought that a thorough study of the absorption



spectra of the fundamental chromophoric system and its hydroxy derivatives using semiempirical calculations and polarized absorption spectroscopy of the recently synthesized hypericin derivative **3** [15] would be appropriate.

Materials and Methods

The long chain homologized hypericin derivative **3** was prepared as described recently [15]. It was dissolved in polyethylene foil (85 μm thickness) by soaking it in a saturated solution of **3** in a $\text{CHCl}_3/\text{CH}_3\text{OH}$ mixture (5/1) for two days at ambient temperature. Afterwards, the foil was washed carefully several times with the same solvent mixture until further washings came off colorless. Spectra of ten layers of this unstretched and fivefold stretched foil were recorded using a Hitachi U-3210 instrument with polarization filters oriented parallel or perpendicular to the stretching direction of the polyethylene foil. A foil treated in the same manner but without dissolved dye served as the reference.

To calculate the absorption spectra of the different tautomers and conformers of hydroxyphenanthroperylene quinones and their deprotonated species, the PPP-PEP method [16] was used. However, the PEP program in its original form did not take into consideration the non-planarity of molecules. Therefore, a subroutine called "torsion" was added to the program which calculated the torsion angle of a series of four atoms by means of vector algebra. The cosine of this angle was multiplied with the Hückel K and the SCF- β parameters for the respective bond. The geometries of the various species were derived by means of the semiempirical AM1 method [17]. The starting geometries for the application of this method were generated by means of force field calculations using the MM2+ system [18]. To test for the reliability of this procedure, the geometry of **1** was calculated; the result showed excellent agreement with the one derived from X-ray crystallography [19].

Results and Discussion

Parameterization

The electronic spectrum of the fundamental chromophoric system, phenanthroperylene quinone (**4**; **I**, $R^1-R^{10} = \text{H}$ [20]) could be nicely reproduced with parameters used before [21] as illustrated in Fig. 1. However, this parameter set (Table 1) had to be extended to cover also the hydroxyphenanthroperylene quinones and their

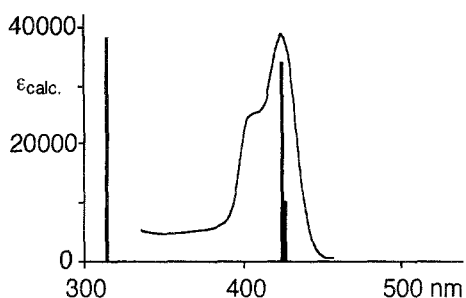


Fig. 1. Experimental (dimethylsulfoxide, arbitrary intensities [20]) and calculated (bars) absorption spectra of **4**

deprotonation products. To describe the various absorption spectra properly, two different parameters for hydroxyl groups had to be introduced: the first one for the *peri* hydroxyl groups (those vicinal to the 7,14-carbonyl groups), and the other one for the hydroxyl groups situated in the bay regions (*e.g.* the positions 3–4 for **1**). The parameters contained in Table 1 were optimized with respect to the positions of the long wavelength absorption bands of the set of compounds contained in Fig. 2.

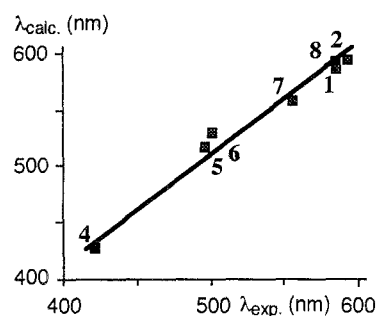


Fig. 2. Experimental vs. calculated long wavelength band positions of **1** [13], **2** [22], **4** [20], **5** (**I**, $R^2 = R^3 = \text{OH}$, $R^1 = R^4 = R^5 - R^{10} = \text{H}$, [13]), **6** (**I**, $R^1 = R^4 = \text{OH}$, $R^2 = R^3 = R^5 - R^{10} = \text{H}$, [13]), **7** (**I**, $R^1 = R^4 = R^5 = R^{10} = \text{OH}$, $R^2 = R^3 = R^6 - R^9 = \text{H}$, [13]), and **8** (**I**, $R^6 = R^9 = \text{iso-propyl}$, $R^1 - R^5 = R^8 = R^{10} = \text{OH}$, [23])

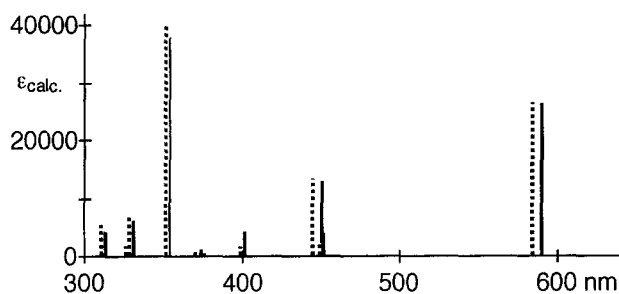
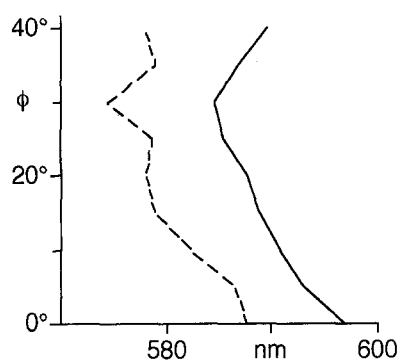
The oxygen parameters for the deprotonated bay hydroxyl group were optimized to reproduce the bathochromic long wavelength band shift of 11 nm observed for **2** [22], the one for the *peri* region was proportionally adjusted, and those describing a C_2 symmetrical phenolate in the bay region by setting them halfway between those of the bay phenol and phenolate values. The isopropyl groups of stentorin (**8**) were approximated using the parameters of methyl groups listed in Table 1. The medium was modelled by setting the effective dielectricity constant $\epsilon = 2.0$.

The Influence of Conformational States and Torsional Deformations upon the Absorption Properties of Hydroxyphenanthroperylene Quinones

From force field calculations, it has been deduced that in hydroxyphenanthroperylene quinones – besides the propeller conformation which has been observed in X-ray crystallographic studies – a conformer of a double butterfly shape could exist [19]. Thus, it seemed to be interesting to investigate the influence of this conformational change upon the absorption spectra. As shown in Fig. 3, the calculated spectra of the two conformers of hypericin (**1**) exhibiting similar intensities were found to be shifted against each other by only a few nanometers. Accordingly, the propeller and

Table 1. Parameters for the calculation of the absorption spectra of phenanthroperylene quinones by means of PEP calculations

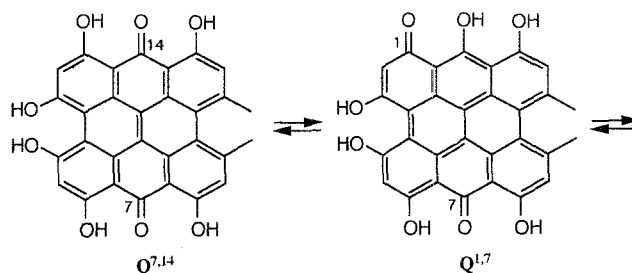
| ν | H | H_ν | γ_ν | K | SCF- β | Z |
|--|-----|---------|--------------|-----|--------------|-----|
| C(=C=) [21] | 0.0 | 10.0 | 9.7 | 1.0 | 2.3 | 1.0 |
| O(C=O) [21] | 0.0 | 17.3 | 14.6 | 1.0 | 3.1 | 1.0 |
| C(CH ₃) [21] | 0.0 | 10.7 | 8.6 | 1.0 | 1.7 | 1.0 |
| H ₃ (CH ₃) [21] | 0.0 | 13.6 | 12.8 | 1.0 | 4.8 | 1.0 |
| -OH (<i>peri</i>) | 0.0 | 22.5 | 14.8 | 1.0 | 2.8 | 2.0 |
| -OH (<i>bay</i>) | 0.0 | 21.5 | 15.3 | 1.0 | 2.4 | 2.0 |
| -O ⁽⁻⁾ (<i>peri</i>) | 0.0 | 20.7 | 14.8 | 1.0 | 2.8 | 2.0 |
| -O ⁽⁻⁾ (<i>bay</i>) | 0.0 | 19.7 | 15.3 | 1.0 | 2.4 | 2.0 |
| -O...H...O- (<i>bay</i>) | 0.0 | 20.6 | 15.3 | 1.0 | 2.4 | 2.0 |

**Fig. 3.** Calculated spectra for the hypericin (**1**) propeller (—) and butterfly (·····) conformers**Fig. 4.** Dependence of the long wavelength band position on the torsional deformation (ϕ ; explained in the text) of the hypericin (**1**) conformers (— propeller, - - - - -butterfly)

butterfly conformers cannot be *a priori* distinguished by absorption spectrometry. Virtually the same result was also obtained for the two conformers of fringelit D (**2**) and stentorin (**8**). Moreover, it should be mentioned that the spectra of isohypericin [9] and isostentorin [23] could also be correctly reproduced by the calculations.

To get an impression of how strong the dependence of the torsional deformation of the phenanthroperylene quinone skeleton would be, the dihedral angles (ϕ) at the two bay regions were varied between 0 and 40°. At the same time, the other various torsional angles of the system were accommodated to the correspondingly induced

stress by means of the MM2+ force field. As illustrated in Fig. 4, the long wavelength bands of the two conformers of hypericin (**1**) were hypsochromically shifted upon this torsional deformation. However, despite the rather large torsional deformation of the system the spectroscopic shifts were found to be rather small.



Scheme 1

Tautomerism

Following the proton displacements indicated in Scheme 1, ten tautomers ($Q^{7,14}$, $Q^{1,7}$, etc.) of hypericin (**1**) are possible in principle, with $Q^{7,14}$ being the most stable one [19]. For fringelit D (**2**) nine and for stentorin (**8**) fifteen tautomers are possible; again the most stable and predominant ones are the $Q^{7,14}$ tautomers [10]. Figure 5 shows the long wavelength band positions of the tautomers of hypericin. It turned out that the $Q^{7,14}$ tautomer was associated with the most hypsochromically positioned band. Accordingly, the spectra of all other hypericin tautomers should be found bathochromically shifted. The situation for the tautomers of **2** and **8** was found to be similar.

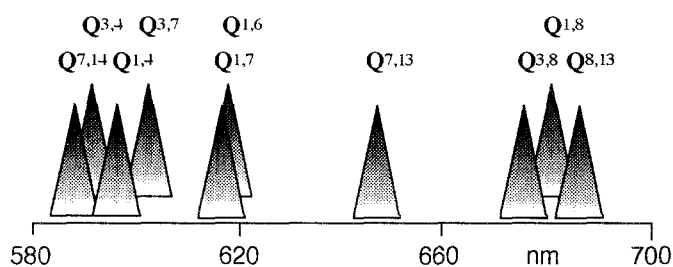


Fig. 5. Positions of the long wavelength absorption bands of the ten hypericin tautomers

In contrast to these results, a hypsochromic shift of about 15 nm has been found experimentally for the $Q^{1,6}$ tautomer of **1** [24]. It is not yet clear how this difference could be interpreted, because this tautomer could by no means be caused to exhibit a hypsochromic shift compared to the $Q^{7,14}$ tautomer in the calculations by variation of the parameters. However, the absolute calculated and experimental differences are rather small and may be caused from solvent shifts and the like.

Deprotonation

Due to their rather low pK_a values for the first deprotonation step in the bay region (in the range of 1–3), hypericin (**1**), the fringelites (*e.g.* **2**), and stentorin (**8**) have been

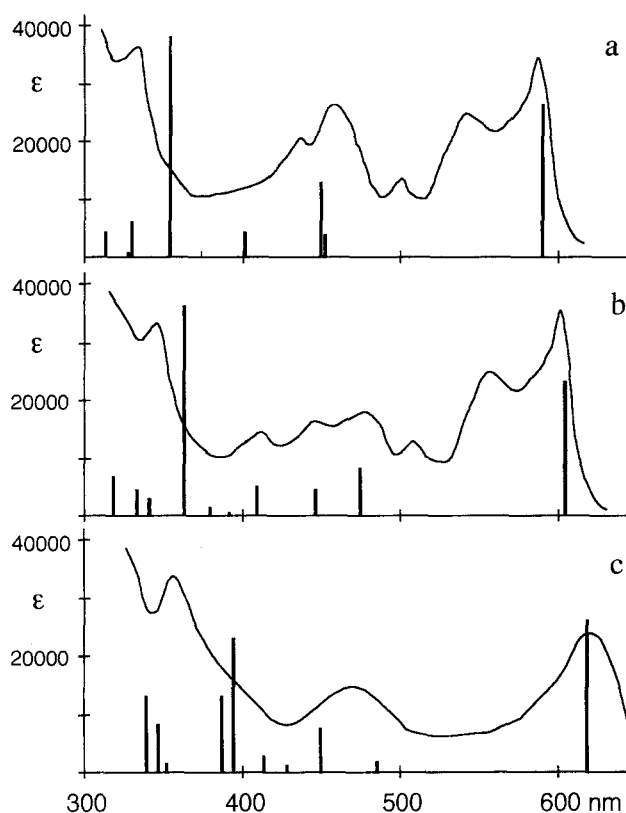


Fig. 6. Experimental spectra for hypericin (a; ethanol, $pH \approx 0.5$), its bay-monoanion (b; ethanol, $pH \approx 6$), and its bay-*peri*-dianion (c; ethanol, $pH \approx 12$), and calculated spectra (bars) of the propeller conformers of **1**, **1**²⁽⁻⁾, and **1**^{3,8(2-)}

found to be present as the deprotonated species under most circumstances [9, 23]. Figure 6 illustrates that the bathochromic shifts of the two main long wavelength bands together with their intensity changes upon deprotonation of **1** could be nicely reproduced by the calculations. The calculated spectra of the propeller and the double butterfly conformers of the bay-anions of **1** were found to be virtually identical.

Following the hypothesis of a C_2 symmetrically structured bay phenolate ion of **1** which has been invoked from ESR and X-ray data [8], the results of the calculations (the parameters of the 3 and 4 oxygen atoms were assumed to reside halfway between those of the bay OH and $O^{(-)}$ substituents; Table 1) with respect to the absorption wavelengths and intensities remained similar. However, in contrast to the nonsymmetrical ion the polarization of the third band was changed (see below).

The calculated spectra of the bay phenolates of **2** and **8** in their propeller and butterfly conformations proved to be indistinguishable and agreed with the experimental data. It should also be noted that according to a AM1 calculation the 3/4 phenolate of **8** was found to be more stable than the 10/11 phenolate with the hydroxyl groups adjacent to the isopropyl groups. Nevertheless, the calculation of the two respective phenolates of **8** gave similar results.

With respect to the second deprotonation step, bathochromic shifted long wavelength bands of the diphenolates of **1** ($O^{(-)}$ in positions 3,1, 3,4, 3,6, 3,8, and 3,13) of 616, 610, 616, 614, and 619 nm were obtained in the calculations. Of course,

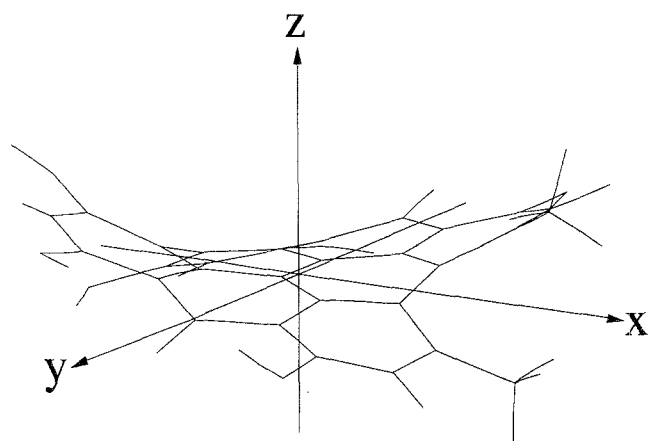


Fig. 7. Orientation of **1** (and, in analogy, of the other phenanthroperylene quinones) for the PEP calculations

the calculated spectra were found to be rather similar and therefore cannot be used to discriminate between the various possibilities. Nevertheless, they were corroborated by the rather broad and featureless experimental spectra (see Fig. 6 for the calculated 3,8-diphenolate ion spectrum and the experimental spectrum of $\mathbf{1}^{2(-)}$ as an example). The experimental spectra of the fringelit D (**2**) and stentorin (**8**) diphenolates were reproduced by the calculated 3,10-diphenolate spectra as well.

Absorption Band Polarization

For the semiempirical PEP calculations, the phenanthroperylene quinone molecules were oriented along the *Cartesian* coordinates as shown in Fig. 7 for the propeller conformer of **1**. It turned out that for the propeller and double butterfly conformers of **1** and **2** as well as for their monodeprotonated species the long wavelength band was polarized along the x-axis, whereas the next two bands to shorter wavelengths were polarized in the y-direction.

To test these results experimentally, the polarized absorption spectrum of **3** dissolved in stretched polyethylene foil was recorded. From a comparison of the normal absorption spectrum of **3** dissolved in the polyethylene foil (Fig. 8) and the

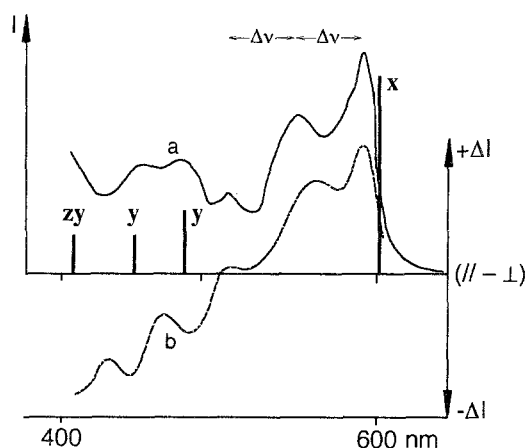


Fig. 8. Absorption spectrum (a) and polarized absorption spectrum (b; this trace was obtained by subtracting the absorption obtained with the polarizer oriented parallel, //, to the stretching direction from the one where the polarizer was oriented perpendicular, \perp , to the stretching direction) of $\mathbf{3}^{(-)}$ embedded in stretched polyethylene foil together with the calculated absorption spectrum (polarizations x, y according to Figs. 7 and 9) of $\mathbf{1}^{(-)}$

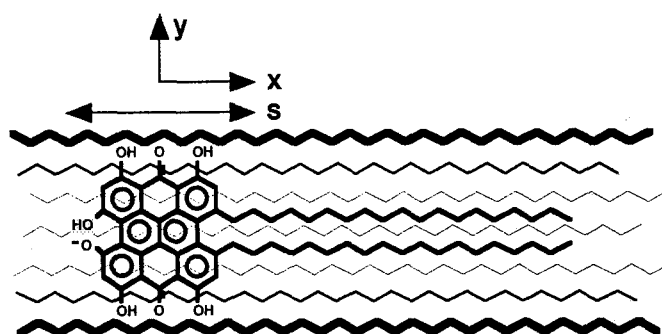


Fig. 9. Schematic representation of the mean alignment and orientation of $3^{(-)}$ dissolved in polyethylene stretched along the s -direction

absorption spectra of **1** and $1^{(-)}$ (Fig. 6; see especially the shape and relative intensity of the “second absorption band system”!) it was concluded that **3** was actually dissolved in polyethylene in its *mono*-dissociated form $3^{(-)}$. From the interaction of the long chain appendices with the polymer chains, the phenanthroperylene quinone chromophore became partially oriented upon stretching with its x -axis mainly aligned to the stretching direction (s) as indicated in Fig. 9. Accordingly, the x -polarized band should absorb more strongly with the polarizer oriented parallel to the stretching direction compared to the perpendicular orientation. For the y -polarized band, the absorption should be less intense upon the same conditions (compare Refs. [25, 26]).

As can be deduced from Fig. 8, the long wavelength band of $3^{(-)}$ was experimentally found to be x -polarized, whereas the next two bands to shorter wavelengths were y -polarized. This was in agreement with the calculated band polarizations of **1** and $1^{(-)}$ if an unsymmetrically structured bay phenolate was assumed. However, if a symmetrical bay phenolate was assumed in the calculation, the three first absorption bands were deduced to be x -, y -, and x -polarized in contrast to the experimental results. Therefore, one can conclude that in solutions the bay phenolate adopts an unsymmetrical structure.

From a comparison of the absorption and polarized absorption spectra of $3^{(-)}$ it was evident that the dichroic ratio is more or less constant throughout the long wavelength band system. Thus, it could be concluded that the three bands of this system found for hydroxyphenanthroperylene quinones and their *mono*-deprotonation products constitute a vibrational progression with a spacing of $\Delta\nu \approx 1320 \text{ cm}^{-1}$. This finding was also corroborated by the observation of a similar vibrational progression in the fluorescence spectra of **3** and $3^{(-)}$ [15].

With respect to the calculated dipole moments of ground and excited states it was found that they were rather similar. Thus, for example, the ground state dipole moment of the propeller conformer of hypericin (**1**) was calculated to amount 2.58 D, with a moment of 4.48 D of the first excited singlet state and a transition moment of 2.13 D. For **2** and the monodeprotonated species $1^{(-)}$ and $2^{(-)}$ and their respective butterfly conformers, similar results were obtained. These rather small differences between ground and excited state moments were in agreement with the rather small solvent shifts observed for **1**, **2**, **3**, **8**, and other hydroxy-phenanthroperylene quinones as well as of their deprotonated species [9, 15, 23].

Acknowledgement

Financial help of the *Fonds zur Förderung der Wissenschaftlichen Forschung* (Project No. P10680-CHE) is gratefully acknowledged. We are also very grateful to Doz. Dr. K. Grubmayr (Linz) for helpful discussions, and to the LIZENS (*Linzer Zentrum für Supercomputing*) for allocation of computer time on the Convex C-3440.

References

- [1] Roth L (1990) *Hypericum – Hypericin: Botanik·Inhaltsstoffe·Wirkung*. Ecomed, Landsberg
- [2] Blumer M (1951) *Mikrochemie* **36/37**: 1048; (1965) *Science* **149**: 722; (1962) *Geochim Cosmochim Acta* **26**: 225; (1960) *Nature* **188**: 1100; (1976) *Scientif Amer* **234**: (3) 34; Falk H, Mayer E, Richter AE (1994) *Microchim Acta* **117**: 1
- [3] Song P-S, Häder D-P, Poff K (1980) *Photochem Photobiol* **32**: 781; Kim I, Rhee JS, Huh JW, Florell S, Faure B, Lee KW, Kahsai T, Song P-S, Tamai N, Yamazaki T, Yamazaki I (1990) *Biochim Biophys Acta* **1040**: 43; Tao N, Orlando M, Hyon J-S, Gross M, Song P-S (1993) *J Amer Chem Soc* **115**: 2526; Wynn JL, Kim JH, Tao N, Dai R, Song P-S, Cotton TM (1995) *J Phys Chem* **99**: 2208
- [4] Preliminary structure according to a personal communication of P-S Song
- [5] Jardon P, Gautron R (1989) *J Chim Phys* **86**: 2173; Hadjur C, Jenuet A, Jardon P (1994) *J Photochem Photobiol B* **26**: 67
- [6] Cotton TM (1992) *Photochem Photobiol* **56**: 157; Wynn JL, Cotton TM (1995) *J Phys Chem* **99**: 4317
- [7] Gai F, Fehr MJ, Petrich JW (1994) *J Phys Chem* **98**: 5784, 8352
- [8] Weiner L, Mazur Y (1992) *J Chem Soc Perkin Trans II* 1992: 1439; Malkin J, Mazur Y (1993) *Photochem Photobiol* **57**: 929; Michaeli A, Regev A, Mazur Y, Feitelson J, Levanon H (1993) *J Phys Chem* **97**: 9154; Freeman D, Frolow F, Kapinus E, Lavie D, Lavie G, Meruelo D, Mazur Y (1994) *J Chem Soc Chem Commun* 1994: 891; Gerson F, Gescheidt G, Häring P, Mazur Y, Freeman D, Spreitzer H, Daub J (1995) *J Am Chem Soc* **117**: 11866
- [9] Falk H, Schoppel G (1991) *Monatsh Chem* **122**: 739; Falk H, Schoppel G (1992) *Monatsh Chem* **123**: 931; Falk H, Schmitzberger W (1993) *Monatsh Chem* **124**: 77; Falk H, Meyer J, Oberreiter M (1993) *Monatsh Chem* **124**: 339; Falk H, Vaisburg AF, Amer AM (1995) *Monatsh Chem* **126**: 993
- [10] Etlzstorfer C, Falk H, Müller N (1993) *Monatsh Chem* **124**: 431; Etlzstorfer C, Falk H (1993) *Monatsh Chem* **124**: 1031; Etlzstorfer C, Falk H (1994) *Monatsh Chem* **125**: 955
- [11] Arabei SM, Galaup JP, Jardon P (1995) *Chem Phys Lett* **232**: 127; Hadjur C, Jardon P (1995) *J Photochem Photobiol B* **29**: 147
- [12] Angerhofer A, Falk H, Meyer J, Schoppel G (1993) *J Photochem Photobiol B* **20**: 133; Pschierer H, Friedrich J, Falk H, Schmitzberger W (1993) *J Phys Chem* **97**: 6902
- [13] Falk H, Meyer J, Oberreiter M (1992) *Monatsh Chem* **123**: 277
- [14] Walker EB, Lee TY, Song P-S (1979) *Biochim Biophys Acta* **587**: 129
- [15] Falk H, Tran TNH (1996) *Monatsh Chem* **127**: 717
- [16] PEP, U, M Westerhoff, QCPE program 11,167 based on: Brearly N, Bloor JE (1965) *Can J Chem* **43**: 1761; Pariser R (1955) *J Chem Phys* **24**: 250; Pariser R (1956) *J Chem Phys* **25**: 1112; Pople JA (1954) *J Chem Phys* **23**: 81
- [17] Version MOPAC 6.0 DEC-3100 Edition 1990; Frank J Seiler Research Laboratory, United States Air Force Academy CO80840; Zoebisch EG, Dewar MJS, Healy EF, Stewart JJP (1985) *J Am Chem Soc* **107**: 3902; Dieter KM, Dewar MJS (1986) *J Am Chem Soc* **108**: 8075; Stewart JJP (1990) *J Comp Aided Mol Design* **4**: 1
- [18] MM2-Molecular Mechanics Version 2 (77-Force-Field) for localized electron systems (QCPE #395): Lifson S, Ermer O (1973) *J Am Chem Soc* **95**: 4121; Allinger NL (1977) *J Am Chem Soc* **99**: 8127; Tai JC, Sprague JT, Yuh Y, Allinger NL (1987) *J Comp Chem* **8**: 581

- [19] Falk H, Etlzstorfer C, Müller N, Schmitzberger W, Wagner UG (1993) *Monatsh Chem* **124**: 751
- [20] Falk H, Vaisburg AF (1995) *Monatsh Chem* **126**: 361
- [21] Höllbacher G, Falk H (1978) *Monatsh Chem* **109**: 1429
- [22] Falk H, Mayr E (1995) *Monatsh Chem* **126**: 699
- [23] Falk H, Mayr E (1995) *Monatsh Chem* **126**: 1311
- [24] Etlzstorfer C, Falk H, Oberreiter M (1993) *Monatsh Chem* **124**: 923
- [25] Michl J, Thulstrup EW (1986) *Spectroscopy with Polarized Light*. Verlag Chemie, Weinheim; Brotin T, Waluk J, Desvergne J-P, Bouas-Laurent H, Michl J (1992) *Photochem Photobiol* **55**: 335
- [26] Falk H, Vormayr G, Margulies L, Metz S, Mazur Y (1986) *Monatsh Chem* **117**: 849

Received January 31, 1996. Accepted February 9, 1996