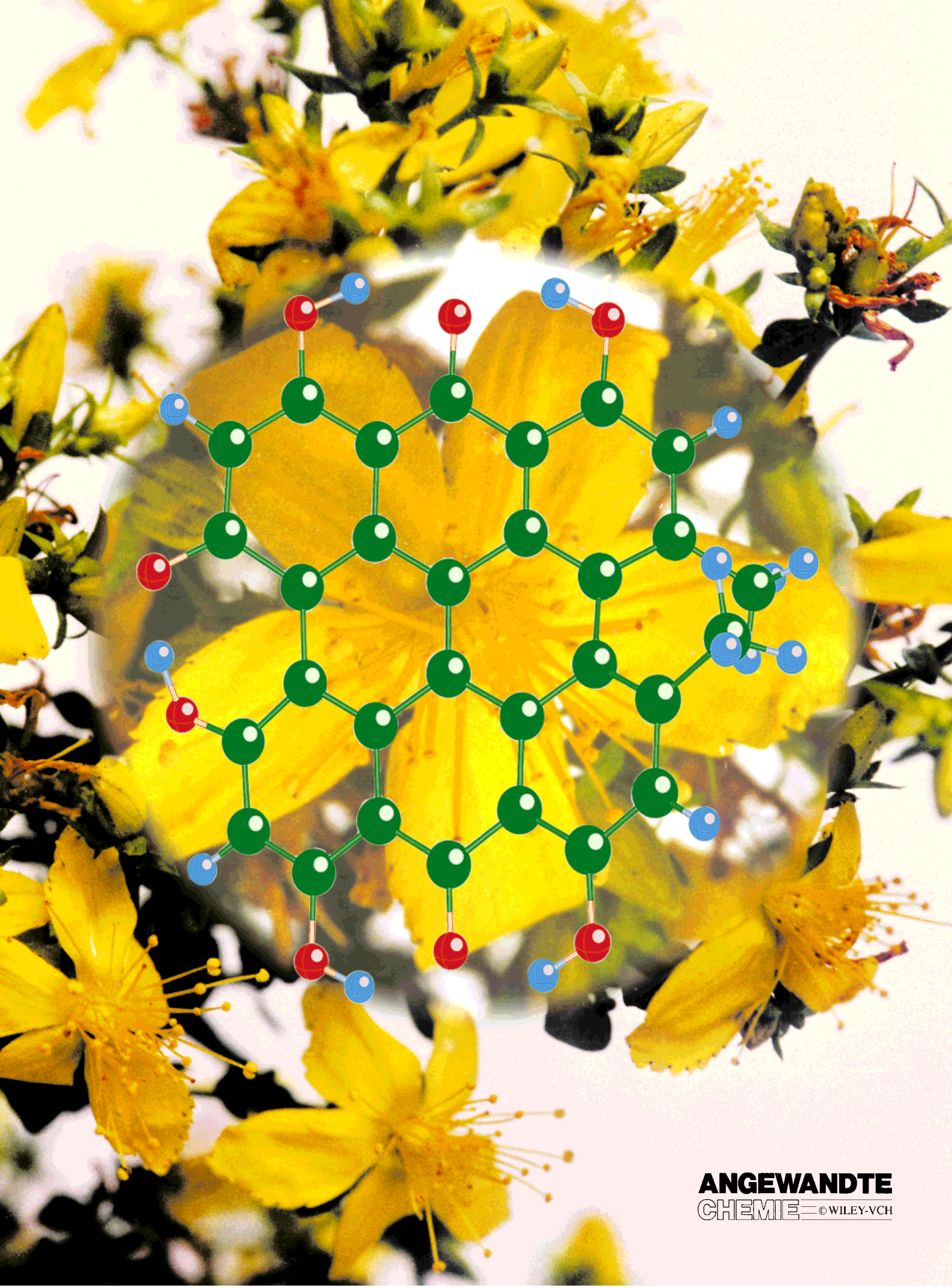

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From the Photosensitizer Hypericin to the Photoreceptor Stentorin—The Chemistry of Phenanthroperylene Quinones

Heinz Falk*

The pursuit of the chemistry of natural compounds containing phenanthroperylene quinones substituted with hydroxyl and alkyl groups dates back nearly half a century. It experienced a renaissance within the last decade when it turned out that one of these compounds, hypericin isolated from St. Johns wort—a phytotherapeutic drug known since antiquity—does not only exhibit ingestion deterrence, but also antiviral, photodynamically useful, and sedative properties. The fact that this group of phenanthroperylene quinones also contains the photosensory pigments of protozoa, such as stentorin, has additionally contributed to this new interest in this class of compounds. However, it is also the wealth of chemical and physical problems that spurred the curiosity of scientists to probe the phenanthroperylene quinones in more detail. These problems are mainly a result of the network of tautomerism, dissociation, conformation, and association equilibria and the structural complexity thus caused by them. In keeping with the broad array of interdisciplinary investigations, which reach from synthetic organic chemistry and spectroscopy to physiology and medicine, this review will focus on a picture of the chemical aspects of this fascinating class of molecules framed by the background of its biological aspects.

Keywords: chirality · natural products · quinones · photochemistry · tautomerism

1. Introduction

- After having ingested a ubiquitous proliferating weed from the genus *Hypericum*, Australian sheep die under a burning sun, bleating miserably.
- A malignant tumor recedes after application of one of the compounds isolated from such plants on irradiation with laser light.
- A protist, such as *Stentor coeruleus*, retreats with vigorous whips of its flagellum after being hit at its photoreceptor system from a sun ray.

The chromophoric system of the phenanthroperylene quinones (**1**; Figure 1) is responsible for all these consequences. Multiple substitution with hydroxyl and partially also with alkyl groups forms the fundamental system of the photosensitizing as well as the photosensory pigments.

As seen from the historical point of view, the roots of our knowledge about these systems date back to antiquity. Even back in those days several species of the genus *Hypericum* were distinguished and used for therapy. The earliest mention might date back to Nikandros of Kolophon who lived in the second century B.C. He suggested that "Cheirons root" could be used against intoxication in animals.[1a] Although *Hypericum* species may be found worldwide, and in particular, in the Mediterranean and in northern Africa, no mention is found that the ancient Egyptians knew or used this plant.[2] Additional mentions occur in the works of Celsus, Andromachus, and Dioskorides written in the epochs that followed.[1b] From these Greek and Roman authors through Galenus up to Paracelsus and Matthiolus,[1c] "St. Johns wort" was considered a highly valued drug. Its applications were numerous, and

1 **2**

Figure 1. Constitution and numbering of the fundamental phenanthro[1,10,9,8-*opqra*]perylene-7,14-dione (**1**), occasionally named in the literature as "*meso*-naphthodianthrone", and the constitution of hypericin (**2**).

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reached from magical applications and its use as an antidepressant to wound healing.[1c,d] Since the first pharmacopoeias *Herba hyperici* and later on also *Olio hyperici* it has been valued with respect to applications for various indications.[1e] Nowadays it is also available in a broad palette of pharmaceutical specialties.[1a] In most recent times it was also discussed for use against alcohol addiction.

The photodynamic effects of *Hypericum* were linked at the beginning of this century to their causes, which were collectively called the "hypericism" syndrome.[3] But only in the middle of the century was the compound that caused hypericism, hypericin (**2**; Figure 1), isolated by Brockmann in a pure form from *Hypericum perforatum* and *hirsutum*, and its constitution assigned by total synthesis.[4] The name hypericin was coined after its first isolation by Cerny in 1911.[1f] After this "classical period", investigations of this class of compounds are only scarcely encountered in literature.

The renaissance in the pursuit of phenanthroperylene quinones started about a decade ago when it turned out that **2** had an antiviral effect. Moreover, photoreceptors, such as stentorin and blepharismin, which are responsible for the phototaxis of certain protozoa, display structural analogy with hypericin.[5a–c] Upon investigating such phenomena of **2** the question of the mechanism of action was raised. It transpired that the fundamental chemistry of the phenanthroperylene quinones was not as simple and clear-cut as could be inferred from a superficial contemplation of the structural formulas of Figure 1. This review will start with an overview of the occurrence and importance of the main members of this class of compounds and then proceed to give mainly a picture of the chemistry of the phenanthroperylene quinones—at the very least to stimulate its further development.

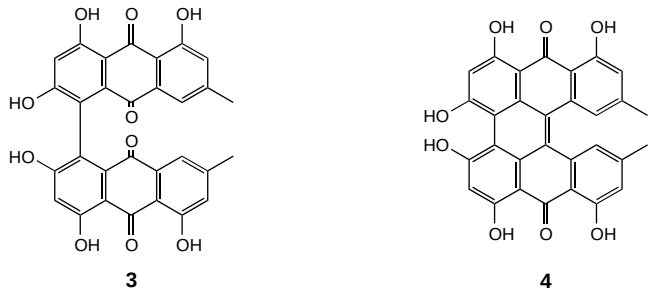
Figure 2. *Hypericum perforatum* Linné; bottom left is shown a microscopic view of a dark petal granule (diameter approximately 100 μm) from which a red liquid—its color being due to the hypericinate ion—is extruded upon applying soft pressure.

2. Occurrence and Importance of Phenanthroperylene Quinones

2.1. Hypericin and Hypericin Derivatives

Hypericin (**2**) has been identified both in the plant and animal kingdoms. Its widest distribution is found in the more than three hundred species of the genus *Hypericum* of the plant family Guttiferae.[1a] It is contained in the plants in the form of dark colored granules (Figure 2) and is present in the form of its *bay*-hypericinate, with potassium as the counterion.[6] This fact could be deduced by means of counter-current droplet distribution and contradicted the earlier presumptions that it should occur as a glycoside.[6a] The content of **2** in dried plant material ($<0.05\,\%$) varies from species to species and is sometimes also found to vary within the same species, being dependent on location and even harvesting time.[1a] In the first instance, **2** was also assumed to occur in the fungus *Dermocybe austroveneta*.[7] However, careful isolation in the dark afforded only skyrin (**3**) and protohypericin (**4**; Figure 3), with the latter cyclizing under the influence of light to yield **2**.[7b] Such intermediates were also observed in *Hypericum*.[1a] As mentioned above, **2** is used by the plants as an ingestion deterrent, which induces edemas and eventually death of the

Heinz Falk was born in St. Pölten. He studied chemistry at the University of Vienna and completed his PhD in 1966 with Prof. K. Schlögl with a thesis on the configuration of optically active ferrocenes. After a postdoc at the ETH Zürich with Prof. A. Eschenmoser he completed his habilitation at the University of Vienna in 1972 where he worked on stereochemical investigations of chiral metallocenes. From 1975 to 1979 he worked as professor of physical organic chemistry at the University of Vienna and was appointed as full professor of organic chemistry at the Johannes Kepler University Linz in 1979. The research interests in his group focus on synthesis, structure–property relations, and spectroscopic investigations of natural products, such as bile pigments and condensed quinones, relevant in photobiology.



3 **4**

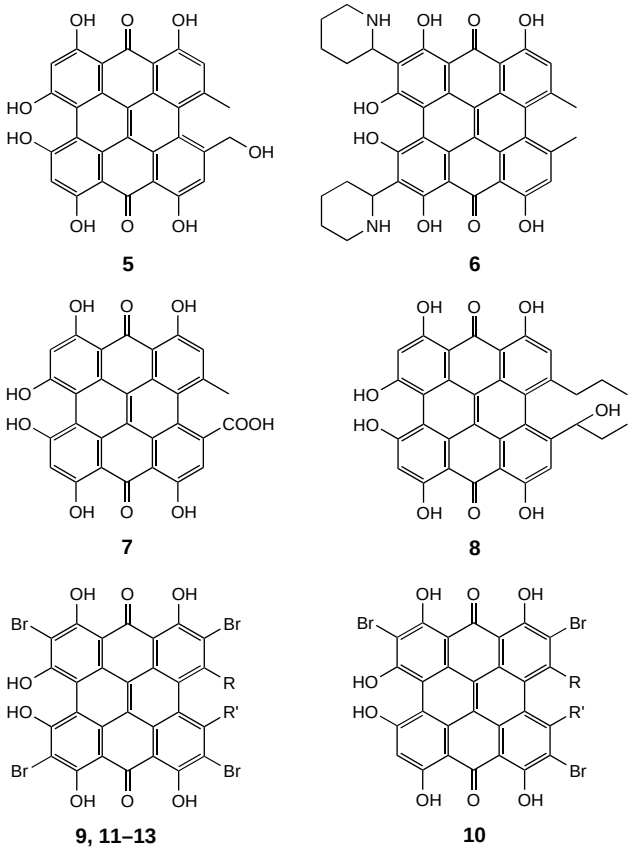
Figure 3. Constitution of skyrin (**3**) and protohypericin (**4**) obtained from the fungus *Dermocybe austroveneta*.

grazing animals under the influence of sunlight (hypericism).[3] Hypericin (**2**) was also shown to act as an insecticide against the tobacco parasite *Manduca sexta*.[8] Interestingly enough, several insects, such as *Platynota flavedana*, have developed a strategy that allows them to escape the fatal influence of light. These insects curl up the leaves of *Hypericum* to make them into sunshades.[8b]

In the animal kingdom **2** was found in the Australian bug *Nipaecoccus aurilanatus* MASKELL.[7a] In this case it is certain that it is genuinely contained in this organism and is not derived from ingested food. The function of **2** in these animals is not known, but it is suspected that it protects the organism against sunlight by contributing to the dark pigmentation of the epidermis![7b]

It was clear that the photodynamic activity that **2** displays under the influence of light could be used for therapy. Thus, the light-induced antiviral activity of **2** was recognized and used rather early on.[9] For example, it turned out that it acted as a virucidal agent against sindbis,[9b,c,o] cytomegalo,[9b,c,o] hepatitis B,[9d] herpes,[9c,l] equine anemia,[9j] papilloma,[9m] and vesicular stomatitis virus.[9p] This effect can be of interest for the virus-decontamination of transfusion blood and blood derivatives by irradiating them in the presence of **2**.[10] Hopes that **2** might be an agent for the photodynamic therapy of AIDS, which were spurred on by the in vitro activity against retroviruses, were in vain.[11] However, the photodynamic therapy of topical cancers has gained an effective weapon in the photosensitizer hypericin (**2**).[12] Moreover, it was shown that this compound enhances the radiolytic sensitivity of tumor cells,[12d, 12n] and there are indications that **2** is also effective in the case of T-lymphocyte-mediated diseases, such as multiple sclerosis, myasthenia gravis, rheumatoid arthritis, scleroderma, polymyositis, pemphigus, psoriasis, and transplant rejection.[13] Results for the molecular basis of all these effects are available from various studies.[14] Accordingly, for example, it was found that enzymes such as reverse transcriptase,[11a] monoaminoxidase,[14a] succinoxidase,[14c] and several protein kinases were inhibited.[14b,e,i] The photodynamic action of **2** could either be attributed to the sensitized formation of singlet oxygen or to an acidification by light-induced deprotonation of the pigment.[14n,o] Interestingly, the electron transfer in the photosystem II of cyanobacteria is inhibited by **2**.[14k] The hypericism syndrome might be mainly a consequence of photohemolysis.[14p] For **2** to be used as a drug it was of utmost importance that no genotoxicity could be found.[15] Recently, several studies on the use of *Hypericum* as an antidepressant were published.[16] It is not clear at the moment if, of the several compounds present in this plant, **2** is the active principle. However, since it was shown that **2** interacts with the serotonin and muscarin receptors this might be a good guess.[16b]

As well as **2**, the ω-hydroxylated hypericin derivative pseudohypericin (**5**; Figure 4) also predominates in several *Hypericum* species.[17] This pigment also seems to exhibit physiological properties comparable to those of **2**.[17d]



5 **6** **7** **8** **9, 11–13** **10**

Figure 4. Constitution of pseudohypericin (**5**), fagopyrin (**6**), hypericinic acid (**7**), Lamprometra pigment (**8**), the gymnochromes A – D (**9**: R = (*S*)-CH(OH)CH_3, R′ = (*R*)-CH(OH)$(CH_2)_2$)CH_3; **10**: R = (*S*)CH(OH)CH_3, R′ = (*R*)-CH(OH)$(CH_2)_2CH_3$ or R′ = (*S*)-CH(OH)CH_3, R = (*R*)-CH(OH)$(CH_2)_2CH_3$; **11**: R = (*S*)-CH(OH)CH_3, R′ = (*S*)-CH(OSO_3H)CH_3; **12**: R = R′ = (*R*)-CH(OSO_3H)$(CH_2)_2CH_3$), and isogymnochrome D (**13**), the atropisomer of **12**.

In 1943 fagopyrin (**6**; Figure 4) was isolated from the blossoms of the red flowering variety of buckwheat, *Fagopyrum esculentum*, a plant that has been known since 1833 to also induce a light-dependent disease in grazing animals (called fagopyrism).[18a,b] Its constitution was deduced only in 1979.[18c] Thereby, it has been one of the longest known natural products of unknown constitution.

Hypericinic acid (**7**; Figure 4) is derived by oxidation of one of the methyl groups of hypericin (**2**). It is found as a by-pigment of **2** in the flour beetle *Nipaecoccus aurilanatus* mentioned above.[7a] The molecular skeleton of hypericin is also found in pigments that occur in sea lilies. Thus, **8** (Figure 4) was isolated from *Lamprometra palmata gyges*

Bell and several other related crinoids.[19b] In addition, the gymnochromes **9**–**13** (Figure 4), a family of brominated and ω-substituted hypericin derivatives, were found in the deep sea crinoid *Gymnocrinus richeri*.[20a,b] Interestingly, other halogen derivatives, such as 2,5-dichloro- and 2,5,9,12-tetrachlorohypericin, could be isolated from lichens (for example, *Nephroma laevigatum*).[20c,d] All these compounds, as well as their precursors of biogenesis, seem to function mainly as ingestion deterrents.[19a]

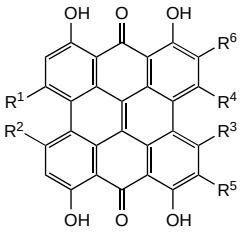


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Figure 5. Constitution of stentorin (**14**: $R^1=R^2=R^3=R^4=OH$, $R^5=R^6=CH(CH_3)_2$) and of fringelite D (**15**: $R^1=R^2=R^3=R^4=OH$, $R^5=R^6=H$), E (**16**: $R^1=R^2=R^4=OH$, $R^3=R^5=R^6=H$), F (**17**: $R^1=R^2=OH$, $R^3=R^4=R^5=R^6=H$), and H (**18**: $R^1=R^2=R^3=R^4=R^5=R^6=H$).

2.2. Stentorin and Fringelites

The photoreceptor pigment stentorin (**14**; Figure 5), which is responsible for the actions of the flagellated protozoon *Stentor coeruleus* (Figure 6), was discovered in the last century (1873) when searching for the pigment responsible for the "green oysters", which were then en vogue.[21a] The constitution **14** of this pigment, formerly called marennin according to the oysters source, which is attached to a protein was isolated by Song in 1993, and was later on proven by synthesis.[21b, 23] Despite intensive efforts the molecular basis of the photosensory signal is still an open question.[22] A photo-induced proton or electron transfer might be regarded as the primary process.[22d, 22g]

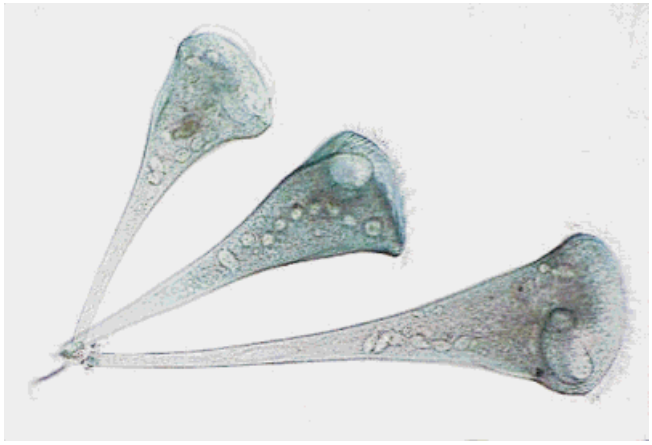


Figure 6. *Stentor coeruleus* (length of the organism approximately 200–400 μm; picture courtesy of D. C. Wood).

An echo of the chromophoric system of **14** from prehistoric times still reverberates in the fringelites (**15**–**19**; Figure 5). These pigments were discovered by Blumer in the Jurassic sea lily *Millericrinus munsterianus* Orbigny (Figure 7) found in Fringeli in the Swiss Jura hills.[24] The pigments are, as shown by a screening of fossils, widely distributed with respect to their ages and organisms.[25] The fringelites, and in particular, fringelite D (**15**), have been found as early as the Devonian

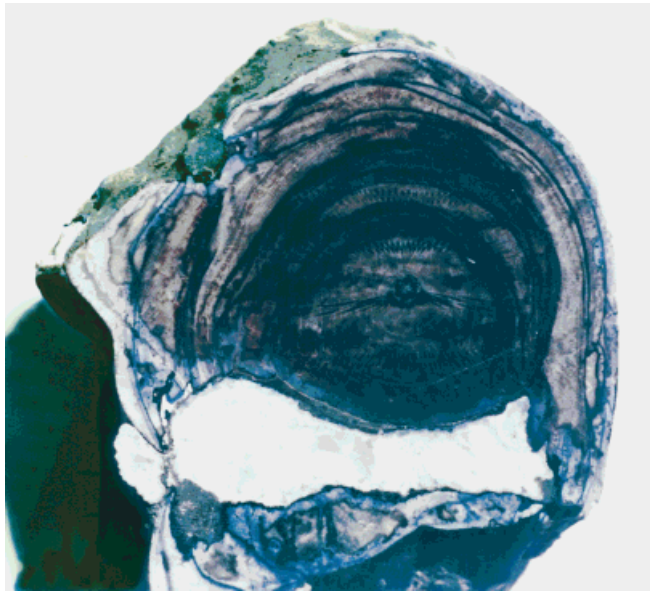


Figure 7. Section through the roots (diameter approximately 5 cm) of the fossilized sea lily *Millericrinus munsterianus* Orbigny from the Upper Jurassic of Liesberg/Switzerland.

age in animal as well as plant fossil specimens—a surprising manifestation of the chemical stability of these organic pigments.[25d] Clearly, they are stabilized against washing out from the fossil material through the formation of their calcium salts and transition metal chelates.[25c] It is still questionable what the physiological significance of the fringelites or their precursors is. However, their function as ingestion deterrents might not be too far fetched.

2.3. Blepharismins

Most of the almost fifty species of the flagellated protozoan genus *Blepharisma* (Figure 8) contain a pigment, which depending on light intensity may be present as a reddish or

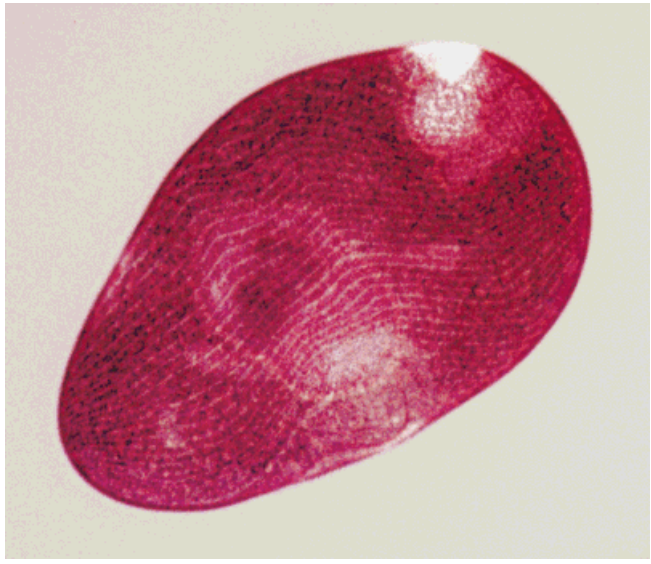


Figure 8. *Blepharisma japonica* (length of the organism approximately 300–400 μm; picture courtesy of F. Lenci).

blue colored form. The pigment is contained in small membrane-coated granules and is bound there to a protein.[26] This pigment, called blepharismin (formerly zoopurpurin), has been known since 1905.[26a] Analysis with respect to the chromophore of this pigment system revealed that it consisted of at least five constitutionally related, ring-homologous phenanthroperylene quinones. The constitution of the blepharismins 1–5 (**19**–**23**; Figure 9) was deduced only recently

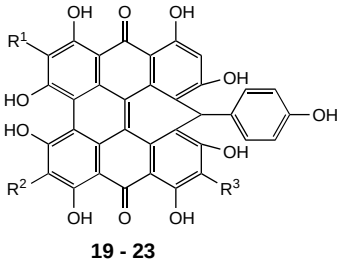


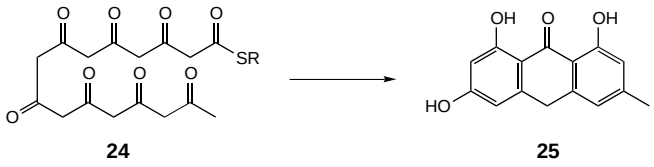
Figure 9. Constitution of blepharismin 1 (**19**: $R^1 = R^2 = C_2H_5$, $R^3 = H$), 2 (**20**: $R^1 = C_2H_5$, $R^2 = CH(CH_3)_2$, $R^3 = H$), 3 (**21**: $R^1 = R^2 = CH(CH_3)_2$, $R^3 = H$), 4 (**22**: $R^1 = C_2H_5$, $R^2 = CH(CH_3)_2$ or $R^1 = CH(CH_3)_2$, $R^2 = C_2H_5$, $R^3 = CH_3$), and 5 (**23**: $R^1 = R^2 = CH(CH_3)_2$, $R^3 = C_2H_5$); for the systematic nomenclature of the ring system see ref. [27c].

by two research groups; **21** constitutes the main pigment.[27] The constitution of "oxyblepharismin" originating from a rearrangement of the blepharismins was recently assigned. It comprises the 4-hydroxybenzaldehyde-*bay*-acetal of **14**.[28n]

The photobiology of *Blepharisma* has been investigated thoroughly.[26b,c, 28] The photosensory signal for the "step-up" photophobic response of the pigment system could originate in a similar manner to the one of stentorin (**14**), with the primary process being a photoinduced proton ejection or an electron transfer reaction.[28g,k]

3. Biogenesis and Synthesis

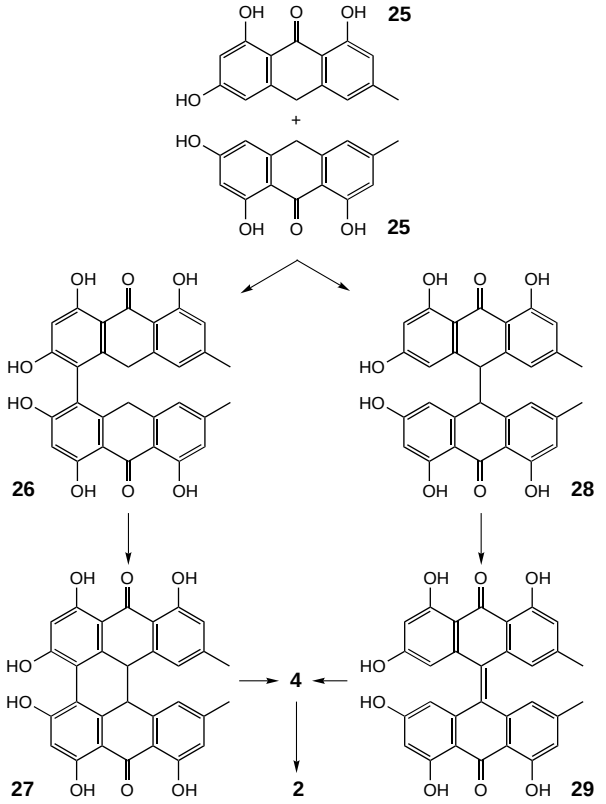
Biogenesis of the phenanthroperylene quinones, which was mostly studied for hypericin (**2**), may be viewed as two consecutive reaction cascades. The first one consists of the biosynthesis of the two anthraquinone "halves" of the phenanthroperylene quinone (Scheme 1). For this purpose,



Scheme 1. Biogenesis of emodin anthrone (**25**).

the well understood acetate malonate pathway (1 acetyl coenzyme-A + 7 malonyl coenzyme-A) that occurs in many organisms of the animal and plant kingdoms is used. It is well known with respect to its enzyme systems, and in this case leads to the octaketide **24**.[29] Multiple aldol cyclization, which was also impressively demonstrated to proceed in chemical synthesis, then results in emodin anthrone (**25**).[29c–e] This compound could also be detected in plant material, which documented its role as the precursor of the second reaction cascade.[30]

In principle, the oxidative dimerization of **25** that forms the second cascade can proceed by two pathways (Scheme 2). In contrast to the well established biogenesis of the precursor,



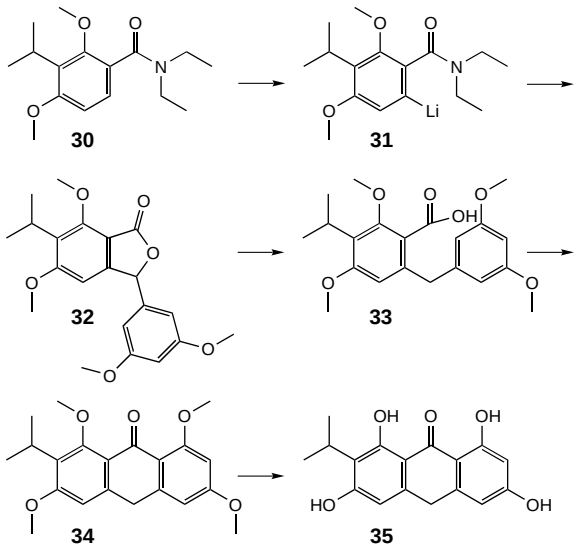
Scheme 2. The two pathways of the biosynthetic dimerization of emodin anthrone (**25**) that yield hypericin (**2**).

the formation of the phenanthroperylene quinone is only speculated.[4a, 4e, 30] The first possible way starts with a phenol coupling to yield penicilliopsin (**26**). This then undergoes a second oxidative coupling to provide the diastereomers of **27**, which is followed by dehydrogenation to give protohypericin (**4**). Photooxidation of **4** eventually leads to hypericin (**2**). However, as shown by the gymnochromes (**9**–**13**; Figure 4),[20] which originate in the darkness of the oceans, the formation of the phenanthroperylene skeleton can also take place without light. For the second possibility, dimerization starts from the central methylene group of the anthrone **25** and yields the diastereomers of **28**. These undergo dehydrogenation to afford **29**, which is then cyclized by oxidative phenol coupling to provide **4**. Of all these intermediates only **26** and **4** could be isolated from the fungus *Penicillium brunneum*,[31] and in particular, from the plant *Hypericum*.[30] As an alternative, skyrin (**3**), identified in *Dermocybe austroveneta*,[7b] constitutes a possible hypericin precursor molecule. Currently, these rather scarce facts and the chemical (in vitro) syntheses form the basis of hypotheses on the biogenesis of phenanthroperylene quinones from the anthracenoid octaketide precursor. Moreover, the question as to whether these

reactions are catalyzed enzymatically or not is still unanswered. The first results and hypotheses on the biogenesis of the *Stentor* and *Blepharisma* pigments were published recently.[28n]

The chemical synthesis of the phenanthroperylene quinones follows the pattern of biogenesis. A rich arsenal of methods is available to prepare the anthraquinone or anthrone precursors.[32] The best strategies for the synthesis of anthracene derivatives substituted in an arbitrary way are based on a Friedel–Crafts cyclization of the correspondingly substituted diphenylmethane carboxylic acid and on a Diels–Alder cycloaddition. This is demonstrated by the example of the two emodin anthrones **35** and **41**, which served as precursors for the synthesis of stentorin (**14**).

To prepare **35** (Scheme 3) the benzamide **30** is regioselectively metalated to provide **31**.[23c] This method was developed by Snieckus et al. and has also been employed successfully in

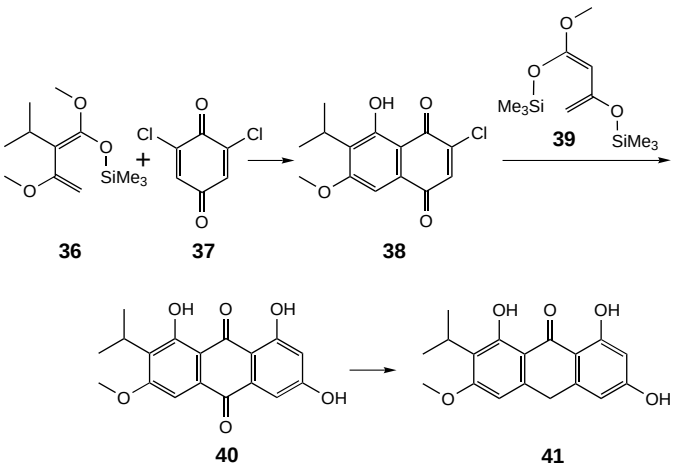


Scheme 3. Synthesis of **35**.

the synthesis of a variety of natural compounds.[33] The reaction of **31** with 3,5-dimethoxybenzaldehyde yields after work-up the lactone **32**, which is hydrogenated to **33**. This acid is then cyclized by means of a Friedel–Crafts reaction to give **34**. Upon its demethylation the desired anthrone **35** is produced.

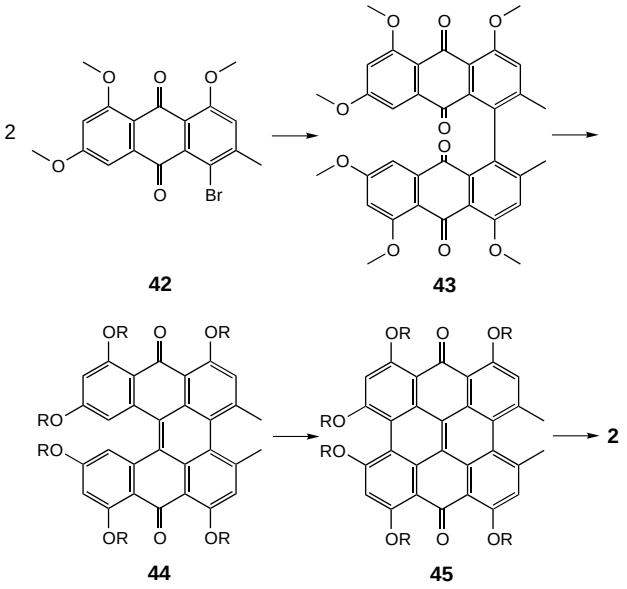
The Diels–Alder pathway leading to **41** (Scheme 4) makes use of the doubly activated ene component of quinone **37**. This is cyclized with the silyl-diene component **36** to give **38** and then this is cyclized with the disilyl derivative **39** to yield the anthraquinone **40**.[23a,b,d] The regioselective reduction of **40** with Sn/HCl finally provides the anthrone **41**.

Emodin anthrone (**25**), the most favored precursor for the preparation of **2**,[34a] is obtained from the reduction of emodin. This can be easily isolated from the bark of the breaking buckthorn (Cortex *frangulae*).[34b] However, the very elegant synthesis sequence corresponding to the biomimetic polyketide formation (Scheme 1)[29c,d] did not reach importance in the synthesis of anthrones, such as **25**, **35**, and **41**, mainly because of the demanding protecting group strategy needed.



Scheme 4. Synthesis of **41**.

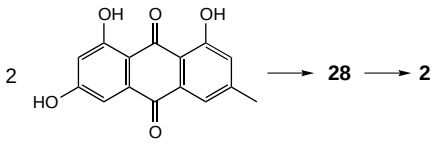
The dimerization of anthraquinone or anthrone precursors to phenanthroperylene quinones may be achieved in several ways. The classical route that led to the first chemical synthesis of hypericin by Brockmann[4d] is based on the regioselective Ullmann coupling of the bromoanthraquinone **42** to the bianthraquinone **43** (Scheme 5). Its reductive



Scheme 5. Synthesis of hypericin (**2**) by Ullmann dimerization of the bromoanthraquinone **42**. **44**, **45**: $5 \times R = CH_3$, $1 \times R = H$.

cyclization to **44** (copper powder in acetic acid/HCl) proceeds smoothly. However, one of the methoxy groups is cleaved in this reaction. Oxidative photocyclization to **45** and demethylation eventually leads to hypericin (**2**). The yields are in most steps satisfactory, however, the disadvantage of this reaction sequence is the demanding and low yielding synthesis of the starting material (**42**). Nevertheless, this methodology was successfully adapted to the synthesis of stentorin (**14**).[23b]

The dimerization of the anthraquinone precursor emodin (**46**; Scheme 6) under alkaline conditions in the presence of hydroquinone as the reducing agent and under nitrogen and



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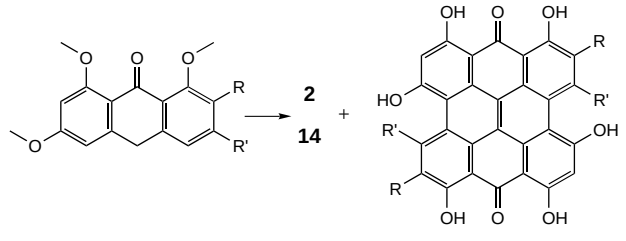
Scheme 6. Synthesis of hypericin (**2**) by dimerization of the anthraquinone **46**.

with the exclusion of light proceeds via the dibenzoperylene derivative **4** (Scheme 2). Photochemical cyclization of the latter then leads to **2**.[35] This procedure is of principal interest for hypotheses about its biogenesis, however, the reaction conditions (20 days in a sealed tube at 100 °C) and its yield (< 30 %) render this method rather unattractive for preparative purposes.

Therefore, procedures relying on the dimerization of anthrone derivatives were used rather early on. Thus, in 1955 Brockmann succeeded in fusing two molecules of emodin anthrone (**25**) through a biomimetic sequence via **28**, **29**, and **4** into **2** (Scheme 2).[31] This synthesis strategy was improved recently with respect to the reagents (hexacyanoferrate(III),[36a] pyridine-*N*-oxide/piperidine/iron(II)-sulfate[36b]) and conditions used. Moreover, several intermediates, such as the diastereomers of **28**, and the bianthrone **29** could be characterized.[36a] These improvements led to an universally applicable strategy that allowed for the synthesis of a series of hypericin derivatives,[37] of fringelite D (**15**),[38] and stentorin (**14**).[23a,c,d]

The regioselectivity of the primary cyclization of **28** (or **29**) in the case of the unsymmetrically substituted anthrone precursors is caused by the difference in reactivity between the two benzene rings flanking the central moiety.[36c] Thus, the difference in electronic activation between the methyl and hydroxyl groups in **25** is sufficient to steer the reaction regioselectively to produce **2**. However, starting from the emodin anthrone trimethyl ether (**47**), dimerization after demethylation affords the mixture of hypericin (**2**) and isohypericin (**49**; Scheme 7).[39] Besides a selective synthesis for the production of isostentorin (**50**),[23a,d] this route was also used for the synthesis of **50** starting from **48**.[23c]

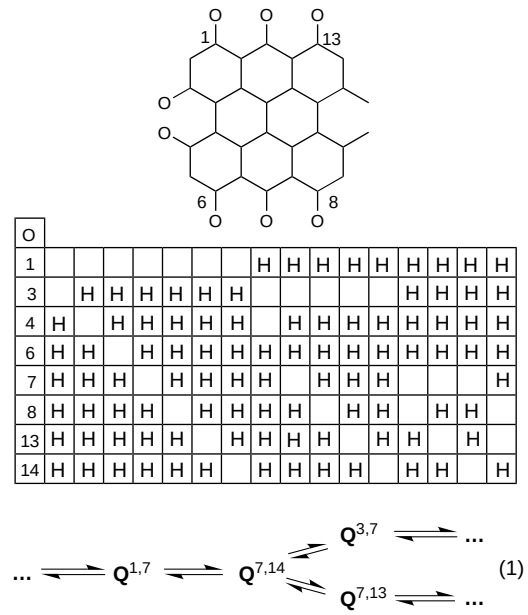


47, **48** **49**, **50**

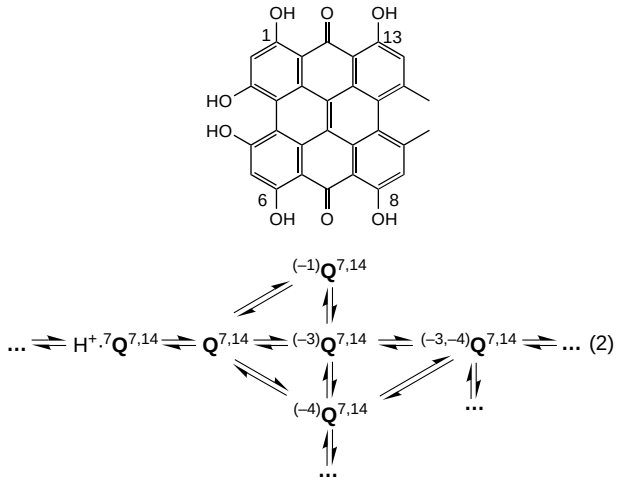
Scheme 7. Synthesis of hypericin (**2**), isohypericin (**49** R = H, R′ = CH_3), stentorin (**14**), and isostentorin (**50** R = $CH(CH_3)_2$, R′ = OH) by nonregioselective dimerization of the corresponding trimethoxyanthrone **47** (R = H, R′ = CH_3) and **48** (R = $CH(CH_3)_2$, R′ = OH).

4. Structural Complexity Resulting from the Intertwining of Equilibria

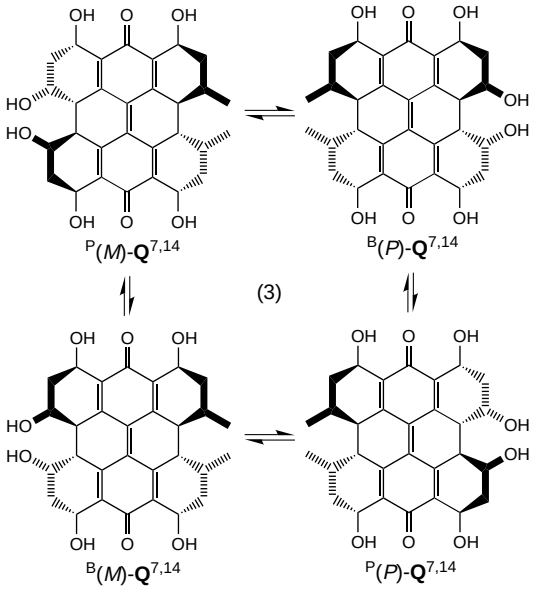
Investigation of the structural aspects of multiple hydroxyl-substituted phenanthroperylene quinones shows that these compounds are more complicated than their simple structural formulas suggest, and are involved in four correlated equilibria of tautomerism (Scheme 8, equilibrium (1)), dissociation (Scheme 9, equilibrium (2)), torsional isomerism (Scheme 10, equilibrium (3)), and homoassociation (Scheme 11, equilibrium (4)), which leads to a high degree of structural complexity. Upon joining these four structural aspects, the example of **2** shows that there are ten tautomers that might in principle all be present as four species (singly protonated, neutral, and the respective energetically most favored single and double deprotonated forms). This adds up



Scheme 8. Partition diagram for the distribution of six protons upon the eight oxygen atoms of hypericin (**2**) and the resulting partial tautomeric equilibrium (1); within this scheme, for example, $\mathbf{Q}^{7,14}$ denotes the tautomer with the carbonyl groups in positions 7 and 14—this one corresponds to the last column of the partition diagram.



Scheme 9. Protonation and deprotonation equilibria (2) of the tautomer $\mathbf{Q}^{7,14}$ of **2**; herein, for example, $^{(-3)}\mathbf{Q}^{7,14}$ denotes the tautomer $\mathbf{Q}^{7,14}$, which is deprotonated in position 3, and $H^+ \cdot {}^7\mathbf{Q}^{7,14}$ then denotes the tautomer $\mathbf{Q}^{7,14}$ protonated at the carbonyl group in position 7.



Scheme 10. Conformation equilibrium (3) of the undissociated tautomer $\mathbf{Q}^{7,14}$ of **2**; herein, for example, $^{P}(M)$-$\mathbf{Q}^{7,14}$ denotes the propeller conformation of the tautomer $\mathbf{Q}^{7,14}$ of configuration (*M*).

$$^{P}(P)\text{-}\mathbf{Q}^{7,14} + {}^{P}(P)\text{-}\mathbf{Q}^{7,14} \rightleftharpoons [{}^{P}(P)\text{-}\mathbf{Q}^{7,14}]_2 \overset{+\,{}^{P}(P)\text{-}\mathbf{Q}^{7,14}}{\rightleftharpoons} \ldots \quad (4)$$

Scheme 11. Homoassociation equilibrium (4) of the propeller conformer of configuration (*P*) of tautomer $\mathbf{Q}^{7,14}$ of **2**, $^{P}(M)$-$\mathbf{Q}^{7,14}$.

to forty such species. Since every one of these species may occur as four torsional stereoisomers the total number of species reaches 160. Eventually, these species may be involved in oligomerization equilibria, for example, in the form of H- or J-aggregates,[40] which renders the number of possible species more or less innumerable. The following sections will focus upon experimental as well as semiempirical or quantum chemical results that clarify the important parts or knots in this complex equilibrium system under certain conditions.

4.1. Tautomerism

The ten canonically structured tautomers of hypericin (**2**; see Scheme 8) are involved in a one proton interconversion network, in which one proton at a time is transferred from a hydroxyl oxygen to a carbonyl oxygen atom. This high complexity of the system has so far more or less prohibited an experimental approach. However, as shown in Figure 10, it could be analyzed with respect to its thermodynamic aspects by means of semiempirical molecular modeling. From such calculations, regardless of the method (HMO, MM2+ force field,[41a] AM1,[41b,e] ab initio with a 6-31G basis set[41d,c]), it is found that the tautomer $\mathbf{Q}^{7,14}$ (in the gaseous state) is at least 40 kJ mol^{-1} more stable than all the others. This is a consequence of the stability of its π system; the highest stability as given by the aromaticity index can even be derived by simple graphical theoretical reasoning.[42] Moreover, the highest number of possible Kekulé structures for all the

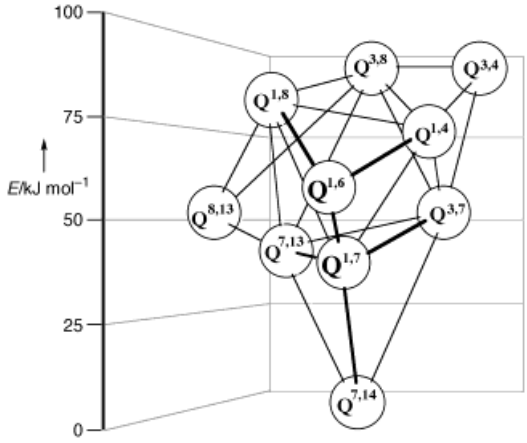


Figure 10. One proton interconversion net and relative binding enthalpies of the tautomers of hypericin (**2**) as deduced from semiempirical (AM1) calculations (for the meaning of designations $Q^{x,y}$ see the legend of Scheme 8).

tautomers of **2** (or of the fundamental system **1**) are derived for the $\mathbf{Q}^{7,14}$ tautomer.[41a,e] The corresponding analyses were also carried out for the ten tautomers of isohypericin (**49**), the sixteen of stentorin (**14**), the fifteen of isostentorin (**50**), and the nine of fringelite D (**15**), and in all cases the pronounced stabilization of the $\mathbf{Q}^{7,14}$ tautomer was found.[43] The activation barrier for the intramolecular transfer of a proton within the *peri* region ($\mathbf{Q}^{7,14} \rightarrow \mathbf{Q}^{1,7}$) was estimated by means of ab initio calculations on **2** to amount to about 41 kJ mol^{-1}, and for the reverse process approximately 7.5 kJ mol^{-1}.[41d]

However, experimental evidence for the presence of defined tautomers under certain conditions is scarce. Moreover, the tautomers are strongly related to the state of dissociation, and thus they will be discussed together with the latter phenomenon. (Section 4.5.).

4.2. Dissociation (Protonation and Deprotonation in Ground and Excited States)

With regard to the protonation of hydroxyphenanthroperylene quinones, such as hypericin (**2**; Figure 18), both the hydroxyl and carbonyl groups have in principle to be considered. The latter ones are usually more basic than the first ones, and their protonation leads to a characteristic change in the chromophore (see Section 5.1.). Therefore, one to two protonation steps can be anticipated for such compounds and for aromatic ketones these steps should be found in the region of $pK_a < -5$. In fact, about 70 % aqueous sulfuric acid has to be used in the spectrophotometric titration of the unsubstituted quinones **1** to produce the monoprotonated form, **1**-H$^+$. This result corresponds to a pK_a value of -6, and for the second carbonyl group a $pK_a = -7$ is found.[44b] The monoprotonation pK_a values are found within the same pK_a region for **2**,[44a] **14**,[23c] and **15**.[38a] The application of a Förster cycle reveals that the basicity of the carbonyl group in the excited state is somewhat higher. Thus, for example, the pK_a^* of **2** is determined as -3.2.[44a]

Deprotonation of hydroxyphenanthroperylene quinones is possible in principle at the phenolic hydroxyl groups in the *peri* and *bay* positions. However, these groups were found to display dramatically different acidities. Whereas a *bay*-hydroxyl group with a pK_a value of 1.8 for **2** approximately matches the acidity of picric acid, the *peri*-hydroxyl groups are deprotonated only within or above the region of common phenols, whereby one (p$K_a \approx 9$) to two (p$K_a \approx 12$) deprotonation steps may be observed. This was shown to be the case for phenols in aqueous solvents and water by means of spectrophotometric titrations,[23c, 38a, 44a, 44c] NMR spectroscopy,[6, 23c, 41a] electrophoresis,[44c] electrospray mass spectrometry,[44d] X-ray structural analysis,[41a, 45a] and comparison with model compounds partially methylated at the hydroxylic groups.[44e] Figure 11 summarizes the results of these studies

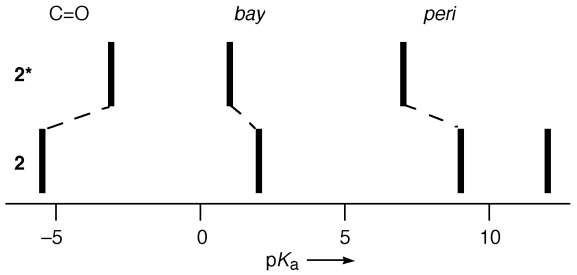


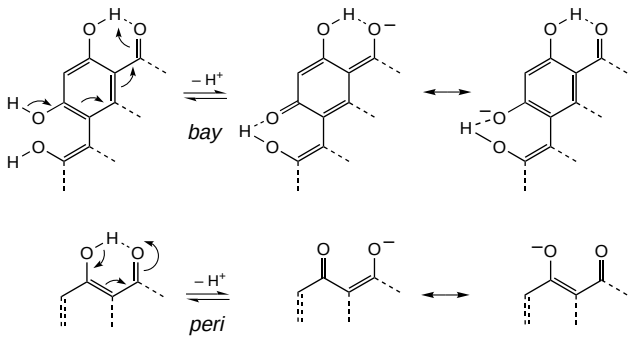
Figure 11. Protonation (C=O) and deprotonation steps (*bay* and *peri* region) of hypericin in the ground state (**2**) and excited state (**2***).

for the example of **2**. It should be stressed here that numerous data published for **2** actually refer to the hypericin-3-ate ion since *bay*-hydroxy derivatives are completely dissociated in polar solvents at low concentrations.[38a, 44c]

The reason for these different acidities of the hydroxyphenanthroperylene quinones in the two regions can be rationalized in the case of their *bay*-ionization by formation of a vinylogous carboxylic acid.[38a, 44c] The resulting phenolate ion $^{(-3)}$**2** produced in this way is stabilized by a very strong, yet unsymmetrical hydrogen bond.[37b, 45] This was deduced from the unsymmetrical distribution of C–C bonding distances,[45a] polarization spectroscopy,[37b] hole-burning experiments,[45b] isotope effects,[45c] and ab initio calculations.[45c] Proton transfer that leads to the formation of the identical tautomeric *bay*-phenolate $^{(-4)}$**2** is extremely fast in solution. Moreover, as derived from X-ray structural analyses, NMR investigations, and intercepting reactions, the *bay*-hypericinate ion is present in the crystalline state as well as in solution as its $\mathbf{Q}^{7,14}$ tautomer.[6, 23c, 41a, 45a,e] In contrast to this, a phenolate ion in the *peri*-region is destabilized by interaction of the lone pairs on the carbonyl oxygen atoms (Scheme 12). The application of a Förster cycle to the excited state yields an acidification of the *bay*- and *peri*-hydroxylic groups by 1 to 4 pK_a units (Figure 11).[23c, 38a, 44a]

4.3. Torsional Isomerism

Substitution of the *bay* region of phenanthroperylene quinones with hydroxyl, and in particular with alkyl groups,



Scheme 12. Hydroxyphenanthroperylene quinone: vinylogous carboxylic acid and stabilization upon deprotonation of the *bay* region, and destabilization upon deprotonation of the *peri* region.

as found in the natural products **2**–**23**, leads to a significant dihedral deformation of the unsubstituted parent compound (**1**), which itself displays an only slightly distorted molecular skeleton. Conformational analysis by means of force field models is shown for hypericin (**2**) in Figure 12.[37d, 41a, 43a, 43b] Besides two energetically similar (± 5 kJ mol^{-1}), but distinct

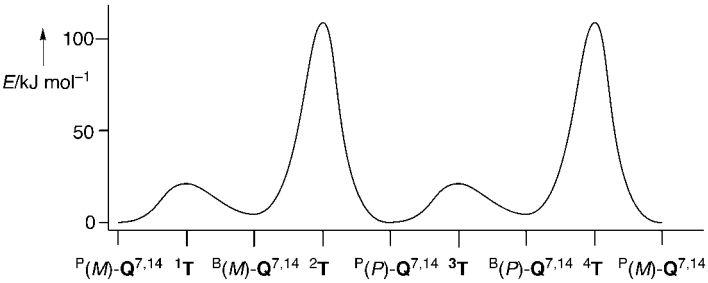


Figure 12. Section through the energy hypersurface of hypericin (**2**); for the designation of the various species see Scheme 10 and Figure 13.

minima pairs, $^{P}(M)/^{P}(P)$ and $^{B}(M)/^{B}(P)$, two quite different (≈ 30, ≈ 100 kJ mol^{-1}) extremal pairs, ^{1}**T**/^{3}**T** and ^{2}**T**/^{4}**T** are obtained. The first pair correspond to the propeller and butterfly conformers shown in Figure 10, while the latter ones are highly strained transition conformations between the energy valleys (Figure 13). The principal situation as given in Figure 12 is only slightly modulated by dissociation, tautomerism, and substitution at the aromatic rings and substituents. This is supported by experimental results.

Accordingly, the dihedral propeller symmetry of the skeleton is clearly revealed in the X-ray structure of two crystal forms of pyridinium hypericin-3-ate.[41a, 45a] The dihedral angles at the hydroxyl and the methyl *bay* regions, $\Theta_{3,\,3a,\,3b,\,4}$ and $\Theta_{10,\,10a,\,10b,\,11}$, amount to 19.6 and 31.9°, respectively.[45a] The results of semiempirical,[41a,b] and in particular from ab initio calculations,[41c,d] are virtually superimposable with the experimental structural data. Thereby, such calculations expand and extrapolate our knowledge beyond that currently accessible from experimental investigations (for example, despite numerous efforts crystals of hypericin itself that are suitable for X-ray structure determination have not yet been obtained). The three orthogonal images of the hypericin-3-ate ion shown in Figure 14 illustrate the degree of distortion of the phenanthroperylene quinone skeleton.

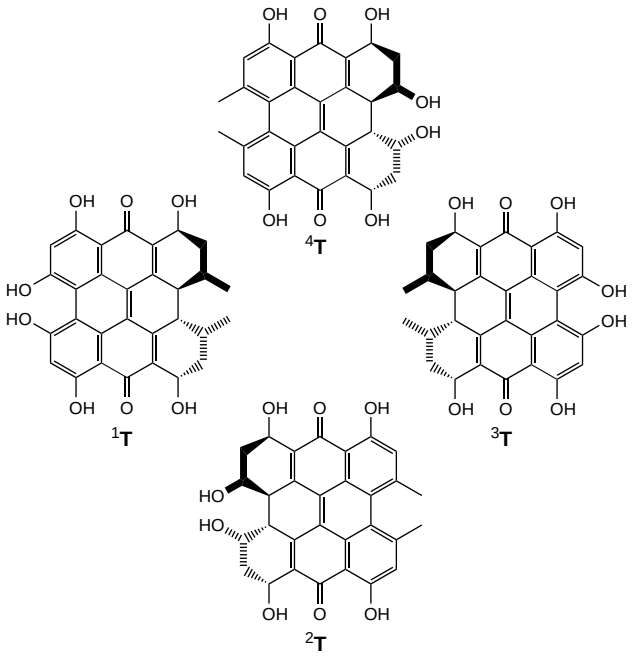


Figure 13. Transition conformations ^{1}**T**–^{4}**T** in the conformational cycle of **2**.

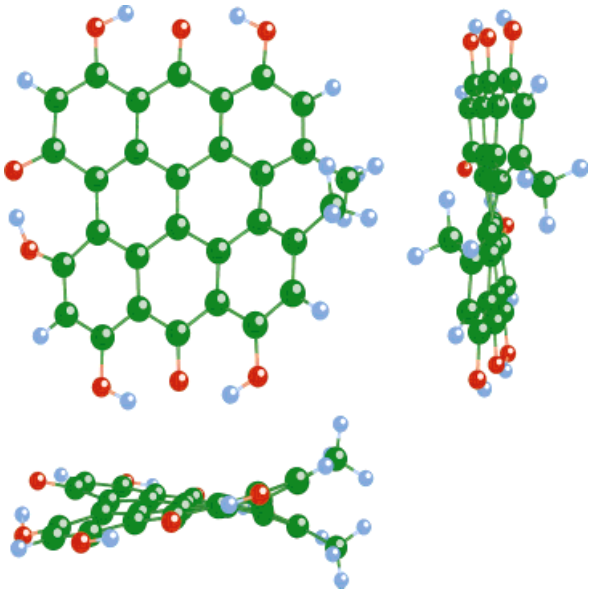


Figure 14. Ball and stick views[46] of the hypericin-3-ate (results from an ab initio calculation; 6-31G basis set).[45c]

With respect to the energetic situation of *bay*-substituted phenanthroperylene quinones an estimate of a lower limit of the enantiomerization barrier (^{2}**T** in Figure 12) of 80 kJ mol^{-1} was determined from the temperature dependent ^{1}H NMR spectrum of pseudohypericin (**5**).[41d] Eventually, the synthesis of the two hypericin derivatives **51** and **52** (Figure 15) bearing enantiomerically pure (*R*)-menthyl residues in one or both side chains, respectively, allowed for more significant results to be obtained.[37d] Measurements of the diastereomerization kinetics of these two thermally easily equilibrating diastereomer pairs at various temperatures afforded Arrhenius activation energies for the inversion of the propeller configurations of 83 ± 3 and 89 ± 3 kJ mol^{-1}. As is clear from the demanding space requirements of the menthyl moieties of **51** and **52** on the one hand, and the concomitant small difference of their activation barriers on the other hand, the thus derived height of the activation barrier represents the intrinsic barrier of *bay*-alkyl-substituted phenanthroperylene quinones. Moreover, this height is in good agreement with the results of semiempirical calculations, thereby also strengthening their respective results.[37d, 41] The very strong chiroptical effects observed for these derivatives (see Section 6) provide further evidence of the chromophore helicity and even corroborates its propeller conformation.

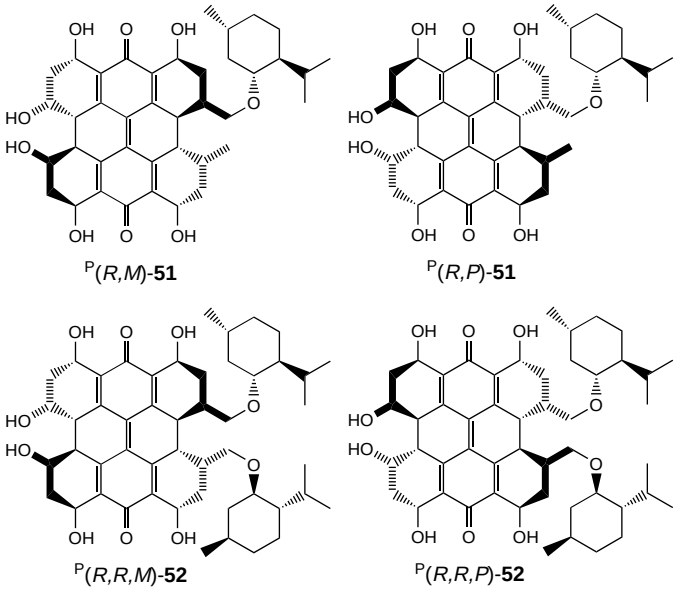


Figure 15. The respective diastereomeric (*R*)-menthylhypericin derivatives **51** and **52**.

4.4. Homoassociation

Besides their dissociation to their *bay*-phenolate ions (see Section 4.3.), hypericin (**2**) and its analogues are monomolecularly dissolved in polar organic solvents, such as dimethylsulfoxide or alcohols. This is proven by compliance with the Beer–Lambert law and results obtained from vapor pressure osmometry.[47a] Upon addition of water to such solutions a dramatic change in their absorption and emission properties,[47a] as well as their photodynamic behavior is observed.[47b] This is a result of the formation of dimers at low concentrations;[47b] at higher concentrations H-aggregates are formed.[40, 47a]

By applying the exciton theory to these dimers an arrangement with parallel ring planes and orthogonal dipole axes is derived.[47b] From measurements of nuclear Overhauser effects (NOE) for strategic ^{1}H NMR signals and of spin-lattice relaxation times H-aggregates were identified that consisted of at least four molecules with their C_2 axes rotated against each other by about 180° (Figure 16).[47a] Polymeric aggregates precipitate upon storage of aqueous hypericin solutions, which can even be viewed by means of electron

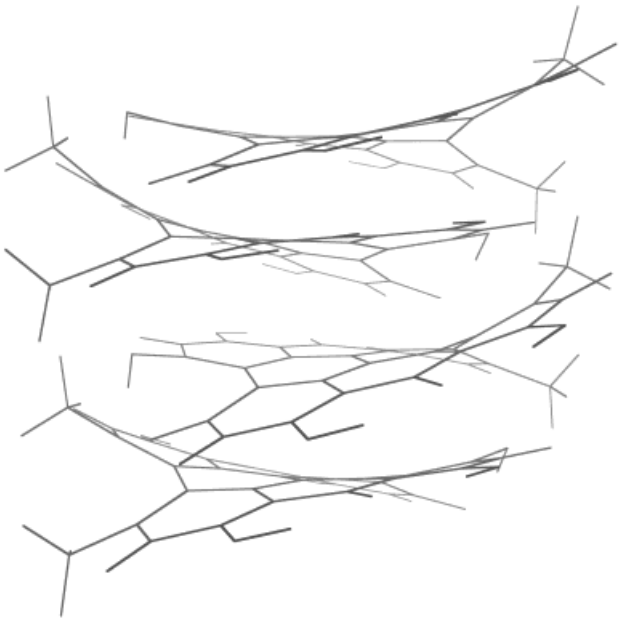
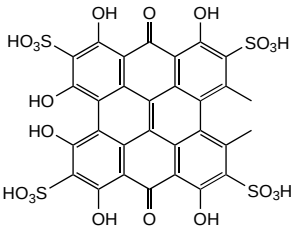


Figure 16. Schematic representation[46] of a hypericin-3-ate-H-aggregate.



53

Figure 17. The formation of J-aggregates at concentrations above 10^{-4} M is possible for the water-soluble hypericin derivative **53**.

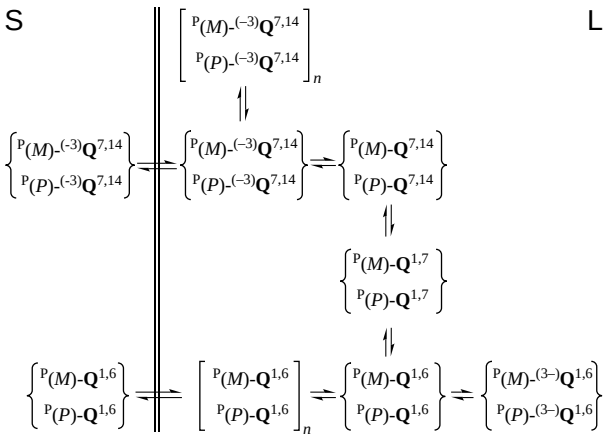
microscopy.[47c] The formation of J-aggregates was possible in the case of the water-soluble hypericin derivatives **53** (Figure 17) at concentrations above 10^{-4} M.[47d]

4.5. Synopsis

In conclusion, from these detailed investigations of the complex equilibrium system discussed above for the phenanthroperylene quinones, particularly for hypericin (**2**), the experimentally determined equilibrium diagram shown in Scheme 13 is obtained. Thus, according to X-ray analysis the hypericinate ion is present in the solid state (S) as the racemate $\{^{P}(M)\text{-}^{(-3)}\mathbf{Q}^{7,14} + {}^{P}(P)\text{-}^{(-3)}\mathbf{Q}^{7,14}\}$ of the propeller conformer of the $\mathbf{Q}^{7,14}$ tautomer.[38b, 41a]

In solutions (L) the dissociation and homoassociation equilibria of this system will be dependent on solvent, concentration, and proton concentration, and this will govern the presence of certain species.[23c, 38a, 44c, 45c,d, 47a] Moreover, high concentrations in aprotic, slightly polar solvents, such as tetrahydrofuran or aliphatic esters, as well as addition of Lewis acids favors tautomerization via the $\mathbf{Q}^{1,7}$ tautomer to yield the extremely sparingly soluble $\mathbf{Q}^{1,6}$ tautomer. This property may be used to enrich or isolate this tautomer.[45e, 48a] The latter is also involved in dissociation and association equilibria. In addition, according to several investigations it is very probable that undissociated crystalline **2** is present as its $\mathbf{Q}^{1,6}$ tautomer.[45e, 48a, 48b]

All these results suggest that extreme care should be exercised during investigations of the physiological and



Scheme 13. The complex equilibrium system of phenanthroperylene quinones, from experimental results mainly for **2**. Winged brackets denote racemic mixtures. Square brackets indicate associates of *n* molecules, S denotes the crystalline state, and L the solution state.

physical properties of the phenanthroperylene quinones with respect to the presence of certain species under certain experimental conditions.

5. Light Absorption and Emission—Excited States

The extended conjugation system of **1** renders its derivatives as colorful dyes that possess unique properties in their excited states. Thus, it is not surprising that nature has selected them during evolution for a variety of functional roles as photosensitizers and photoreceptors.

5.1. Absorption

The singlet absorption band of the fundamental chromophores of **1** at about 420 nm is bathochromically shifted by substitution with hydroxyl and alkyl groups and is observed at about 600 nm for the highly substituted derivatives such as **2** (Figure 18). This absorption band, and also its phenotype, varies in a characteristic way for the various positions of the equilibria within the complex structural system. Tautomerization and conformational changes (torsion resulting from the influence of substituents, butterfly and propeller conformers) have minor influence on the absorption bands, however, deprotonation, protonation, and association may lead occasionally to dramatic changes in the absorption behavior.[37b, 45e, 48a] Accordingly, protonation and deprotonation normally cause a bathochromic shift. The intensity and form of the second absorption band system around 450 nm (Figure 18) is highly diagnostic for the dissociation of hydroxyl groups. A characteristic feature of H-aggregates is their absorption spectrum, which displays a lower intensity and is hypsochromically shifted relative to the monomer and shows the anticipated exciton splitting (Figure 18).[47a]

This phenomenological behavior of the light-absorbing properties of phenanthroperylene quinones can be nicely rationalized and understood by means of semiempirical

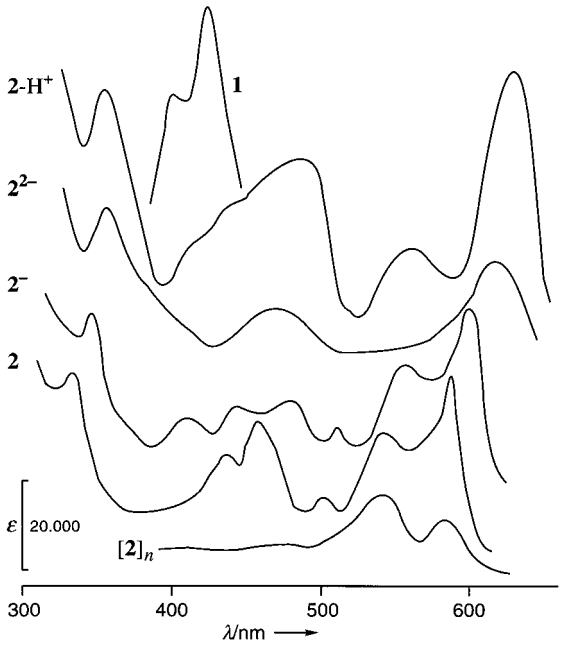


Figure 18. UV/Vis spectra of the fundamental phenanthroperylene quinone (**1**) and of hypericin (**2**) in 80% ethanol, as well as its protonation (**2**-H^+; 80% ethanol, pH ≈ 0.5), *bay*-deprotonation ($\mathbf{2}^-$; 80% ethanol, pH ≈ 6), *bay,peri*-dideprotonation product ($\mathbf{2}^{2-}$; 80% ethanol, pH 12), and of an H-aggregate ($[\mathbf{2}]_n$; H_2O).

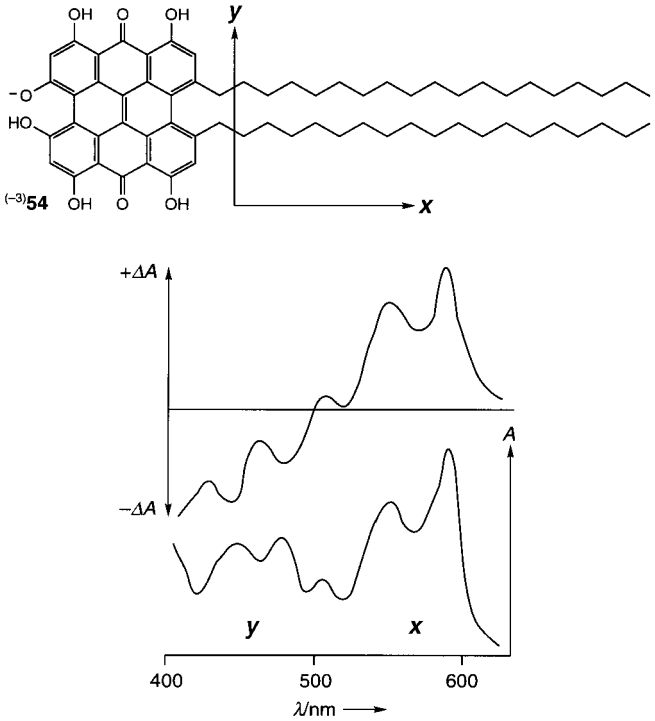


Figure 19. Absorption (A) and polarization spectrum (ΔA) of the hypericin derivative $^{(-3)}$**54** incorporated into polyethylene and then stretched. The polarization curve is obtained by subtracting the absorption obtained with the polarizer oriented perpendicularly to the stretching direction from that of the corresponding parallel orientation. The polarization of the two band systems is then x and y.

quantum chemical calculations of the PPP type.[37b] The distinct structuring of the absorption bands results from vibrational progressions.[37b, 49a] The polarization of the absorption bands derived from such calculations are corroborated by an analysis of the emission polarization,[49b] and moreover, by direct measurements of their polarization.[37b] For the latter experiment, the hypericin derivative $^{(-3)}$**54** (Figure 19) was incorporated into a polyethylene foil, which after being stretched was investigated by means of polarized light.[37b] Thus the obtained polarization of the absorption band obtained shows that the short wavelength band system is rather sensitive to changes in the *bay* region.

5.2. Emission

5.2.1. Fluorescence

Solutions of hydroxyphenanthroperylene quinone derivatives display a significant fluorescence with medium to high quantum yields, which is characterized by only small Stokes shifts and pronounced vibrational progression. The fluorescence is slightly dependent on the ionization of the hydroxyl groups or the tautomeric situation.[23c, 37b,c,d, 38a, 39, 44b, 48a, 50a] However, it is quenched completely upon formation of H-aggregates and it is also dampened by bromine substitution of the aromatic rings.[47a, 50b] As an illustration the intensely studied *bay*-hypericinate ion $^{(-3)}$**2** is used.[51, 52] Its fluorescence band at 595 nm is Stokes shifted by 5 nm (ethanol), and is followed at room temperature by a second band at 640 nm whose intensity is about 80% of the first one. As could be expected, the elusive vibrational structure is sharpened at 77 K and complemented by a band at 700 nm.[52e] Finally, at 1.2 K the vibrational structure is completely developed with sharp bands being observed at 595 (100), 610 (30), 644 (26), 663 (7, shoulder), and 702 (9) nm (relative intensity).[50a] The quantum yield of the fluorescence amounts to 0.2 to 0.3;[47a, 53] its monoexponential decay is characterized by a mean lifetime of 5 ns.[22g, 52a] The fluorescence is self-quenched.[53b] Moreover, it is also quenched by electron donors and electron acceptors.[22g, 52d,g] The latter phenomenon stresses the point that this class of compounds may act either as electron acceptors of the quinone type or as electron donors of the aryl ketone type.[52g] H-aggregates are virtually nonfluorescent.[47a] The emission of the first singlet state is built up with a delay of 10 ps, which is attributed to an intramolecular proton transfer.[9k]

5.2.2. Phosphorescence

Information on the phosphorescence and the triplet state of hydroxyphenanthroperylene quinone derivatives is currently only available for the *bay*-hypericinate ion $^{(-3)}$**2** and isohypericin (**49**).[22g, 49b, 50a, 52e, 53a, 54] The band form of the phosphorescence of $^{(-3)}$**2** is similar to that of fluorescence:[51] at 77 K and 1.2 K a vibrational progression from 755 (100), 779 (70), 836 (33), and 936 (10) nm (relative intensity) is obtained in ethanol.[52e, 50a] The phosphorescence quantum yield is of the order of $\leq 10^{-3}$.[53a] The triplet state is very effectively populated from the singlet state with a quantum yield of this intersystem crossing of about 0.7.[54a] The triplet state decays monoexponentially with a mean lifetime of a few millisec-

onds.[50a, 52e, 53a] The various possibilities of energy transfer originating in the triplet state of $^{(-3)}$**2** have been investigated intensely. These results will be discussed in more detail within the discussion of photochemical reactions (Section 10.2.).[22g, 53a, 54b]

6. Chiroptical Properties

Bay-substituted phenanthroperylene quinones are chiral as a result of the C_1 or C_2 symmetry of their propeller conformers. Thus, in principle, they may be resolved into their antipodes. Since the activation barrier of racemization in many derivatives is rather low, a detailed investigation of their chiroptical properties and an assignment of their absolute configuration had to be postponed until the diastereomers **51** and **52** (Figure 15) became available. These display pronounced opposite Cotton effects in the regions of their two main absorption band systems. These effects are virtually identical for the derivatives $^{(-3)}$**51** and $^{(-3)}$**52**, with the respective two diastereomers of each having a mirror image relation (Figure 20).[55a] This fact corroborates the assumption

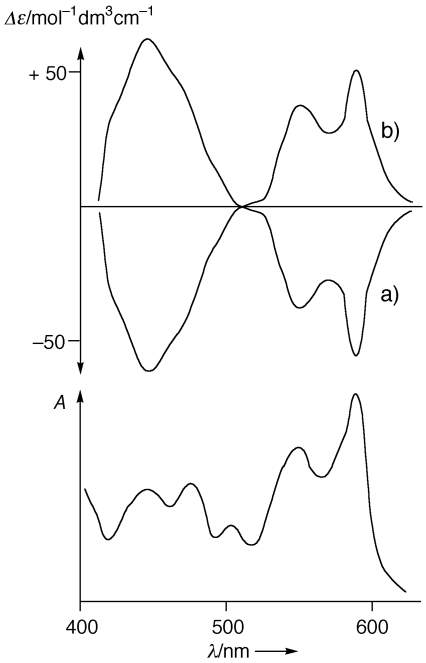


Figure 20. Circular dichroism ($\Delta\varepsilon$) and absorption spectra (A; identical) of the two diastereomers (R,P)-$^{(-3)}$**51** (a) and (R,M)-$^{(-3)}$**51** (b) in ethanol.

that the observed chiroptical effects are caused by the intrinsic helicity of the phenanthroperylene quinone chromophore and are not induced by the menthyl moieties.

Chromophores with formal or intrinsic C_2 symmetry are properly described by means of the Wagnière C_2 rule.[56] From its application the diasteromer with a negative Cotton effect of its long wavelength absorption band is assigned the absolute configuration (P) (Figure 21).[55a] Investigations on **51** and **52** under conditions that stabilize certain species of the complex equilibrium system (see Section 4.5.) do not reveal significant changes in the chiroptical signals. This observation

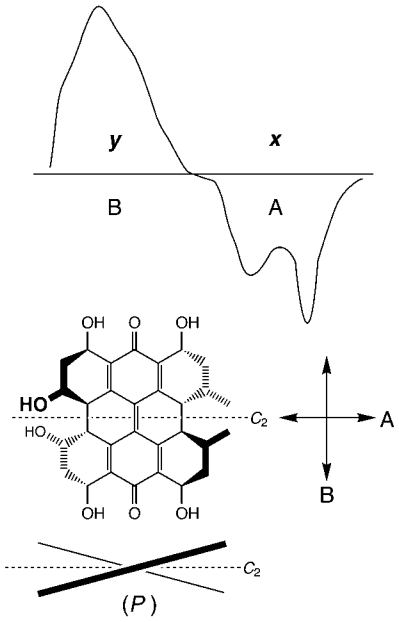


Figure 21. Application of the C_2 rule[56] to the hypericin chromophore: for x and y polarization of the long and short wavelength band (Figure 19) for the point group C_2 the corresponding transitions are of symmetry A and B. Accordingly, the absolute configuration (P) is assigned from the negative Cotton effect of the long wavelength band.[55a]

indicates that the intrinsic helicity and, accordingly, the chiroptical properties of the chromophore are similar for the *bay*-ionized, nonionized, and tautomeric forms.[55a] Semiempirical calculations corroborate the absolute configuration derived by means of the C_2 rule. Moreover, they demonstrate that the intense chiroptical signals have the propeller conformation as a prerequisite[55a] and that the butterfly conformation would display only marginal chiroptical effects.[55b]

The absolute configuration thus deduced for the hydroxyphenanthroperylene chromophore allows that of the related stentorin chromophore of **14** (Figure 5) to be deduced. From the observed sequence of positive and negative Cotton effects of the long and short wavelength absorption band systems of the native stentorin protein complex an absolute configuration (M) is assigned.[22f, 55a] Accordingly, the gymnochromes B and D (**10** and **12**;[20a] Figure 4) are assigned an absolute configuration (P) and isogymnochrome D (**13**;[20a] Figure 4) an absolute configuration (M).

7. Vibrational and NMR Spectra

Several infrared spectra of hydroxyphenanthroperylene quinone derivatives may be found in the literature.[6, 37a,c, 38a, 39, 45d, 47d, 50b] In addition, particularly in the case of hypericin (**2**), Fourier transform resonance Raman spectrometry (FTRR),[48b, 57a] fluorescence and fluorescence excitation spectroscopy,[49a] as well as surface enhanced Raman scattering (SERS) have been used to obtain structurally relevant vibrational data.[48b, 57b] However, the assignment of all vibrational bands beyond the characteristic hydroxyl and carbonyl vibrations has yet to be achieved. Currently, the 156

and 153 normal vibrations expected for **2** (54 atoms) and its phenolate ion $^{(-3)}$**2** (53 atoms), respectively, are mostly unassigned. Nevertheless, vibrational spectroscopy could provide a valuable tool for clarifying structural details of the phenanthroperylene quinones, which are otherwise inaccessible.[48b]

Although the notoriously low solubility of many phenanthroperylene quinones limits the application of ^{1}H and ^{13}C NMR spectroscopy to some degree, it is nevertheless an indispensable tool for structural analysis in this class of compounds. Characteristic chemical shifts, as shown for the hypericinate ion ($^{(-3)}$**2**) in Figure 22, as well as two dimensional correlation spectroscopy can be used successfully to assign constitution, tautomerism, ionization, hydrogen bonding, and exchange processes of hydroxyphenanthroperylene quinone derivatives. This is illustrated with the following examples.

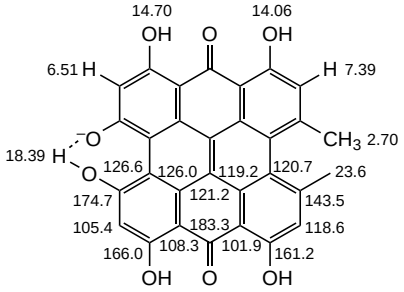


Figure 22. Chemical shifts of the ^{1}H and ^{13}C nuclei of the hypericinate ion ($^{(-3)}$**2**) in [D$_6$]dimethylsulfoxide (according to data from ref. [6]).

The assignment of constitutions to stentorin (**14**; Figure 5) and isostentorin (**50**; Scheme 7) is achieved unequivocally from a comparison of the ^{1}H NMR spectra of the two *bay*-diphenolates $^{(-3,-10)}$**14** and $^{(-3,-10)}$**50**: as shown in Figure 23, the two hydrogen-bonded *bay* protons of $^{(-3,-10)}$**14** undergo fast exchange and are placed on the formal C_2 symmetry axis. Thus, they have two different chemical environments and yield two distinct signals. In contrast to this, a C_2 axis perpendicular to the ring system in $^{(-3,-10)}$**50** causes two identical *bay* protons and accordingly, only one signal is observed.[23c]

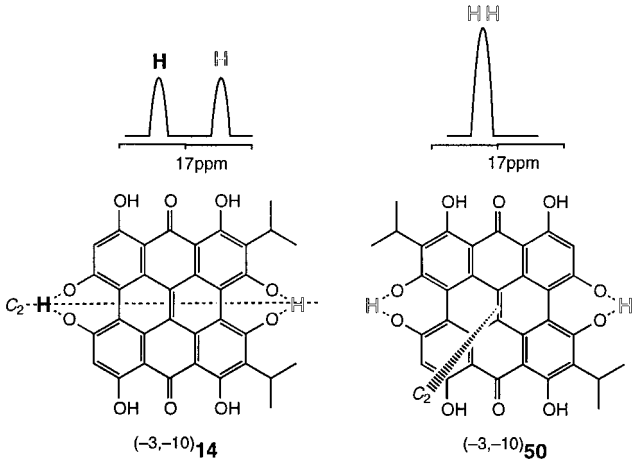
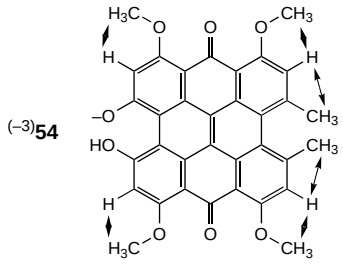


Figure 23. The constitution of stentorin (**14**) and isostentorin (**50**) is derived from the ^{1}H NMR spectra of their di-*bay*-phenolates.

As shown in Figure 24 two dimensional ^{1}H NOE correlation spectroscopy allows the assignment of the tetramethyl derivative **54**, which was formed by methylation of the **Q**^{7,14} tautomer.[44e]



$^{(-3)}$**54**

Figure 24. NOE correlations of the protons of **54**.

Finally, the region and number of signals in the ^{1}H and ^{13}C NMR spectra of the hypericinate ions ($^{(-3)}$**2**) as shown in Figure 22 immediately allows the deduction that the ionization of a hydroxyl group leads to a hydrogen-bonded phenolate, which when viewed through the methods time window is shown to be involved in a very fast exchange. An investigation of secondary isotope effects (H/D) showed the presence of an unsymmetrical *bay* hydrogen bridge in $^{(-3)}$**2**,[45c] which is in accord with other results[37b, 45a,b,c].

8. Heteroassociation

Considering the pronounced tendency of hydroxyphenanthroperylene quinones to undergo homoassociation (see Section 4.4.) it is not surprising that they also display a strong tendency to associate with other molecules. The first effect to mention is specific solvation, which is observed upon addition of benzene to a solution of **2**. As shown by means of ^{1}H nmr experiments, the benzene molecules associate in stacks above and below the mean plane of the hypericin ring system.[47a] Whereas strong electron acceptors, such as tetracyanoethylene, are not involved in the formation of donor–acceptor complexes,[47a] such complexes are encountered in the case of donors, such as duroquinone or ferrocene.[22g] Interestingly, for the example of hypericin (**2**) or $^{(-3)}$**2** two patterns of behavior are observed. On the one hand pigment H-aggregates are solubilized by binding to a host molecule, and on the other hand defined complexes are formed in which the pigment is associated monomolecularly to the host, and might even be associated in a specific orientation.[47a]

Accordingly, the absorption spectrum together with the absence of fluorescence of **2** and $^{(-3)}$**2** in the presence of aqueous solutions of γ-cyclodextrin, lysozyme, basic pancreatic trypsin inhibitor, melittin, polylysine, and DNA displays the characteristic bands of H-aggregates as illustrated in Figure 18 for $[\mathbf{2}]_n$.[47a] The pronounced solubilization encountered in these cases are interpreted as an association of the H-aggregates to the corresponding host molecule. With lipoproteins, such as HDL and LDL, heteroassociates with very high hypericinate content are found.[9i]

As inferred from the absorption spectra of several solution systems, monomolecular association occurs in the case of the solubilization of hydroxyphenanthroperylene quinones by means of the formation of micelles and vesicles.[14n, 53a,b, 58a,b,c] As to whether covalent or noncovalent bonding is present between the apoproteins and the pigments of stentorin and blepharismin is not yet known with certainty. However, experiments to form Schiff bases between a carbonyl group of hydroxyphenanthroperylene quinones and the ε-lysine amino group in analogy to the visual pigments failed.[58d] Therefore it may be assumed that in the native pigment system a specific, but noncovalent association is formed.

A special situation is encountered in the case of the specific heteroassociation of **2** ($^{(-3)}$**2**) to serum albumin, which is of the utmost importance for the transport and availability of this pigment under physiological conditions. The 1:1 complex formed between human serum albumine and $^{(-3)}$**2** (binding constant $K_b = 7.5 \times 10^4 M^{-1}$)[58e] may be easily isolated.[47] It is easily dissolved in water, and has absorption and fluorescence properties differing only slightly from those of homogeneous solutions of $^{(-3)}$**2**. Its fluorescence polarization corresponds to that of solutions of $^{(-3)}$**2** in low temperature glasses or glycerol, which points to a relatively rigid positioning of the chromophore with respect to the albumine molecule.[47a] With respect to the site of association it was shown by means of competition experiments with diazepam, warfarin, and bilirubin that $^{(-3)}$**2** is selectively bound to the active site of domain IIIA of human serum albumine.[47a] In addition, resonance Raman and SERS data point to an interaction of the pigment with the tryptophane residue of domain IIA,[58f] which is close to domain IIIA and forms a common surface.[59a, 59b] As can be inferred from the X-ray structural analysis of this protein, hydrophobic interactions are mainly responsible for bonding in this case.[59a,b] Hole-burning experiments point to a relatively flat bonding geometry and a pronounced accessibility of the solvent to the pigment.[45b] The long wavelength absorption band of bound $^{(-3)}$**2** displays a Cotton effect with $\Delta\varepsilon_{600}$ of $+19$.[47a] It can be inferred from the chiroptical properties of the chromophore (see Section 6) that there is only a partial preference of the propeller enantiomer with configuration (*M*). Such low partial chiral discrimination was also deduced from hole-burning experiments.[45b]

Resonance Raman spectroscopy indicated a sequence-specific interaction of $^{(-3)}$**2** with oligonucleotides having a partial sequence corresponding to the HIV "rev" gene resonance.[47e]

9. Salts and Coordination Complexes

The pronounced acidity of the *bay*-region hydroxyl groups of phenanthroperylene quinones make salt formation a definite possibility. Thus, as mentioned in Section 2.1, hypericin (**2**) is present in the plant material mainly as its potassium salt.[6a] For **2** salts with monovalent counterions are occasionally prepared because of their enhanced solubility.[13b, 41a, 45d, 48a, 59c,d] In addition, in the *bay*-hydroxylated fringelites (Figure 5) salt formation with divalent ions, such as Ca^{2+}, yields polymeric systems, which because of their extreme insolubility are highly stabile in fossils.[25c]

The *peri*-hydroxyl groups situated in the neighborhood of the carbonyl groups display the best prerequisites to form chelates with transition metal ions. Such coordination complexes could be characterized in the case of fringelite D and Zn^{2+}. This binding principle allows for the formation of a network of the just mentioned Ca^{2+} salt chains by coordination with the transition metal ions, which eventually leads to the extreme stability of this organic pigments in fossil material.[25c] Chelate formation with Al^{3+}, Fe^{3+}, Cu^{2+}, Gd^{2+}, and Tb^{2+} with the *peri*-hydroxyl groups of $^{(-3)}$**2** was inferred from the bathochromic shifts in the corresponding absorption spectra.[59e]

10. Reactions

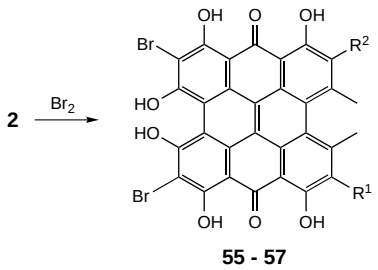
Only a few reactions that are suitable for the derivatization of phenanthroperylene quinones on a preparative scale are known. The two following sections will deal with the most important reactions involving compounds in ground and excited states.

10.1. Reactions of the Ground State

Protonation and deprotonation reactions as viewed from the structural viewpoint were discussed in Section 4.2. Defined reaction products from the chemical or electrochemical reduction or oxidation of hydroxyphenanthroperylene quinones, which would correspond to hydroquinone or bisquinone derivatives, could not be isolated so far. As demonstrated by ESR, electron nuclear double and triple resonance experiments, electrochemical or chemical (metallic potassium) reduction of the hypericinate ion ($^{(-3)}$**2**) in aprotic solvents leads to the radical dianion $^{(-3,\text{-}peri)}$**2**$^{\bullet}$, which is stable for days.[60b] However, the radical anion $^{(-3)}$**2**$^{\bullet}$ derived in the same way from undissociated **2** is short-lived and rapidly forms the aforementioned radical dianion $^{(-3,\text{-}peri)}$**2**$\cdot$. Reducing cyclic voltammetry between -1.0 and -2.2 V points to a step involving $^{(3-)}$**2** and perhaps also $^{(4-)}$**2**.[60b] A viologen-mediated, photoinduced electron transfer from **2** to a modified gold electrode was documented recently.[60e] Oxidative cyclic voltammetry of $^{(3-)}$**2** in dimethylsulfoxide displays only a insufficiently defined, nonreversible oxidation process involving several electrons in the region of $+0.9$ V.[60a]

As could be expected, electrophilic substitution is favored at the aromatic positions of hydroxyphenanthroperylene quinones. Reaction of hypericin (**2**) with bromine thus affords the tetrabromo derivative **57** via the di- and trisubstitution products **55** and **56**, which can be isolated (Scheme 14).[50b] In analogy, sulfonation of **2** yields the tetrasulfonic acid **53** via the corresponding hypericin di- and trisulfonic acids (Figure 17).[47d]

Nucleophilic substitution cannot be observed because of the pronounced activation of the aromatic system. However, the phenolic hydroxyl groups, or their respective phenolate ions, may act as nucleophiles. Thereby the *bay*-hydroxyl



Scheme 14. Electrophilic bromination of hypericin to **55** ($R^1 = R^2 = H$), **56** ($R^1 = Br$, $R^2 = H$), and **57** ($R^1 = R^2 = Br$).

groups react preferably in alkylation or acylation reactions.[39, 44e, 45d] Radical-catalyzed reactions of **2**, for example, the ω-halogenation of methyl groups, were unsuccessful.[60c] Hypericin **2** was also found to be largely inert in cycloaddition reactions with singlet oxygen, dienes, and dienophiles.[47a]

The mass spectrometric fragmentation of hydroxyphenanthroperylene quinones, in peculiar those with ionized *bay*-hydroxyl groups, such as $^{(-3)}$**2**, by electrospray ionization is rather characteristic. The structural aspects and mechanism of the reactions taking place in this case have been discussed in detail,[60d] but such discussions are beyond the scope of this review.

10.2. Photochemical Reactions

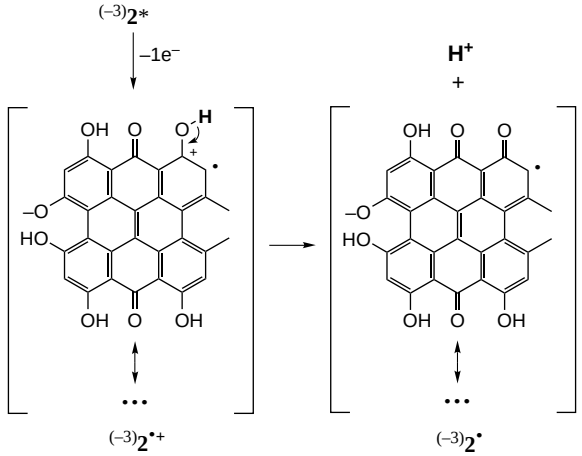
Electron transfer reactions involving the excited singlet state of hydroxylated phenanthroperylene quinones can be verified from fluorescence quenching by electron acceptors and ESR detection of the resulting radical species.[22g]

The triplet state is an ideal starting point for chemical processes because of the efficient intersystem transfer. However, the triplet state can function both as the source of excitation transfer, as is typical for quinones and other electron acceptors, and as an electron donor, as is characteristic of phenols. Most of these investigations were carried out using hypericin **2** as an electron acceptor and its *bay*-phenolate ion $^{(-3)}$**2** as an electron donor.[14c,n, 52a, 54c, 58a,b,c, 61] Such reactions are mainly of interest in regard to the application of the phenanthroperylene quinones as agents for photodynamical therapy (Section 2.1.), and moreover, with respect to their function as photoreceptors (Sections 2.2. and 2.3.).

In these cases excitation transfer for the formation of singlet oxygen ($^3\mathbf{2} + O_2 \rightarrow \mathbf{2} + {}^1O_2$; quantum yield $\Phi_\Delta \approx 0.4$[61f]) is of utmost importance. This photochemical behavior is classified as a type II reaction of photosensitized reactions.[62] The photochemically induced disproportionation ($^3\mathbf{2} + \mathbf{2} \rightarrow \mathbf{2}^{\bullet-} + \mathbf{2}^{\bullet+}$) is also possible and has been documented by ESR experiments, and even observed to a small extent in solutions of very high concentration, that is, with homoaggregates in the ground state.[61c] This reaction is comparable to the formation of the superoxide radical by direct reaction of the triplet species with oxygen ($^3\mathbf{2} + O_2 \rightarrow \mathbf{2}^{\bullet+} + O_2^{\bullet-}$).[61c,d] The latter could also be derived from a reaction starting from the anion radical ($\mathbf{2}^{\bullet-} + O_2 \rightarrow \mathbf{2} + O_2^{\bullet-}$).[61d] These reactions are classified as photosensitizing reactions of type I.[61d, 62]

The excited state of hydroxyphenanthroperylene quinones can also act as the source of a light-induced proton transfer, which is of eminent importance for their functional aspects (Sections 2.1.–2.3.). Epifluorescence microscopy investigations on $^{(-3)}$**2** incorporated together with a fluorescence pH indicator into a vesicle demonstrated that after excitation a proton is transferred within a few 10 μs to the indicator.[58c, 63] As indicated by studies on partially O-alkylated hypericin derivatives this proton mainly originates in the *peri* region of the pigment.[63]

With respect to the reaction mechanism, one can assume that the acidification of the molecule in its singlet or respective triplet state is insufficient to bridge the rather large pK_a gap between the proton donor and acceptor. However, by means of electron transfer from the excited singlet or triplet state $^{(-3)}$**2*** (see above) the cation radical $^{(-3)}\mathbf{2}^{\bullet+}$ (Scheme 15) could be formed, which should be much



Scheme 15. Light-induced proton transfer in the hypericinate ion $^{(-3)}$**2**.

more acidic than the ground state of $^{(-3)}$**2**.[22g] This is in analogy to the case of tyrosine for which after excitation and electron transfer its acidity was found to be increased by ten orders of magnitude.[64] The species thus formed is sufficiently long lived to account for the time characteristics observed for this reaction.[63]

11. Summary and Outlook

Hydroxyl-substituted phenanthroperylene quinones form a fascinating group of natural products. Some of its representatives, such as hypericin or stentorin, display important physiological properties. These reach from therapeutically useful photosensitizations to their function as photoreceptors. Their chemistry is mostly characterized by a complex intertwined network of tautomerism, dissociation, torsional, and association equilibria, which account for their chemical, physical, reactive, and accordingly, also the dependence of their physiological properties on environmental parameters.

Although our knowledge of the chemistry of this class of compounds has expanded considerably in the last decade, there are still many gaps present which have to be bridged as a result of the importance of these molecules for physiology and medicine. Thus, detailed investigations on the structures of the tautomers under physiological conditions are still lacking. The detailed photochemistry of the photosensory pigments is, as is evident from the discussions above, only marginally known. In addition, the bonding of these chromophores to their respective proteins as well as the reaction cascades that lead to the physiological signal are mostly unclear. Further pursuit of the chemistry of the phenanthroperylene quinones still remains a challenge!

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[51] It should be stressed that the data given here are the ones originally published for hypericin (**2**). However, judged from the conditions and absorption spectra displayed in these papers—see the discussions in Section 4—it is present as the *bay*-hypericinate ion $^{(-3)}$**2**.

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