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Concerning the Question of Covalent Bonding in Hypericin-Chromoproteins: Schiff Base Formation?

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Summary. Depending on the reaction conditions, *peri*-hydroxy substituted anthraquinones like 1,8-dihydroxyanthraquinone and 1,4-dihydroxyanthraquinone could be derivatized with ammonia, propylamine, isopropylamine, and a lysine derivative to yield a variety of imino and amino substitution and addition products. However, hypericin resisted such derivatization under a variety of reaction conditions. Therefore, the hypothesis that hypericin is bound to its apoprotein in photopigments *via* a Schiff base to the ε-amino group of a lysine residue or a terminal amino group seems to be rather unlikely.

Keywords. Hypericin; Stentor; Schiff base; Anthraquinone imines; Aminoanthraquinones.

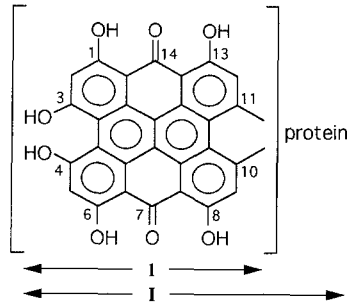
Zur Frage der kovalenten Bindung in Hypericin-Chromoproteiden: Schiff-Basenbildung?

Zusammenfassung. Abhängig von den Reaktionsbedingungen gaben *peri*-hydroxylsubstituierte Anthrachinone, wie 1,8-Dihydroxyanthrachinon oder 1,4-Dihydroxyanthrachinon, mit Propylamin, Isopropylamin und einem Lysinderivat eine Reihe von Imino- und Amino-Substitutionsprodukten oder Addukten. Allerdings widerstand Hypericin unter Variation der Reaktionsbedingungen einer solchen Derivatisierung. Deshalb ist die Hypothese, daß Hypericin in seinen Photopigmenten über eine Schiffsche Base mit der ε-Aminogruppe eines Lysinrestes oder mit einer terminalen Aminogruppe verknüpft ist, eher unwahrscheinlich.

Introduction

Hypericin (**1**) constitutes the prosthetic group of the photoreceptor pigment which is responsible for the photophobic actions in certain algae [1]. The nature of the bond between **1** and its apoprotein to yield **I** is known, but it has been inferred from its chromotographic, spectroscopic, and chemical behavior to be a rather loose covalent bond [2]. According to Ref. [2] the chromophore is easily cleaved off the protein by means of dilute acid.

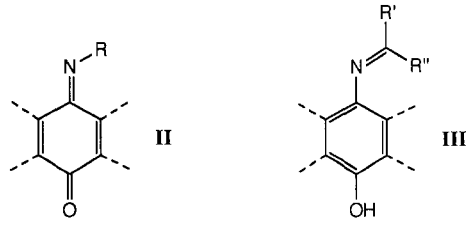
In principle, two kinds of covalent bonds may be imagined between the functional groups of **1** and an apoprotein. The first one would involve an ester linkage between an amino acid residue (a terminal carboxylic group, or the one of an acidic amino acid) and a phenolic hydroxyl group of **1**. This kind of bonding has been suggested to occur in position 6 of the chromophore from a force field investigation of several hypothetical dipeptides of **1** [3]. The second kind of bonding



between **1** and the apoprotein would involve the quinone carbonyl groups in positions 7 and 14. Bonding of a carbonyl group of **1** to a protein could be envisaged to occur by means of a Schiff base formation with a basic amino acid. Such a Schiff base formation between the ε-amino group of lysine and carbonyl groups is a well known bonding motif in the chromoproteins of the rhodopsin family [4]. Moreover, quinone Schiff bases are important with respect to the cofactor PQQ, which is associated with various dehydrogenases and amine oxidases [5]. Therefore, the present study will focus on the general question of Schiff base formation between quinones and primary amines with respect to the hypothesis of hypericin chromoprotein bonding.

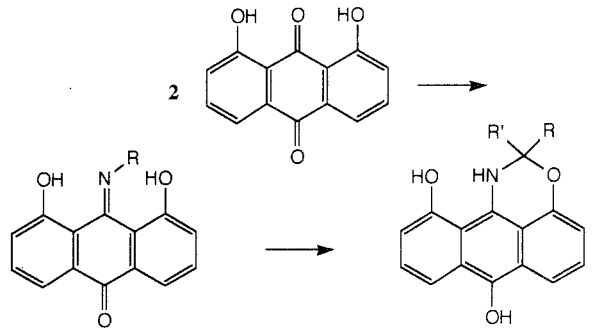
Results and Discussion

In principle, Schiff bases can be derived from quinones and primary amines. Two structural types are known from literature [6, 7]. Their formation is dependent on the nature of the primary amine. Thus, primary amines including ammonia which lack acidic α-protons, will form quinone imines **II**. Derivatives of amines with mobile α-protons could then undergo tautomerization of the primary derivative of type **II** to yield hydroxyarylimino derivatives **III**.



To investigate the possibility of the formation of hypericin type imines, 1,8-dihydroxyanthraquinone (**2**) was chosen as a first model. It displays a similar *peri*-hydroxyl substituted carbonyl region as **1**, but in contrast to **1**, it is available in quantity. It was reacted with ammonia, propylamine and isopropylamine in acetonitrile as the solvent to yield **3**–**5**. The imine **3** had been obtained earlier by heating **2** with methanolic ammonia under pressure, or upon heating with aqueous ammonia containing sodium hydroxide [8]. This study had been undertaken with respect to an analytical procedure called the Bornträger reaction. Upon prolonged reaction of **4** and **5** under their preparation conditions cyclization into **6** and **7**, presumably *via* the corresponding tautomers of type **III**, took place. The latter

compounds were more easily obtained directly by refluxing **2** and the corresponding amine in tetrahydrofuran with potassium fluoride or boron trifluoride etherate as the catalyst. It was interesting to note that **3**–**5** are only very sparingly soluble, whereas **6** and **7** are easily soluble in common solvents. Even using octadecylamine as the reagent did not improve the solubility of the corresponding imino-derivative. A reaction in the direction from **6** or **7** back to **4** or **5** could not be achieved under a variety of reaction conditions.



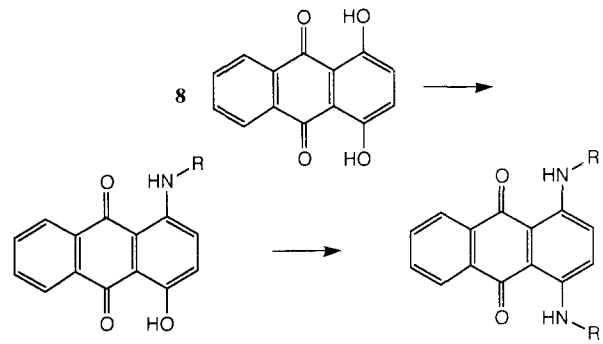
R = H **3**
R = $CH_2CH_2CH_3$ **4**
R = $CH(CH_3)_2$ **5**

R' = H, R = CH_2CH_3 **6**
R' = R = CH_3 **7**

According to PCMODEL [9] MMX force field calculations, tautomerization of **4** and **5** into their type **III** systems was thermodynamically unfavourable by approximately 80 kJ/mol. This was mainly due to torsional energy and non-bonded interaction terms. Thus, the reaction seemed to be driven by the high stability of the resulting six membered aminal rings of **6** and **7**. From these calculations it followed also that the formation of the 9-imino-derivative **5** was favored over the corresponding 10-imino derivative by about 3 kJ/mol, whereas the 10-aminoisopropylidene system was found to be even less stable by 80 kJ/mol.

The structures of **3**–**7** were elucidated from their spectroscopic data. The proton NMR spectra did not allow unambigous distinction between alkylidene structures of type **III** and their cyclization products **6** and **7**. However, the chemical equivalence of the methyl ^1H- and ^{13}C-shifts in **7** together with a ^{13}C chemical shift of approximately 90 ppm, which is characteristic of an acetal or aminal functional group, suggested the constitutions of **6** and **7**. Moreover, a long range $^1H/^{13}C$-correlation experiment proved the interaction of the methyl proton signal and the ^{13}C signal at 90 ppm in **7**. Of course, an important difference between the two sets of compounds was a rather large bathochromic shift of more than 120 nm in the visible absorption bands of **3**–**5** compared to **6** and **7**. This bathochromic shift also nicely corroborated the more extensive conjugation. It should be mentioned that the imines **3**–**5** were found to be rather weak bases ($pK_a \approx 2.5$), and even weaker ones in their excited states ($pK_a^* \approx -7$).

In general, anthraquinones with *peri*-hydroxyl groups are known to react with ammonia to yield imines of type **II** in which the hydroxyl group is placed next to the imino group [6]. However, upon treatment with propyl- or isopropyl amine under a variety of reaction conditions neither 1- nor 2-hydroxy-anthraquinone resulted in Schiff bases of type **II** or **III**. Therefore, quinizarin (**8**) was used as the second model; its structure could be envisaged as a "compressed" form of **1**.



Amination of **8** has a long standing tradition in colour chemistry as it provided access to a wealth of important industrial dyes [6]. However, in order to achieve reactivity of **8** with amines, it had first to be reduced to yield dihydroquinizarin. This has then been easily reacted with one or two moles of amine. Reoxidation of these condensates eventually resulted in 1-amino-4-hydroxy- and 1,4-diamino-anthraquinone derivatives [6]. The direct amination of quinizarin and its derivatives has been achieved recently by treatment of **8** with aqueous ammonia [10]. This derivative has then been used as the starting material for the synthesis of imino-antibiotics of the daunomycin type [11]. However, the latter reaction seemed to be also restricted to the case of ammonia because attempts to derivatize **8** with primary amines under these reaction conditions failed.

However, reaction of **8** with propylamine and isopropylamine in tetrahydrofuran or acetonitrile under reflux provided **9** and **10**. Upon prolonged treatment and providing additional amounts of the corresponding amine the disubstituted products **11** or **12** were also obtained.

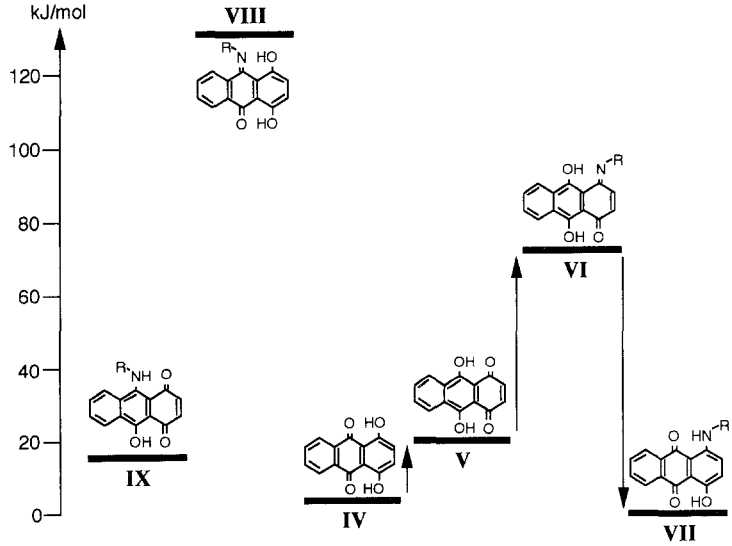
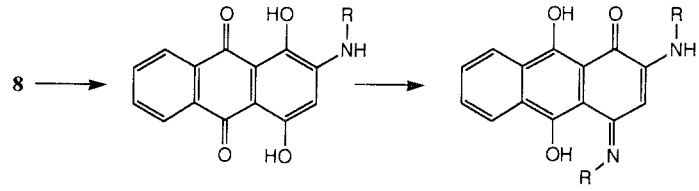


Fig. 1. Relative energies of the quinizarin (**8**) tautomers **IV** and **V** and their amino and imino derivatives **VII** and **IX**, and **VI** and **VIII** (R = $CH(CH_3)_2$) as derived from PCMODEL calculations

From a mechanistic point of view these results could be interpreted as a series of reaction steps. **8** had first to tautomerize into the 9,10-dihydroxy-1,4-anthraquinone. The latter could then yield the corresponding 1-quinone imine, which upon tautomerization afforded the 1-amino-4-hydroxy-anthraquinone **9** or **10**. An analogous reaction cascade starting from **9** and **10** could then lead to **11** and **12**.

PCMODEL calculations of the two primary addition products of isopropylamine to the two tautomers of **8** indicated that the 9-amino-9-hydroxy system is more stable than the 1-amino-1-hydroxy system by 5 kJ/mol. Accordingly, a kinetically controlled reaction sequence should have led to a nitrogen attachment in position 9. However, calculations of the various possible products revealed (Fig. 1) that the reaction cascade starting from the most stable quinizarin tautomer **IV** via **V** and **VI** to **VII** is thermodynamically controlled. The corresponding 9-amino system **IX** was found to be less stable than **VII** by 16 kJ/mol, and was therefore indeed energetically unfavorable.

In contrast to the reaction conditions which led to **9–12**, reaction of **8** with primary amines in the presence of Lewis acids like $BF_3 \cdot O(C_2H_5)_2$ or $AlCl_3$ and tetrahedrofuran as the solvent, resulted in the 2-aminoquinizarins **13–15**. Prolonged reaction of **13–15** under these conditions yielded only in the case of **14** the quinone imine **16**, whereas **13** did not react any further, and **15** gradually decomposed. The constitutions of these derivatives were assigned from their spectroscopic data.



R = $CH_2CH_2CH_3$ **13**
R = $CH(CH_3)_2$ **14**
R = $(CH_2)_4CH(NHTos)COOEt$ **15**

R = $CH(CH_3)_2$ **16**

From the mechanistic point of view the formation of **13–15** could be understood as a reaction sequence starting with tautomerization of **8** to its 1,4-quinoid form. The latter could then undergo an addition with the amine, a reaction type which is well known for the chemistry of quinones [12]. The resulting 2-amino-9,10-dihydroxy-1,4-anthraquinones eventually could be tautomerized to the 2-amino-anthraquinones **13–15**. Formation of **16** could then proceed along the mechanistic lines advanced for **10**. However, the resulting 2-amino-9,10-dihydroxy-1,4-quinoid system was obviously more stable than the tautomeric 1-hydroxy-2,4-diamino-anthraquinones. Judged from PCMODEL calculations the 9,10-dioxo tautomer of 14 was more stable than the 1,4-dioxo-tautomer by 33 kJ/mol. These calculations also predicted the 1,4-quinoid tautomer of **16** as the most stable one. The corresponding 9,10-dioxo-tautomer was calculated to be less stable by 48 kJ/mol. Other possible tautomers were found to be even more destabilized.

In contrast to the above results on the amino derivatization of *peri*-hydroxy-anthraquinone model compounds, neither the more advanced model compound 1,6-dihydroxyhelianthrone, nor hypericin (**1**) itself could be brought to react with amines like ammonia, propylamine, isopropylamine, benzylamine, or aniline under

a variety of reaction conditions. On the one hand, this behavior could be rationalized in terms of a strong delocalization of the quinoid structure in polycondensated systems. As could be inferred from formula **1**, the carbonyl reactivity of the system might be approximated by the strongly attenuated reactivity of a benzophenone. On the other hand, due to the high acidity of the 3,4-hydroxyl groups of **1** [13], the molecule will be deprotonated by the amine. The resulting system might then be envisaged as a vinylogous carboxylate, which of course, would not undergo typical carbonyl reactions like Schiff base formation. The same arguments hold for the 1,6-tautomer of **1** which has been recently produced by base catalyzed tautomerization of **1** [14].

Conclusions

Depending on the reaction conditions, several *peri*-hydroxy substituted anthraquinones allowed derivatization with primary amines to yield a variety of imino and amino derivatives. However, highly condensated ring systems like the phenanthroperylenequinone **1** did not react with such amines under even rather non-physiological reaction conditions. Therefore, the hypothesis that hypericin (**1**) is bound to its apoprotein in photosensory pigments of certain algae *via* a Schiff base to the terminal amino group or the amino group of a lysine residue was derived to be rather unlikely.

Experimental Part

Melting points (uncorrected) were taken by means of a Kofler hot stage microscope (Reichert, Vienna). ^{1}H-, ^{13}C-, IR-, UV-VIS-, fluorescence-, and mass-spectra were recorded using the Bruker-AC-200-, and WM-360-, Biorad-FT-IR-45-, Hitachi-U-3210-, F-4010-, and HP-5989MS-instruments. For absorption and fluorescence spectroscopy 95% ethanol of "für die Spektroskopie" and "für die Fluoreszenzspektroskopie" grades (Merck) were used. The solutions were degassed by bubbling with argon. However, no influence of air was detected on the fluorescence quantum yields. Rhodamine B was used as the quantum yield standard. The pK_a values were determined spectrophotometrically using 80% aqueous ethanol as the solvent and sulfuric acid as the titrant. Excited state pK_a^* values were derived by application of the Förster cycle (see [13]).

1,6-Dihydroxyhelianthrone, **1**, **3**, 1-hydroxyanthraquinone, and 2-hydroxyanthraquinone, were prepared according to [8, 13, 15, 16, 17,]; **2** and **8** were of commercial origin (Aldrich, Merck).

1,8-Dihydroxy-9-iminoanthraquinone [**3**; $C_{14}H_9O_3N$]

Preparation of **3** according to Ref. [8] using the $NaOH/NH_3$ method, yielded 25%; m.p. not until 300 °C. ^{1}H-NMR (200 MHz, δ, *DMSO-d*$_6$): 5.86 (s, broad, =NH), 7.27 (X-part of ABX system, $J_{AX} \approx J_{BX} = 4.8$ Hz, H-3,6), 7.63 (AB-part of ABX system, H-2,4,5,7), 13.58 (s, broad, OH-1,8) ppm. ^{13}C-NMR (90 MHz, δ, *DMSO-d*$_6$): too insoluble to record data. IR (KBr): $\nu = 2875$, 1667, 1616, 1653, 1451, 1419 cm^{-1}. MS (32 eV, 150 °C) *m/e* (%) = 239 (30; M^+), 183 (3), 69 (15), 57 (21), 55 (25), 45 (74), 43 (100), 41 (67). UV-VIS (ethanol, $c = 2 \cdot 10^{-5}$ mol/l): $\lambda_{max.} = 535$ (11 000) nm (ε). Fluorescence (ethanol, room temp.): $\lambda = 631$ nm, $\Phi_f = 0.012$ ($\lambda_{ex.} = 540$ nm). Spectrophotometric titration (ethanol/water, 80%): $pK_a = 2.4 \pm 0.05$, $pK_*^a = -7.8$, $\lambda_3 = 537$ nm, $\lambda_{3H^+} = 432$ nm, $\varepsilon_\lambda/\varepsilon_{\lambda^+} = 1.4$.

1,8-Dihydroxy-9-propyliminoanthraquinone [**4**; $C_{17}H_{15}O_3N$]

60 mg (0.25 mmol) **2** were dissolved in 30 ml acetonitrile (p. A.), 4.3 ml (50 mmol) propylamine were added and the mixture boiled under reflux for 24 h. Solvent and amine were evaporated on a rotatory

evaporator, and the residue was chromatographed on silica with ethylacetate/methanol = 8/1. Yield 15%; m.p. not until 300 °C. ^{1}H-NMR (200 MHz, δ, *DMSO-d_6*): 0.98 (t, $J = 7.8$ Hz, $-CH_2CH_3$), 1.54 (tq, $J_1 = 7.8$ Hz, $J_2 = 6.5$ Hz, $-CH_2CH_3$), 2.7 (t, $J = 6.5$ Hz, $-CH_2-N=$), 6.99 (A-part of AMX system, $J_{AM} = 8.1$ Hz, $J_{AX} = 1$ Hz, H-2,7), 7.38 (X-part of AMX systems, $J_{AX} = 1$ Hz, $J_{XM} = 7.2$ Hz, H-4,5), 7.47 (M-part of AMX system, $J_{AM} = 8.1$ Hz, $J_{XM} = 7.2$ Hz, H-3,6), 14.23 (s, broad, OH-1,8) ppm. ^{13}C-NMR (90 MHz, δ, *DMSO-d_6*): too insoluble to record data. IR (KBR): $\nu = 2876, 1648, 1609, 1455, 1406\ cm^{-1}$. MS could not be recorded. UV-VIS (ethanol, $c = 2 \cdot 10^{-5}$ mol/1): $\lambda_{max.} = 536$ (10 200), 287 (9 800) nm (ε). Fluorescence (ethanol, room temp.): $\lambda = 632$ nm, $\Phi_f = 0.012$ ($\lambda_{ex.} = 540$ nm). Spectrophotomeric titration (ethanol/water, 80%): $pK_a = 2.7 \pm 0.05$, $pK_a^* = -6.8$, $\lambda_4 = 540$ nm, $\lambda_{4H^+} = 434$ nm, $\varepsilon_\lambda/\varepsilon_{\lambda^+} = 1.6$.

1,8-Dihydroxy-9-isopropyliminoanthraquinone [**5**; $C_{17}H_{15}O_3N$]

Prepared in analogy to **4** in 20% yield; m.p. not until 300 °C. ^{1}H-NMR (200 MHz, δ, *DMSO-d_6*): 1.72 (s, $2CH_3$), 6.73 (A-part of AMX system, $J_{AM} = 8.5$ Hz, $J_{AX} = 1.2$ Hz, H-2,7), 7.05 (X-part of AMX system, $J_{AX} = 1.2$ Hz, $J_{XM} = 7.2$ Hz, H-4,5), 7.23 (M-part of AMX system, $J_{AM} = 8.5$ Hz, $J_{XM} = 7.2$ Hz, H-3,6), 14.62 (s, broad, OH-1,8) ppm. ^{13}C-NMR (90 MHz, δ, *DMSO-d_6*): too insoluble to record data. IR (KBr): $\nu = 2936, 1648, 1604, 1565, 1443, 1414\ cm^{-1}$. MS could not be recorded. UV-VIS (ethanol, $c = 1 \cdot 10^{-5}$ mol/l): $\lambda_{max.} = 536$ (9 800), 284 (8 400) nm (ε). Fluorescence (ethanol, room temp.): $\lambda = 630$ nm, $\Phi_f = 0.02$ ($\lambda_{ex.} = 540$ nm). Spektrophotometric titration (ethanol/water, 80%): $pK_a = 2.4 \pm 0.05$, $pK_a^* = -6.9$, $\lambda_5 = 537$ nm, $\lambda_{5H^+} = 433$ nm, $\varepsilon_\lambda/\varepsilon_{\lambda^+} = 1.5$.

(racem.)-2-Ethyl-1,2-dihydro-3-oxa-1-aza-benzo[d,e]anthracene-7,11-diol [**6**; $C_{17}H_{15}O_3N$]

a) 100 mg (0.41 mmol) **2** were dissolved in 25 ml tetrahydrofuran, and 1.5 g KF and 1.5 ml (17 mmol) propylamine were added. After boiling under reflux for 10 h KF was filtered off and thoroughly washed. Evaporation of the solvent and the amine on a rotatory evaporator resulted in a raw product which was chromatographed on alumina (neutral, activity II–III) with dichloromethane as the solvent. b) Instead of KF, 3 drops of $BF_3 \cdot O(C_2H_5)_2$ were added. Yield 35%; m.p. 113 °C. ^{1}H-NMR (200 MHz, δ, $CDCl_3$): 1.15 (t, $J = 7.0$ Hz, CH_3), 2.11 (dq, $J_1 = 7.0$ JHz, $J_2 = 6.0$ Hz, CH_2), 5.86 (t, $J = 6.0$ Hz, CH), 7.16 (X-part of AMX system, $J_{XM} = 8.0$ Hz, $J_{AX} = 1.0$ Hz, H-2,7), 7.52 (M-part of AMX system, $J_{AM} = J_{MX} = 8.0$ Hz, H-3,6), 7.78 (A-part of AMX system, $J_{AM} = 8.0$ Hz, $J_{AX} = 1.0$ Hz, H-4,5), 13.40 (s, OH) ppm. ^{13}C-NMR (90 MHz, δ, $CDCl_3$): 8.5 (CH_3), 29.4 (CH_2), 88.9 (N–C–O), 114.1 ($C_{ar.}$), 114.7 ($C_{ar.}$), 119.1 ($CH_{ar.}$), 119.9 ($CH_{ar.}$), 121.5 ($CH_{ar.}$), 123.3 ($CH_{ar.}$), 130.3 ($C_{ar.}$), 132.9 ($C_{ar.}$), 133.3 ($CH_{ar.}$), 134.8 ($CH_{ar.}$), 154.1 ($C_{ar.}$), 157.1 ($C_{ar.}$), 161.0 ($C_{ar.}$), 182.6 ($C_{ar.}$) ppm. IR (KBr): $\nu = 2969, 2873, 1666, 1625, 1563, 1487, 1457\ cm^{-1}$. MX (70 eV, 90 °C) *m/e* (%) = 279 (14; $M^+ - 2H$), 251 (17), 250 (100), 139 (12), 81 (4), 69 (26), 41 (22). UV-VIS (ethanol, $c = 10^{-5}$ mol/l): $\lambda_{max.} = 394$ (6 200), 279 (13 200), 265 (12 600) nm (ε). Fluorescence (ethanol, room temp.): $\lambda = 608$ nm, $\Phi_f = 0.025$ ($\lambda_{ex.} = 400$ nm). The spectrophotometric titration in ethanol/water (80%) was not possible due to insufficient shifts and intensity differences of the absorption spectra upon protonation.

2,2-Dimethyl-1,2-dihydro-3-oxa-1-aza-benzo[d,e]anthracene-7,11-diol [**7**; $C_{17}H_{15}O_3N$]

Prepared in analogy to **6** in 25% yield; m.p. 178 °C. ^{1}H-NMR (200 MHz, δ, $CDCl_3$): 1.74 (s, $2CH_3$), 7.15 (X-part of AMX system, $J_{XM} = 8$ Hz, $J_{AX} = 1$ Hz, H-2, 7), 7.48 (M-part of AMX system, $J_{AM} = J_{MX} = 8$ Hz, H-3,6), 7.77 (A-part of AMX system, $J_{AM} = 8$ Hz, $J_{AX} = 1$ Hz, H-4,5) 13.40 (s, OH) ppm. ^{13}C-NMR (90 MHz, δ, $CDCl_3$): 28.61 (CH_3), 90.7 (N–C–O), 113.4 ($C_{ar.}$), 115.5 ($C_{ar.}$), 119.0 ($CH_{ar.}$), 119.6 ($CH_{ar.}$), 121.8 ($CH_{ar.}$), 123.8 ($CH_{ar.}$), 130.2 ($C_{ar.}$), 132.9 ($C_{ar.}$), 133.2 ($CH_{ar.}$), 134.8 ($CH_{ar.}$), 153.1 ($CH_{ar.}$), 155.4 ($C_{ar.}$), 160.9 ($C_{ar.}$), 182.6 ($C_{ar.}$) ppm, ^{1}H/^{13}C-correlation experiment according to Ref. [18]: 1.74 (^{1}H)↔90.7 (^{13}C). IR (KBr): $\nu = 2970, 1662, 1631, 1594, 1485\ cm^{-1}$. MS (70 eV, 90 °C) *m/e* (%) = 280 (2; $M^+ - H$), 279 (28), 265 (18), 264 (100), 139 (6), UV-VIS (ethanol, $c = 10^{-5}$ mol/l):

$\lambda_{max.}$ = 393 (6 800), 280 (14 600), 264 (13 600) nm (ε). Fluorescence (ethanol, room temp.): λ = 596 nm, Φ_f = 0.02 ($\lambda_{ex.}$ = 400 nm). The spectrophotometric titration in ethanol/water (80%) was not possible due to insufficient shifts and intensity differences of the absorption bands upon protonation.

1-Propylamino-4-hydroxy-anthraquinone [**9**; $C_{17}H_{14}O_3N$]

500 mg (2.1 mmol) **8** were dissolved in 250 ml acetonitrile (p. A.) and 42 ml (0.5 mol) propylamine was added. After boiling for 14 h under reflux solvent and amine was evaporated on a rotatory evaporator and the residue consisting mainly of **9** + **11** was chromatographed on silica with dichloromethane. R_f (**9**) = 0.8, R_f (**11**) = 0.5. Yield 5%; m.p. 120 °C. ^{1}H-NMR (360 MHz, δ, $CDCl_3$): 1.04 (t, J = 7.2 Hz, $-CH_2\mathbf{CH_3}$), 1.70 (tq, $J_1 = J_2$ = 7.2 Hz, $-CH_2\mathbf{CH_2}CH_3$), 3.18 (dt, J_1 = 7.2 Hz, J_2 = 4.6 Hz, NH–$\mathbf{CH_2}CH_2$–), 7.01 (AB-system, J_{AB} = 7.0 Hz, H-2,3), 7.65 (AA'-part of AA'BB' system, H-6,7), 8.16 (BB'-part of AA'BB' system, H-5,8), 10.12 (t, J = 4.6 Hz, NH), 13.60 (s, OH-4) ppm. ^{13}C-NMR (90 MHz, δ, *DMSO-d*$_6$): 11.7 (CH_3), 22.7 (CH_2), 44.6 (CH_2), 108.1 ($C_{ar.}$), 113.5 ($C_{ar.}$), 123.7 ($CH_{ar.}$), 126.1 ($CH_{ar.}$), 126.5 ($CH_{ar.}$), 128.6 ($CH_{ar.}$), 132.2 ($CH_{ar.}$), 132.5 ($C_{ar.}$), 133.8 ($CH_{ar.}$), 135.3 ($C_{ar.}$), 147.4 ($C_{ar.}$), 156.5 ($C_{ar.}$), 181.5 ($C_{ar.}$), 187.1 ($C_{ar.}$) ppm. IR (KBr): ν = 2964, 1616, 1585, 1559, 1496, 1459 cm^{-1}. MS (70 eV, 90 °C) *m/e* (%) = 281 (44, M^+), 253 (12), 152 (100), 196 (4), 81 (9), 71 (3), 69 (38), 57 (23), 55 (22), 43 (43), 41 (50). UV-VIS (ethanol, $c = 2 \cdot 10^{-5}$ mol/l): $\lambda_{max.}$ = 599 (10 700), 558 (11 000), 528 (sh, 6 700), 288 (sh, 7 100), 252 (33 200) nm (ε). Fluorescence (ethanol, room temp.): λ = 640 nm, Φ_f = 0.008 ($\lambda_{ex.}$ = 550 nm).

1-Isopropylamino-4-hydroxy-anthraquinone [**10**; $C_{17}H_{14}O_3N$]

Prepared in analogy to **9** (**10** + **12**; chromatography on silica with toluene/hexane = 30/1 as the solvent – R_f (**10**) = 0.5, R_f (**12**) = 0.2) in 15% yield; m.p. 136–137 °C. ^{1}H-NMR (200 MHz, δ, $CDCl_3$): 1.35 (A_6-part of A_6X system, J = 7 Hz, $2CH_3$), 3.90 (X-part of A_6X system, $-\mathbf{CH}Me_2$), 7.23 (s, H-2,3), 7.75 (AD-part of ABCD system, H-6,7), 8.31 (BC-part of ABCD system, H-5,8), 10.45 (s, broad, NH), 13.78 (s, OH) ppm. ^{13}C-NMR (90 MHz, δ, $CDCl_3$): 23.2 (CH_3), 43.8 (CH), 108.1 ($C_{ar.}$), 113.7 ($C_{ar.}$), 124.4 ($CH_{ar.}$), 126.3 ($CH_{ar.}$), 126.5 ($C_{ar.}$), 128.9 ($CH_{ar.}$), 132.3 ($CH_{ar.}$), 132.6 ($C_{ar.}$), 134.1 ($CH_{ar.}$), 135.5 ($C_{ar.}$), 146.9 ($C_{ar.}$), 156.7 ($C_{ar.}$), 181.3 ($C_{ar.}$), 187.3 ($C_{ar.}$) ppm. IR (KBr): ν = 2963, 1619, 1589, 1583, 1707, 1443 cm^{-1}. MS (70 eV, 90 °C) *m/e* (%) = 281 (58, M^+), 266 (100), 248 (46), 182 (9), 139 (18), 77 (26), 43 (68). UV-VIS (ethanol, $c = 2 \cdot 10^{-5}$ mol/l): $\lambda_{max.}$ = 599 (9 900), 558 (10 100), 522 (sh, 5 800), 291 (sh, 5 600), 250 (29 300) nm (ε). Fluorescence (ethanol, room temp.): λ = 640 nm, Φ_f = 0.02 ($\lambda_{ex.}$ = 550 nm).

1,4-Bis-propylamino-anthraquinone [**11**; $C_{20}H_{20}O_2N_2$]

Prepared together with **9** in 30% yield; it was also formed upon treatment of **9** under the reaction condition given above. M.p. 124–125 °C. ^{1}H-NMR (200 MHz, δ, $CDCl_3$): 1.07 (t, J = 7.3 Hz, 2-$CH_2\mathbf{CH_3}$), 1.75 (tq, J_1 = 7.3 Hz, J_2 = 7.3 Hz, 2-$CH_2\mathbf{CH_2}\mathbf{CH_3}$), 3.32 (t, broad, J_1 = 7.3 Hz, 2NH–$\mathbf{CH_2}CH_2$), 7.16 (s, H-2,3), 7.66 (BB'-part of AA'BB' system, H-6,7), 8.32 (AA'-part of AA'BB' system, H-5,8), 10.82 (s, broad, 2N**H**CH_2) ppm. ^{13}C-NMR (90 MHz, δ, $CDCl_3$): 11.7 (CH_3), 22.9 (CH_2), 44.6 (CH_2), 109.6 ($C_{ar.}$), 123.4 ($CH_{ar.}$), 125.9 ($CH_{ar.}$), 131.8 ($CH_{ar.}$),134.5 ($C_{ar.}$), 146.2 ($C_{ar.}$), 182.1 ($C_{ar.}$) ppm. IR (KBr): ν = 2961, 2857, 1643, 1607, 1555, 1520, 1464 cm^{-1}. MS (70 eV, 90 °C) *m/e* (%) = 322 (18; M^+), 293 (100), 247 (15), 236 (3), 43 (22), 41 (32). UV-VIS (ethanol, $c = 10^{-5}$ mol/l): $\lambda_{max.}$ = 642 (19 500), 595 (14 800), 557 (sh, 6 300), 277 (15 200), 256 (29 600) nm (ε). Fluorescence (ethanol, room temp.): λ = 704 nm, Φ_f = 0.03 ($\lambda_{ex.}$ = 550 nm).

1,4-Bis-isopropylamino-anthraquinone [**12**; $C_{20}H_{20}O_2N_2$]

Prepared together with **10** in 25% yield; it was also formed upon treatment of **10** under the reaction conditions given above. M.p. 165–167 °C. ^{1}H-NMR (200 MHz, δ, $CDCl_3$): 1.35 (A_6-part of A6MX system, J_{AM} = 7 Hz, $4CH_3$), 3.90 (M-part of A_6MX system, J_{AM} = 7 Hz, J_{MX} = 7.4 Hz, 2CH), 7.22 (s, H-2,3), 7.66 (BB'-part of AA'BB' system, H-6,7), 8.33 (AA'-part of AA'BB' system, H-5,8), 10.92 (X-part

of A_6MX system, $J = 7.4$ Hz, 2NH) ppm. ^{13}C-NMR (90 MHz, δ, $CDCl_3$): 23.4 (CH_3), 43.7 (CH), 109.6 ($C_{ar.}$), 123.9 ($CH_{ar.}$), 125.9 ($CH_{ar.}$), 131.8 ($CH_{ar.}$), 134.6 ($C_{ar.}$), 145.3 ($C_{ar.}$), 182.0 ($C_{ar.}$) ppm. IR (KBr): $\nu = 2961$, 2869, 1643, 1612, 1592, 1559, 1518, 1460 cm^{-1}. MS (70 eV, 90 °C) *m/e* (%) = 322 (1, M^+), 152 (1), 125 (2), 97 (16), 69 (36), 41 (100). UV-VIS (ethanol, $c = 10^{-5}$ mol/l): $\lambda_{max.} = 640$ (20 300), 594 (16 000), 552 (sh, 6 800), 278 (16 000), 261 (31 700) nm (ε). Fluorescence (ethanol, room temp.): $\lambda = 704$ nm, $\Phi_f = 0.02$ ($\lambda_{ex.} = 550$ nm).

2-Propylamino-1,4-dihydroxy-anthraquinone [13; $C_{17}H_{15}O_4N$]

Prepared in analogy to **9**, however with addition of 0.5 ml $BF_3 \cdot O(C_2H_5)_2$. Yield 20%; m.p. 187–188 °C. ^{1}H-NMR (360 MHz, δ, $CDCl_3$): 1.05 (t, $J = 7.4$ Hz, $-CH_2\mathbf{CH_3}$), 1.75 (tq, $J_1 = J_2 = 7.4$ Hz, $-CH_2\mathbf{CH_2}$-CH_3), 3.23 (*t*, $J = 7.4$ Hz, NH–$\mathbf{CH_2}CH_2$–), 5.8 (s, broad, $-\mathbf{NH}CH_2$), 6.14 (s, H-3), 7.77 (BC-part of ABCD system, H-6,7), 8.34 (AD-part of ABCD system, H-5,8), 14.0 (s, OH-1), 14.3 (s, OH-4) ppm. ^{13}C-NMR (90 MHz, δ, $CDCl_3$): 11.5 (CH_3), 21.9 (CH_2), 44.7 (CH_2), 100.3 ($CH_{ar.}$), 102.9 ($C_{ar.}$), 110.4 ($C_{ar.}$), 126.3 ($CH_{ar.}$), 126.4 ($CH_{ar.}$), 132.2 ($C_{ar.}$), 132.3 ($CH_{ar.}$), 134.0 ($CH_{ar.}$), 134.7 ($C_{ar.}$), 147.7 (C–N), 153.6 ($C_{ar.}$), 166.5 ($C_{ar.}$), 178.0 ($C_{ar.}$), 183.5 ($C_{ar.}$) ppm. IR (KBr): $\nu = 2960$, 1629, 1581, 1513, 1427 cm^{-1}. MS (70 eV, 90 °C) *m/e* (%) = 297 (49; M^+), 169 (16), 168 (100), 69 (2), 57 (4), 55 (2), 43 (16), 41 (20). UV-VIS (ethanol, $c = 10^{-5}$ mol/l): $\lambda_{max.} = 517$ (18 400), 268 (38 700) nm (ε). Fluorescence (ethanol, room temp.): $\lambda = 616$ nm, $\Phi_f = 0.04$ ($\lambda_{ex.} = 520$ nm).

2-Isopropylamino-1,4-dihydroxy-anthraquinone [**14**; $C_{17}H_{15}O_4N$]

Prepared in analogy to **13**; yield 20%. m.p. 171–175 °C. ^{1}H-NMR (360 MHz, δ, $CDCl_3$): 1.30 (A_6-part of A_6MX system, $J_{AM} = 6.5$ Hz, $2CH_3$), 3.71 (M-part of A_6MX system $J_{AM} = 6.5$ Hz, $J_{MX} = 7.9$ Hz, CH), 5.49 (X-part of A_6MX system, $J_{MX} = 7.9$ Hz, $-\mathbf{NH}$–CH), 6.06 (s, H-3), 7.71 (BC-part of ABCD system, H-6,7), 8.25 (AD-part of ABCD system, H-5,8), 13.93 (s, OH-1), 14.25 (s, OH-4) ppm. ^{13}C-NMR (90 MHz, δ, $CDCl_3$): 22.1 (CH_3), 44.2 (CH), 100.4 ($CH_{ar.}$), 102.7 ($C_{ar.}$), 110.3 ($C_{ar.}$), 126.2 ($CH_{ar.}$), 126.4 ($CH_{ar.}$), 132.1 ($C_{ar.}$), 132.2 ($CH_{ar.}$), 134.0 ($CH_{ar.}$), 134.6 ($C_{ar.}$), 146.6 (C–N), 153.7 ($C_{ar.}$), 166.5 ($C_{ar.}$), 177.7 ($C_{ar.}$), 183.3 ($C_{ar.}$) ppm. IR (KBr): $\nu = 2971$, 1632, 1578, 1517, 1457 cm^{-1}. MS (70 eV, 90 °C) *m/e* (%) = 298 (7; M^+), 181 (13), 266 (3), 219 (2), 149 (4), 127 (4), 69 (27), 61 (100). UV-VIS (ethanol, $c = 2 \cdot 10^{-5}$ mol/l): $\lambda_{max.} = 515$ (16 700), 268 (32 700) nm (ε). Fluorescence (ethanol, room temp.): $\lambda = 618$ nm, $\Phi_f = 0.04$ ($\lambda_{ex.} = 580$ nm).

1,4-Dihydroxy-2-(N$_\alpha$-tosyl-L-lysine-ethylester-N$_\varepsilon$-amino)-anthraquinone [**15**; $\mathbf{C_{22}H_{30}O_8N_2S}$]

100 mg (0.41 mmol) **8** were dissolved in 15 ml acetonitrile (p. A.) and 2.5 g (7.6 mmol) N_α-tosyl-lysine-ethylester [19] dissolved in 10 ml acetonitrile were added. After boiling under reflux for 24 h the solvent was evaporated on a rotatory evaporator. The residue was dissolved in 50 ml dichloromethane, extracted three times with 3% HCl, and washed two times with water. The organic phase was then dried with Na_2SO_4 and after evaporation of the solvent chromatographed on solica with toluene/methanol = 6/1. Yield 15%; m.p. 161–163 °C. ^{1}H-NMR (200 MHz, δ, $CDCl_3$): 1.08 (t, $J = 7.2$ Hz, $OCH_2\mathbf{CH_3}$), 1.68 (m, $3CH_2$), 2.40 (s, CH_3), 3.23 (m, NH–CH_2), 3.92 (q, $J = 7.2$ Hz, $O\mathbf{CH_2}CH_3 + CH_\alpha$), 5.23 (d, $J = 8.4$ Hz, NHα), 5.68 (t, $J = 6.4$ Hz, NHε), 6.09 (s, H-3), 7.28 (AA'-part of AA'XX' system, $J = 7.0$ Hz, H-3',5'), 7.71 (XX'-part of AA'XX' system, $J = 7.0$ Hz, H-2',6'), 7.72 (BC-part of ABCD system, H-6,7), 8.25 (AD-part of ABCD system, H-5,8), 13.94 (s, OH-1), 14.24 (s, OH-4) ppm. IR (KBr): $\nu = 2954$, 2952, 2885, 1739, 1600, 1579, 1522, 1435 cm^{-1}. MS (32 eV, 70 °C) *m/e* (%) = 567 (17, M^+), 394 (12), 347 (9), 320 (41), 268 (63), 255 (44), 240 (13), 170 (12), 156 (33), 131 (27), 124 (32), 91 (100), 65 (38). UV-VIS (ethanol, $c = 2 \cdot 10^{-5}$ mol/l): $\lambda_{max.} = 516$ (17 200), 268 (28 800) nm (ε). Fluorescence (ethanol, rom temp.): $\lambda = 616$ nm, $\Phi_f = 0.03$ ($\lambda_{ex.} = 520$ nm).

2-Isopropylamino-4-isopropylimino-9,10-dihydroxy-1,4-anthraquinone [**16**; $C_{20}H_{22}O_3N_2$]

Prepared in analogy to **14** but with stirring for 24 h at room temperature and chromatography on alumina (activity II–III) with dichloromethane. The main product was still **14**, which upon treatment

under this conditions also resulted in further formation of **16**. Yield 5%; m.p. 212–215 °C. ^{1}H-NMR (360 MHz, δ, $CDCl_3$): 1.36 (A_6-part of A_6X system, $J = 6.4$ Hz, $2CH_3$-2), 1.57 (A_6-part of A_6X system, $J = 6.3$ Hz, $2CH_3$-4), 3.87 (X-part of A_6X system $J = 6.4$ Hz, CH-2), 4.68 (X-part of A_6X system $J = 6.3$ Hz, CH-4), 6.03 (s, H-3), 7.26 (s, broad, NH-2), 7.76 (MN-part of AMNX system, H-6,7), 8.23 (A-part of AMNX-system, H-5), 8.62 (X-part of AMNX-system, H-8), 13.16 (s, broad, OH-1,10) ppm. NOE: NH-2↔H-3; CH-4↔OH-10; OH-10↔H-5; H-8↔OH-9. ^{13}C-NMR (90 MHz, δ, $CDCl_3$): 21.9 (CH_3), 24.5 (CH_3), 45.1 (CH), 50.1 (CH), 95.4 ($CH_{ar.}$), 103.5 ($C_{ar.}$), 127.5 ($CH_{ar.}$), 128.3 ($CH_{ar.}$), 128.6 ($C_{ar.}$), 131.1 ($CH_{ar.}$), 132.3 ($CH_{ar.}$), 154.9 ($C_{ar.}$), 155.1 ($C_{ar.}$), 157.5 ($C_{ar.}$), 168.9 ($C_{ar.}$), 170.4 ($C_{ar.}$) ppm. IR (KBr): $\nu = 2972, 2855, 1607, 1557, 1524, 1443\ cm^{-1}$. MS (70 eV, 150 °C) *m/e* (%) = 336 (4; $M^+ - 2H$), 83 (5), 69 (8), 57 (31), 55 (35), 43 (69), 42 (100). UV-VIS (ethanol, $c = 2 \cdot 10^{-5}$ mol/l): $\lambda_{max.} = 564$ (18 000), 527 (12 300), 301 (17 200), 287 (sh, 15 600) nm (ε). Fluorescence (ethanol, room temp.): $\lambda = 652$ nm, $\Phi_f = 0.04$ ($\lambda_{ex.} = 550$ nm).

Note added in proof

The photoreceptor pigment of Stentor was only recently established to be stentorin, a molecule similar to hypericin (N. Tao, M. Orlando, J. Hyon, M. Gross, P.-S. Song (1993) J. Am. Chem. Soc. **115**: 2526). However, the results and conclusions of the present paper apply to stentorin as well as to hypericin.

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