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The Deprotonation and Protonation Equilibria of a Hypericin Derivative in Aqueous Solution

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Summary. A hypericin derivative ω,ω'-appended at the methyl groups with two polyethylene glycol moieties (about 23 units long) and capped with acetyl groups was synthesized starting from emodin. This derivative proved to be soluble in water and was investigated by means of spectrophotometric titrations and electrophoresis experiments. Deprotonation at the *bay*-region hydroxyl group was observed at $pK_a = 1.6$. This was followed by a second deprotonation step of a *peri*-hydroxyl group at a pK_a value of 9.4. This derivative could be protonated at the carbonyl group characterized by a pK_a value of -5.7. From pK_a determinations in water-ethanol mixtures the corresponding pK_a values of hypericin itself determined in such mixtures were extrapolated to the aqueous phase. This resulted in estimated pK_a values of 1.8, 9.2, and -6.

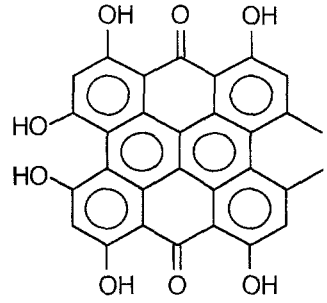
Keywords. Hypericin; ω,ω'-Polyethylene glycol appended hypericin; pK_a values; Spectrophotometric titrations; Electrophoresis.

Deprotonierungs- und Protonierungsgleichgewichte eines Hypericinderivates in wäßriger Lösung

Zusammenfassung. Ein an den Methylgruppen von Hypericin mit endständig acetylierten Polyethylenglykolketten (ungefähr 23 Einheiten lang) ω,ω'-disubstituiertes Derivat wurde ausgehend von Emodin synthetisiert. Dieses Derivat ist wasserlöslich, und es wurde mit Hilfe von spektrophotometrischen Titrationen und Elektrophorese untersucht. Die Deprotonierung der *bay*-Hydroxylgruppe erfolgt bei $pK_a = 1.6$. Diese wird von einem zweiten Deprotonierungsschritt an einer *peri*-Hydroxylgruppe bei einem pK_a-Wert von 9.4 gefolgt. Dieses Derivat konnte an der Carbonylgruppe protoniert werden, was durch einen pK_a-Wert von -5.7 charakterisiert ist. Ausgehend von pK_a-Messungen in Wasser-Ethanol-Mischungen wurden die entsprechenden pK_a-Werte auch für das Hypericin selbst in wäßriger Phase extrapoliert. Dies führte zu geschätzten pK_a-Werten von 1.8, 9.2 und -6.

Introduction

Hypericin (**1**) and several of its structural analogs are confined to aqueous systems with respect to their display of physiological properties [1]. Since the protonation and deprotonation behaviour of these systems is thought to be of fundamental importance for their physiological effects, measurements of the corresponding aqueous pK_a values to characterize these equilibria seem quite desirable. As the extremely low solubility and association behavior of these pigments in water



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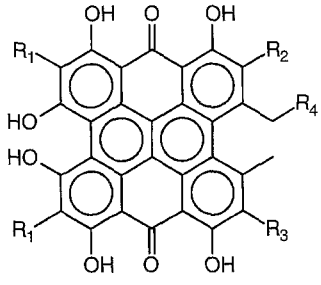
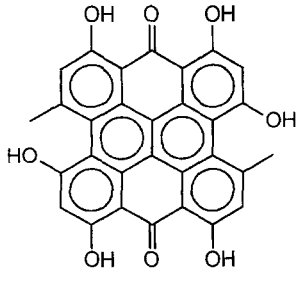
prohibits reliable estimates of these values in aqueous solutions, several efforts have been launched to derive such estimates from solutions of **1** and the analogous compounds **2–12** in solvent mixtures or aqueous micellar systems [2–12]. According to the data presented in Table 1 for convenience, these values differ quite extensively and, moreover, authors disagree about what kind of equilibrium (compare Scheme 1) is actually observed within a certain pK_a range.

A different approach to this problem would be based on a hypericin derivative which is soluble in water and whose essential functionalities (C=O, OH) are left undisturbed from the derivatization. Since for similar motives we have been recently successful to synthesize a hypericin derivative soluble in hydrocarbons by means of appending two linear long chain alkyl residues at the two ω-methyl groups of **1** [13], an analogous approach seemed to be worth pursuing in the



$R_1 = R_2 = R_3 = R_4 = H$ **1**
$R_1 = Br, R_2 = R_3 = R_4 = H$ **2**
$R_1 = R_2 = Br, R_3 = R_4 = H$ **3**
$R_1 = R_2 = R_3 = Br, R_4 = H$ **4**
$R_1 = R_2 = R_3 = H, R_4 = OH$ **5**



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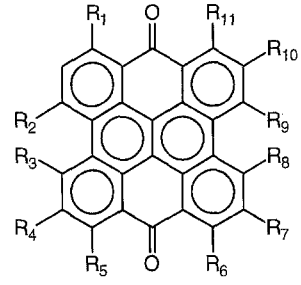
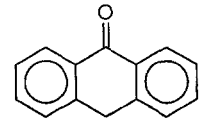


$R_1 = R_2 = R_3 = R_5 = R_6 = R_8 = R_9 = R_{11} = OH,$
$R_4 = H, R_7 = R_{10} =$ isopropyl **7**
$R_1 = R_2 = R_3 = R_5 = R_6 = R_8 = R_9 = R_{11} = OH,$
$R_7 = H, R_4 = R_{10} =$ isopropyl **8**
$R_1 = R_2 = R_3 = R_5 = R_6 = R_8 = R_9 = R_{11} = OH,$
$R_4 = R_7 = R_{10} = H$ **9**
$R_2 = R_3 = R_4 = R_7 = R_8 = R_9 = R_{10} = H,$
$R_1 = R_5 = R_6 = R_{11} = OH$ **10**
$R_1 - R_{11} = H$ **11**



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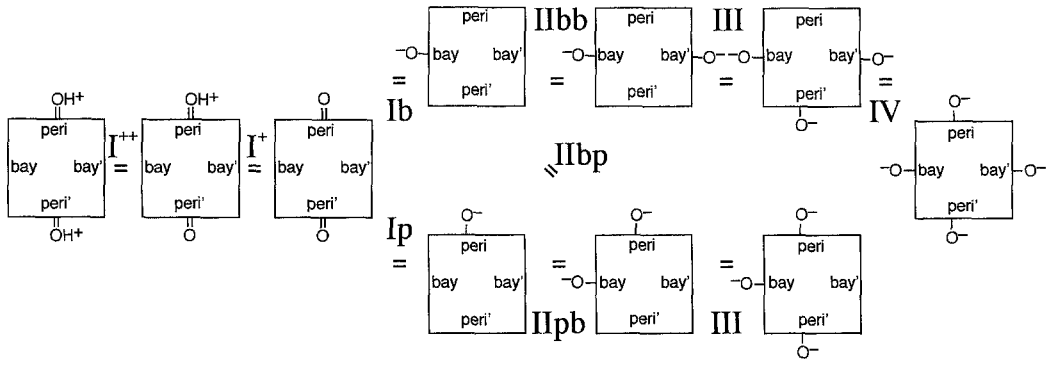
present case. Since **1** ω,ω'-appended with triethylene glycol moieties or glycerol did not significantly improve the solubility in water [14], the synthesis of a hypericin derivative appended at the two ω-methyl groups with polyethylene glycol chains of appropriate lengths and the investigation of its protonation and deprotonation equilibria in aqueous solutions will be advanced in the present communication.

Table 1. pK_a values for the protonation and deprotonation behavior of hypericin (**1**) and analogous derivatives **2** – **12** with **I–IV** denoting the various protonation and deprotonation equilibria according to Scheme 1

	I^{++}	I^{+}	**Ib**	**Ip**	**IIbp**	**IIbb**	**III**	**IV**	System	Ref.
1			7.0						aq. phosphate	[2]
1		−6.0	11.0						aq.H_2SO_4 80% *DMSO*	[3]
1			11.7						aq. Triton	[4]
1			1.7		12.5				80% EtOH	[7]
1		−7.5	1.5		13				aq. H_2SO_4, 80% *DMSO*	[5]
1		1	7		11				aq. *Brij*-35	[6]
2					10.5				aq. H_2SO_4, 80% *DMSO*	[8][a]
3					9.6				aq. H_2SO_4, 80% *DMSO*	[8][a]
4			1.2		8.8				aq. H_2SO_4, 80% *DMSO*	[8][a]
5					11				aq. H_2SO_4 80% *DMSO*	[3][a]
6		−5.9	7						aq. H_2SO_4, 80% *DMSO*	[9]
7		−5.5	0.8			2.9	13.5		aq. H_2SO_4, 80% *DMSO*	[10]
8		−5.1	0.8			3.5	13.0		aq. H_2SO_4, 80% *DMSO*	[10]
9			1.6			3.6	13.2		80% EtOH	[7]
9		−5.3	1.4			3.3	11.0	12.4	aq. H_2SO_4 20% *DMSO*	[5]
			2.2			5.1			80% *DMSO*	
			3.1						*DMSO*	
10		−7.3		13.0					aq. H_2SO_4, 80% *DMSO*	[3]
11	−7.0	−6.0							aq. H_2SO_4, 80% *DMSO*	[11]
12		−5.0							aq. H_2SO_4, 80% *DMSO*	[12]

[a] Position of deprotonation reassigned following the results of Ref. [5].

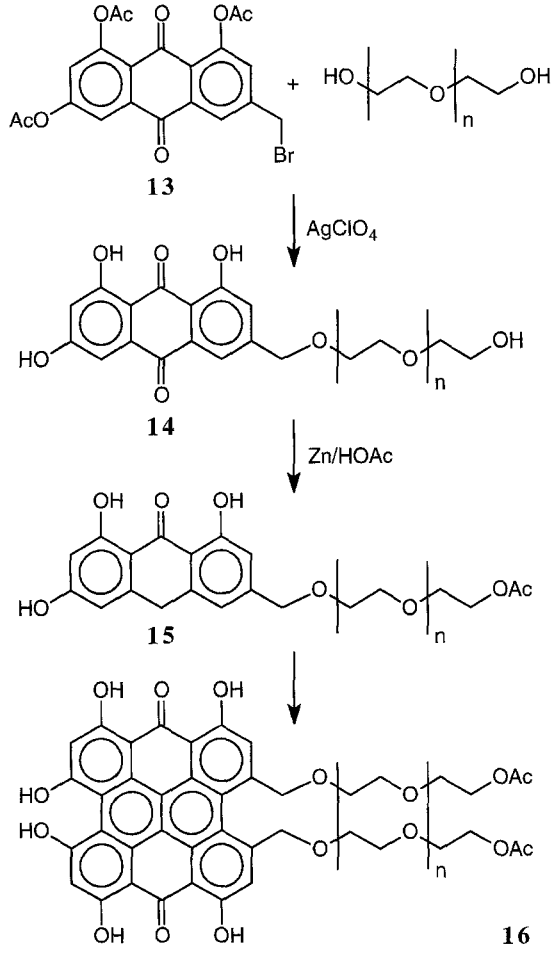


Scheme 1

Results and Discussion

Synthetic aspects

The synthesis of the desired ω,ω'-polyethylene glycol appended hypericin derivative **16** was executed straightforwardly along the recently established route



[14] starting from emodin *via* the easily accessible [14] ω-bromo-triacetoxy-emodin derivative **13**. Nucleophilic displacement of the bromine substituent by polyethylene glycol-1000 in presence of silver perchlorate resulted in the corresponding appended derivative **14** (judged from the relative proton NMR signal intensities, $n \cong 22$), which was reduced to the anthrone derivative **15**. It should be noted that this reduction provided the best yields when run in glacial acetic acid as solvent, and that these conditions resulted also in the acetylation of the polyethylene glycol hydroxylic end group. Conventional oxidative and photochemical dimerization of **15** provided the desired hypericin derivative **16** in fair overall yield.

Association equilibria and the nature of species at different pH values

The ω,ω'-appended derivative **16** could be dissolved in water ($pH \approx 6$) in concentrations up to 10^{-5} mol/l. Upon variation of the concentration of such solutions, it became evident from the absorption spectra shown in Fig. 1 that this derivative was involved in a homoassociation equilibrium. Whereas in the region of $1 \cdot 10^{-7}$ mol/l the absorption spectrum of **16** in water became similar to those recorded in ethanol or dimethylsulfoxide (which were found to be independent of concentration), it showed the characteristics of a homoassociated species [15] for concentrations $> 10^{-6}$ mol/l.

It should also be noted that – in contrast to the behavior of **1** [15] – the highly diluted aqueous solution of **16** (trace *a* of Fig. 1) exhibited fluorescence comparable to those obtained for other solvents (see Experimental). This pointed to the presence of non-associated species of **16**. Moreover, from a comparison of the extinction coefficients of trace *a* of Fig. 1 with those of **16** dissolved in a non-associating solvent like ethanol or dimethylsulfoxide there might still be some association also in highly diluted aqueous solutions. Although this kind of association has been recently attributed to a monomer-dimer equilibrium in the

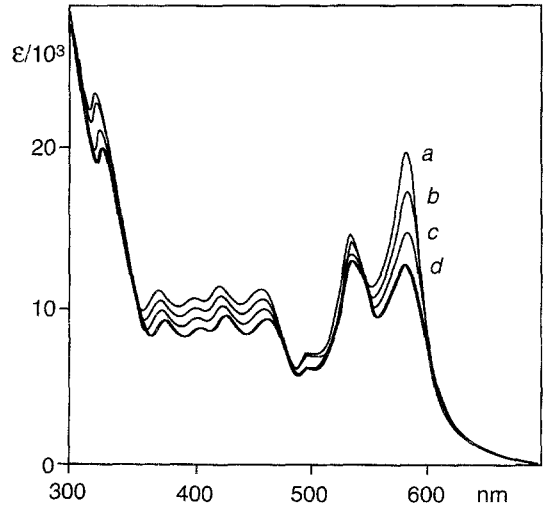


Fig. 1. Absorption spectra of **16** in aqueous solution ($pH \approx 6$) at $1 \cdot 10^{-7}$ (*a*), $5 \cdot 10^{-7}$ (*b*), $1 \cdot 10^{-6}$ (*c*), and $\geq 1 \cdot 10^{-5}$ (*d*) mol/l

case of hypericin [16], our data did not permit to unequivocally assign a specific homoassociation equilibrium.

To account for the various charged or electroneutral species dependent upon the *pH* values of the solutions, an electrophoresis experiment was conducted. Due to the low charge-to-mass value, migration of such species in the case of **16** could not be observed. Therefore, we resorted to use **1** which was subjected to a series of electrophoresis strips with *pH* values varying from 0 to 12 in a water-dimethylsulfoxide mixture (see Experimental). Thus, it turned out that whereas the applied spot did not move at *pH* 0 and 1, it moved towards the anode between *pH* 2 and 12. Accordingly, there was no doubt that anionic species were prevailing in this *pH* region, and the species described in Fig. 1 were actually $\mathbf{16}^-$ and its oligomers.

Deprotonation equilibria

Upon spectrophotometric titration of **16** in a highly dilute aqueous solution it became evident (Fig. 2) that, depending on the *pH* value of the solution, a certain species dominated below *pH* 1, another one prevailed between *pH* 2.5 and 8, and a third one became prominent above *pH* 10. From this experiment, apparent pK_a values of 1.6 and 9.4 could be derived and we were thus left to assign equilibria (*cf.* Scheme 1) to these two steps and to account for the nature of the three species.

From the electrophoresis experiment described above it followed immediately that the species giving rise to the absorption spectra *b* and *c* of Fig. 2 were the mono- and di-deprotonated ones, whereas the one pertaining to trace *a* was to be assigned the non-dissociated electroneutral form. Judged from the shape of this spectrum, which was similar to the one of **16** dissolved in water at high concentrations as given in Fig. 1, it was obviously involved in strong homoassociation. This could be corroborated by a comparison of these data with those of a titration

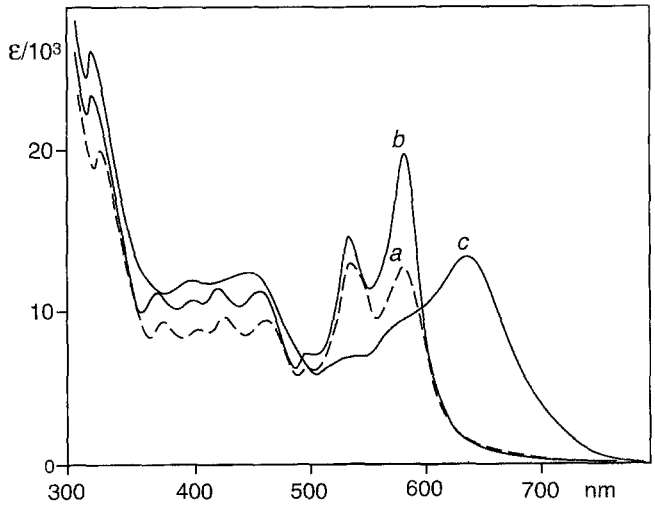


Fig. 2. Absorption spectra of **16** in aqueous solutions ($c = 1 \cdot 10^{-7}$ mol/l) at $pH = 0.2$ (*a*), 5 (*b*), and 12 (*c*)

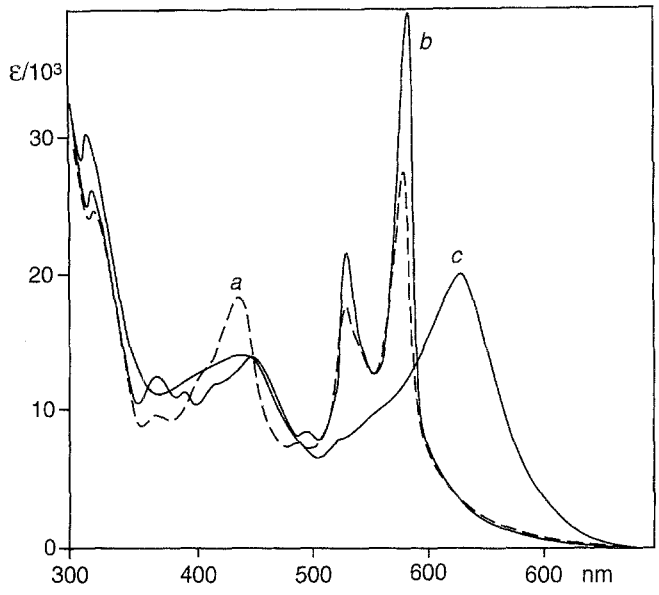


Fig. 3. Absorption spectra of **16** in ethanol ($c = 1.10^{-7}$ mol/l) at $pH = 0.2$ (*a*), 5 (*b*), and 12 (*c*)

of **16** in ethanolic solution as illustrated in Fig. 3. Here the two anionic species of traces *b* and *c* were found to be similar to those of the aqueous system. However, the electroneutral species given by trace *a* displayed the typical monomeric characteristics with a prominent band in the 450 nm region. It should be mentioned that the titration of **16** in *DMSO*/water mixtures like those given for **1** in Table 1 yielded similar results.

According to these experiments, the apparent pK_a value of 1.6 for **1b** was characteristic for the deprotonation of the electroneutral homoassociated species. It was assigned to step **Ib** of Scheme 1 because from a series of studies it has been derived that the *bay*-hydroxyl group of hypericin type molecules is the most acidic one [5, 17–19]. However, the influence of the homoassociation on the pK_a value was found to be rather small. This was inferred from measuring the pK_a values for a series of water/ethanol mixtures as displayed in Fig. 4. It should be mentioned that the nature of the species prevailing between *pH* 2 and 8 was also corroborated by salt formation of **16** with weak bases like pyridine ($pK_a = 5.3$, [20]) which has also been observed for **1** [21]. It should be stressed that this result was also in accordance with the findings that isohypericin (**6**) and emodin, in which the *bay* anion cannot be stabilized by a strong intramolecular hydrogen bond [17], displays *bay*-deprotonation pK_a values of 7 [9] and 8.9, well above the corresponding hypericin value. The second deprotonation step of **16** characterized by a pK_a of 9.4 was assigned to the deprotonation of a *peri*-hydroxyl group according to step **IIbp** of Scheme 1.

The hypericin derivative **16** could also be incorporated into the aqueous nonionic detergent micelles of Triton-X-100. Upon titration, the same spectroscopic characteristics as those given in Fig. 2 were observed, and the corresponding pK_a values did not significantly deviate from those measured for the aqueous system. It should be mentioned that in the vicinity of *pH* 7 a faint sigmoid inflection in the titration absorption intensity curve of the long wavelength band could be observed

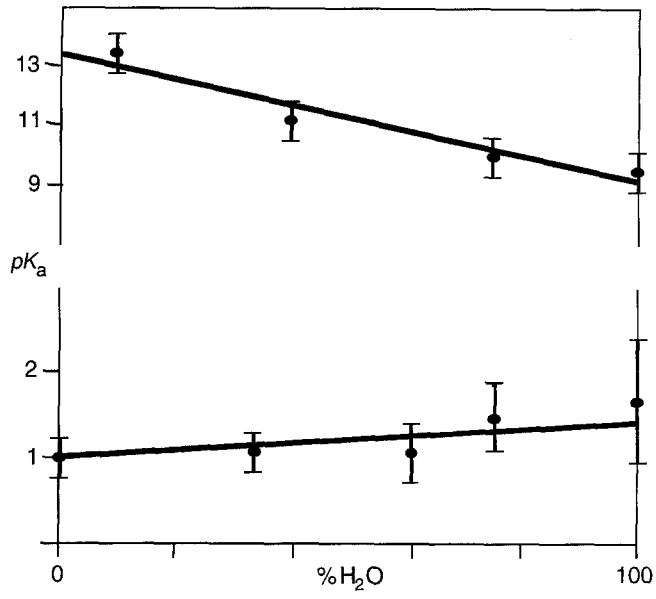


Fig. 4. Apparent pK_a values of **16** dissolved in water/ethanol mixtures

which corresponds to the one at $pH \approx 7$ described by *Jardon et al.* for **1** dissolved in the nonionic aqueous *Brij* detergent micelle [6]. These authors assigned the first deprotonation step of **1** to this rather spurious signal (Table 1). However, from a variation of the ionic strength and the titrants it became clear that this phenomenon had to be ascribed to the influence of the salt formed from the titrants upon association of the hypericinate ion. One should also bear in mind that such association effects will be enhanced in a micellar system, since the effective concentration of the solute is generally orders of magnitude higher within the micelle as compared to experiments with a homogeneous solution.

Protonation equilibria

The spectroscopic characteristics of the homoassociated electroneutral species of **16** as shown by traces *a* of Figs. 2 and 5 remained without significant changes when dissolved in aqueous sulfuric acid ranging in *pH* from 1 to 0, followed by H_0 from 0 to about −4.5. Below this value, the typical changes observed in such spectrophotometric titrations occurred, eventually leading to the absorption spectrum given by trace *b* of Fig. 5. From these data, a pK_a value of −5.7 was derived. The phenomenon was assigned to the protonation of one carbonyl group of **16** (equilibrium $\mathbf{I}^+$ of Scheme 1). It is observed in the same region as the one for simple aromatic carbonyl systems like **12** and the phenanthroperylene quinone parent compound **11** (Table 1). One should bear in mind that to achieve protonation of these systems at a carbonyl group, one would need sulfuric acid with a concentration of more than 50%! Therefore, assignments of pK_a values in the range of +1 (0.1 *N* HCl) to the protonation equilibrium of the electroneutral species of hypericin type systems which have been discussed hitherto several times (*cf.* Table 1) could no longer be accepted.

A second protonation step involving the second carbonyl group could possibly be inferred from the spectroscopic behaviour of **16** in solutions with H_0 values

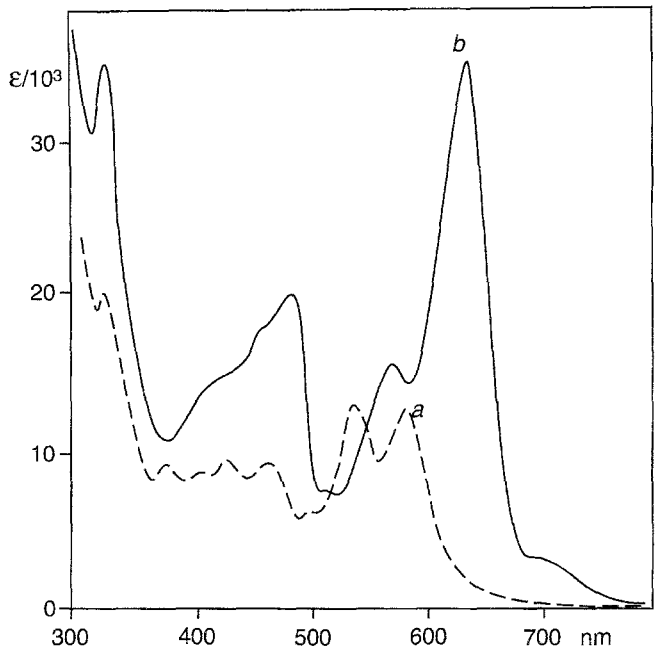
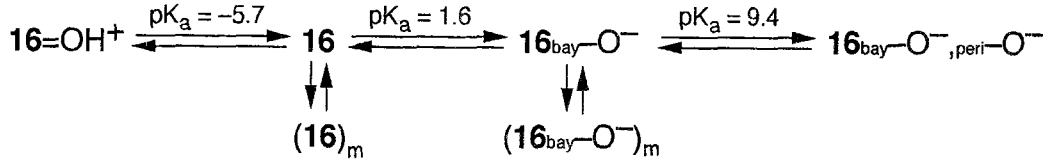


Fig. 5. Absorption spectra of **16** dissolved in water at $pH = 0$ (*a*) and in water/H_2SO_4 of $H_0 = -6.8$ (*b*); ($c = 1 \cdot 10^{-7}$ mol/l)

below −9. However, in contrast to the parent compound **11** (Table 1), the changes were not distinct enough for the calculation of a pK_a value, and it could not be rigorously excluded that the pK_a value of −5.7 derived above was an appearent pK_a value including both protonation steps I^+ and I^{++} (Scheme 1).

Conclusions

From the experiments described above it was concluded that the ω-appended hypericin derivative **16** in aqueous solution is characterized by three main ionization steps which are coupled in part to homoassociation equilibria as shown in Scheme 2. By means of a system of mixed solvents, the latter equilibria were

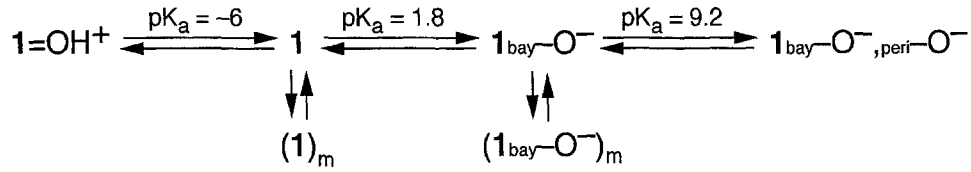


Scheme 2

derived to exert only minor influences upon the intrinsic pK_a values of **16**, provided its concentration was low in such determinations.

These results might then be extrapolated with due care to aqueous solutions of hypericin (**1**) itself as illustrated in Scheme 3. Earlier measurements provided carbonyl group protonation values of −6.0 and −7.5 (Table 1) in the same aqueous

sulfuric acid system as used in the present investigation for the study of **16**. Thus, a protonation pK_a value (steps $\mathbf{I}^+$ or even an overlay of $\mathbf{I}^+$ and $\mathbf{I}^{++}$ of Scheme 1) in the order of −6 seemed to be reasonable.



Scheme 3

For the first deprotonation step **Ib**, values of 1.7 (80% ethanol [7]) and 1.5 (80% dimethylsulfoxide/water [5]) have been reported (*cf.* Table 1). Given the dependence of the pK_a value upon the water concentration in ethanol as exemplified in Fig. 4, one might extrapolate such values to a pK_a value for **1** in the aqueous system of about 1.8. This value was quite similar to that observed for **16** in aqueous solution. However, it has to be kept in mind that **1** and $\mathbf{1}^-$ are quite insoluble in water, and even if they are solubilized they will be present as heavily homoassociated materials. Thus, the extrapolated value in this case will strictly pertain only to a fictitious system made up of a water soluble and slightly associating **1**. In such cases of heavily associated and rather insoluble systems one is strongly reminded of the long standing problem of the dissociation of bilirubin [22]. Much the same arguments will hold for extrapolations from pK_a values determined for dimethylsulfoxide/water mixtures.

The dideprotonation step of **1** (**Ibp**) has been determined to occur at a pK_a value of 12.5 in 80% ethanol [7] (Table 1). Using again the concentration dependence of the pK_a values of **16** in an aqueous ethanolic system as given by Fig. 4, this value could be extrapolated to a pK_a value of 9.2 for aqueous solutions of **1**.

Experimental

Melting points were taken by means of a Kofler hot stage microscope (Reichert, Vienna). ^1H, ^{13}C, IR, UV/Vis, and fluorescence spectra were recorded using Bruker-DPX-200, Biorad-FT-IR-45, Perkin-Elmer IR-710B, Hitachi-U-3210, and F-4010 instruments. Spectrophotometric titrations were carried out as described in Refs. [3, 5, 8]. For fluorescence spectroscopy, 95% ethanol of *für die Fluoreszenzspektroskopie* grade (Merck), otherwise *p.a.* solvents were used. For the determination of the fluorescence quantum yields, Rhodamine B fluorescence ($\Phi_f = 0.69$ ethanol) was used as standard.

The electrophoresis of the various species of **1** (prepared according to Ref. [23]) was carried out using a Reichelt Elektrophoresesystem H-1000 apparatus with cellulose paper as the stationary support and *DMSO*/H_2O= 90/10 (v/v) adjusted to *pH* 0 through 12 in steps of 1.0 by means of HCl/NaOH. Tetrabutylammonium hydrosulfate was used as the supporting electrolyte. The experiments were run at 400 V and 40 mA for 6 h. Whereas at *pH* 0 and 1 the applied spot of **1** did not move within the specified time, it moved by about 20 mm towards the anode between *pH* 2 and 12. As a control, emodin (isolated according to Ref. [23]; $pK_a = 8.9$ as derived from a spectrophotometric titration) was also applied under these conditions; it behaved the same way as **1** but moving only at $pH \geq 10$.

1,3,8-Trihydroxy-6-(ω-hydroxy-polyethoxy-methyl)-anthracene-9,10-dione (**14**)

A mixture of 250 mg (0.53 mmol) 1,6,8-tri-O-acetoxy-3-bromomethyl-anthraquinone (**13**; prepared from emodin according to Ref. [14]), 250 mg (0.97 mmol) $AgClO_4 \cdot H_2O$, and 3 g polyethylene glycol-1000 (Fluka) was stirred for 3 h at 100°C. The reaction mixture was taken up in 250 ml water, filtered, and extracted three times with 100 ml ethyl acetate. The organic phase was washed twice with 100 ml brine, dried over Na_2SO_4, and chromatographed with $CHCl_3$:$CH_3OH = 10 : 1$ over a silica column.

Yield: 415 mg (60%), oil; ^{1}H NMR ($CDCl_3$, δ, 200 MHz): 12.1 and 12.19 (2s, OH-1 and 8), 7.58, 7.17, 7.16, 6.61 (4s, H-ar), 4.55 (s, ar-CH_2-O), 3.5–3.8 (m, O(-CH_2-CH_2-O)$_{n\cong23}$-H) ppm; ^{13}C NMR ($CDCl_3$, δ, 50 MHz): 190.2 (C=O), 181.4 (C=O), 165.2, 165.2, 162.3, 148.0, 135.0, 133.2, 122.0, 118.0, 114.5, 109.7, 109.3, 108.6 (12C, C-ar), 72.4, 72.0 (CH_2-O-CH_2), 70.6, 70.5, 70.4, 70.2 (signal overlap, O-(CH_2CH_2O)$_{n\cong23}$), 61.6 (CH_2OH) ppm; IR (KBr): $\nu = 3390$, 3064, 2870, 1677, 1629 cm^{-1}; UV/Vis (ethanol): $\lambda_{max} = 500$ (6500), 470 (6400), 314 (13300), 256 (20700), 217 (32000) nm (ε).

1,3,8-Trihydroxy-6-(ω-acetoxy-polyethoxy-methyl)-10H-anthracen-9-one (**15**)

To a solution of 200 mg **14** (0.15 mmol) in 50 ml glacial acetic acid, 400 mg of $SnCl_2 \cdot 2H_2O$ (1.77 mol) in 5 ml conc. HCl were added under heating and refluxed for additional 3 h. After cooling the reaction mixture was poured into 300 ml brine, extracted with ethyl acetate, dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica with $CHCl_3$:CH_3OH= 10:1 as the eluent.

Yield: 125 mg (63%); m.p.: 28–29°C; ^{1}HNMR ($CDCl_3$, δ, 200 MHz): 12.41 and 12.34 (2s, OH-1 and OH-8), 8.55 (bs, OH-6), 6.73, 6.71, 6.29 (3s, H-ar), 6.26 (d, $J = 2.0$ Hz, H-ar), 4.46, (s, ar-CH_2O), 4.16 (t, $J = 4.8$ Hz, C*H*$_2$-OCOCH$_3$), 4.09 (s, ar-CH_2-ar), 3.5–3.7 (m, O-(CH_2CH_2O)$_{n\cong23}$), 2.02 (s, $OCOCH_3$) ppm; ^{13}C NMR ($CDCl_3$, δ, 50 MHz): 192.4 (C=O), 170.9 (COO), 165.1, 164.1, 162.4, 146.5, 143.8, 141.2, 113.4, 109.1 (8C, C-ar), 107.2, 101.7, 116.7, 114.5 (4C, CH-ar), 72.1, 70.4, 70.2 (signal overlap), 69.7, 68.9, 63.4, 61.4 (CH_2-O-(CH_2CH_2O)$_{n\cong23}$), 32.7 (ar-CH_2-ar), 20.8 (O-CO*C*H$_3$) ppm; IR (KBr): $\nu = 3300$, 2920, 2850, 1736, 1620, 1599 cm^{-1}; UV/Vis (ethanol): $\lambda_{max} = 362$ (13800), 270 (8200), 260 (8700), 220 (19900) nm (ε).

1,3,4,6,8,13-Hexahydroxy-10,11-bis-(ω-acetoxy-polyethoxy-methyl)-phenanthro-[1,10,9,8-o,p,q,r,a]perylene-7,14-dione (**16**)

125 mg (0.094 mmol) **15** were added to 85 mg (0.94 mmol) pyridine-N-oxide and 9 mg $FeSO_4 \cdot 7H_2O$ dissolved in a mixture of 0.3 ml piperidine and 0.3 ml pyridine and stirred at 100°C for 1h under Ar and protection from light. Then the reaction mixture was acidified with 10% HCl, saturated with NaCl, and extracted three times with 200 ml chloroform. The organic phase was washed twice with 50 ml water and dried over Na_2SO_4. After evaporation of the solvent, the residue was dissolved in 500 ml acetone and irradiated at 20°C for 2 h using a 700 W tungsten lamp. After evaporation of the solvent, the residue was chromatographed on silica plates using $CHCl_3$:CH_3OH= 20:3 as the eluent.

Yield: 24 mg (19%) of a hygroscopic and viscous oil at room temperature; ^{1}H NMR ($CDCl_3$, δ, 200 MHz): 18.43 (bs, OH-3), 14.82 and 14.32 (2s, OH-1,6 and OH-8,13), 7.66 (s, H-9,12), 6.76 (s, H-2,5), 5.06 and 4.6 (AX-system, $J = 11.7$ Hz, ar-CH_2O), 4.18 (t, $J = 4.8$ Hz, 2C*H*$_2$-OCOCH$_3$), 3.0–4.0 (m, 2O-(CH_2CH_2O)$_{n\cong23}$), 2.04 (s, 2$OCOCH_3$) ppm; ^{1}H NMR (D_2O, δ, 200 MHz); 7.40 (s, 2H-ar), 5.90 (s, 2H-ar), 4.25 (m, 2CH_2-$OCOCH_3$), 3.3–3.8 (m, 2O-(CH_2CH_2O)$_{n\cong23}$), 2.11 (s, 2O-$COCH_3$) ppm; ^{13}C NMR ($CDCl_3$, δ, 50 MHz): 184.2 (C=O), 171.0 (COO), 174.9 (C-3 + 4), 169.1 (C-1 + 6), 162.5 (C-8 + 13), 142.6 (C-10 + 11), 127.5 (C-3a + 3b), 126.5 (C-6b + 14b), 121.9 (C-6c + 13c), 120.2 (C-10a + 10b), 119.6 (C-7b + 13b), 117.8 (C-9 + 12), 110.7 (C-6a + 14a), 106.5 (C-2 + 5), 103.1 (C-7a + 13a), 70.4 (signal overlap), 70.3, 70.1 70.0, 69.93, 69.7, 69.2, 69.0, 63.5

(CH_2-(OCH_2CH_2)$_{n\cong 23}$-O), 20.9 ($OCOCH_3$) ppm; IR (KBr): $\nu = 3500$, 2900, 1736, 1584, 1584, 1549, 1460, 1436 cm^{-1}; UV/Vis (methanol; $c = 1 \cdot 10^{-6}$ mol/l): $\lambda_{max} = 592$ (42100), 549 (21900), 511 (10100), 477 (14300), 384 (13000) nm (ε); fluorescence (methanol; $c = 1 \cdot 10^{-6}$ mol/l): $\lambda_{em} = 601$ (1), 657 (0.29) nm (rel. intensities), $\Phi_f = 0.10$; UV/Vis (ethanol; $c = 1 \cdot 10^{-6}$ mol/l): $\lambda_{max} = 592$ (43100), 551 (21500), 512 (9900), 480 (13400), 450 (9600), 414 (9300), 383 (12000), 330 (25500), 287 (33600) nm (ε); fluorescence (ethanol; $c = 1 \cdot 10^{-6}$ mol/l): $\lambda_{em} = 602$ (1), 650 (0.28) nm (rel. intensities), $\Phi_f = 0.13$; UV/Vis (acetone; $c = 1 \cdot 10^{-6}$ mol/l): $\lambda_{max} = 598$ (44400), 554 (21500), 513 (9800), 481 (12900), 387 (12900) nm (ε); fluorescence (acetone; $c = 1 \cdot 10^{-6}$ mol/l): $\lambda_{em} = 605$ (1), 654 (0.28) nm (rel. intensities), $\Phi_f = 0.08$; UV/Vis ($CHCl_3$; $c = 1 \cdot 10^{-6}$ mol/l): $\lambda_{max} = 601$ (41500), 556 (21200), 516 (9500), 486 (13300), 390 (13300) nm (ε); fluorescence ($CHCl_3$; $c = 1 \cdot 10^{-6}$ mol/l): $\lambda_{em} = 609$ (1), 657 (0.29) nm (rel. intensities), $\Phi_f = 0.10$; UV/Vis (CH_2Cl_2; $c = 1 \cdot 10^{-6}$ mol/l): $\lambda_{max} = 600$ (41200), 555 (21000), 516 (9000), 482 (12400), 389 (11500) nm (ε); fluorescence (CH_2Cl_2; $c = 1 \cdot 10^{-6}$ mol/l): $\lambda_{em} = 609$ (1), 657 (0.29) nm (rel. intensities), $\Phi_f = 0.10$; UV/Vis (pyridine; $c = 1 \cdot 10^{-6}$ mol/l): $\lambda_{max} = 604$ (48000), 558 (23500), 519 (11000), 486 (15400), 388 (17800) nm (ε); fluorescence (pyridine; $c = 1 \cdot 10^{-6}$ mol/l): $\lambda_{em} = 611$ (1), 660 (0.28) nm (rel. intensities), $\Phi_f = 0.10$; UV/Vis (*DMSO*; $c = 1 \cdot 10^{-6}$ mol/l): $\lambda_{max} = 602$ (33800), 557 (16200), 512 (7400), 481 (11100), 388 (11100) nm (ε); fluorescence (*DMSO*; $c = 1 \cdot 10^{-6}$ mol/l): $\lambda_{em} = 609$ (1), 657 (0.36) nm (rel. intensities), $\Phi_f = 0.08$; UV/Vis (H_2O; $c = 1 \cdot 10^{-7}$ mol/l, other concentrations see Fig. 1): $\lambda_{max} = 592$ (19000), 552 (12350), 512 (6700), 474 (10500) nm (ε); fluorescence (H_2O; $c = 1 \cdot 10^{-7}$ mol/l): $\lambda_{em} = 601$ (1), 646 (0.29) nm (rel. intensities), $\Phi_f = 0.06$; fluorescence (H_2O; $c = 4 \cdot 10^{-6}$ mol/l): $\lambda_{em} = 601$ (1), 646 (0.29) nm (rel. intensities), $\Phi_f = 0.008$.

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