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UV/Vis and fluorescence study on anthralin and its alkylated derivatives

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Abstract

Anthralin **1** and some of its C-10 or *O*-alkylated derivatives were investigated by UV/VIS- and fluorescence spectroscopy in different solvents and buffer systems, respectively. The effects of substituents on the formation of anthralin anion as well as the constitution of the resulting anions confirm that C–H acidity at position 10 is necessary for the formation of a fully aromatic anionic form. It is concluded that the resulting anion **1a** is the pharmacologically active species of the antipsoriatic anthralin. Tautomerism of the neutral molecule is not observable.

Keywords: Anthralin; UV/Vis spectroscopy; Fluorescence spectroscopy; Alkylated derivatives

1. Introduction

Anthralin (1,8-dihydroxy-9(10*H*)-anthracenone, **1**) is a potent antipsoriatic drug and has been used for over 80 years for treatment of the skin disease psoriasis [1]. The proposed mechanism for the pharmacological action of anthralin involves deprotonation to form the anthralin anion [2–4], followed by a one-electron transfer from the anion to oxygen to afford the anthralin-10-yl radical and superperoxide radical anion [5]. Furthermore, anthralin anion is a photosensitizer which can activate oxygen to singlet oxygen [6,7].

In order to minimize undesired side effects, e.g., irritation and staining of the healthy skin, attempts have been made to derivatize anthralin. The resulting analogues exhibit lower irritancy and optimal efficacy at lower dose [8]. Some of these derivatives have been clinically tested [9,10].

In order to obtain further information on the physicochemical properties of anthralin and on the effect of substitution on the formation of the anthralin anion, experiments were performed using absorption and fluorescence spectroscopy. The effect of solvents and pH on the spectra of anthralin and its alkylated derivatives was the subject of these studies. Furthermore, it was of interest whether the pharmacologically active species described by Raab and Gmeiner [11] comprises the anion only, or whether the tautomeric 1,8,9-trihydroxy anthracene (for discussion of tautomerism in this field cf. Ref. [12]) or the phototautomeric form play a role as well.

2. Materials and methods

The chemicals used were of the purest commercially available grade. Anthralin (**1**) [13], 1-methoxy-8-hydroxy-9(10*H*)-anthracenone (**5**) [14,15], and 1,8-dimethoxy-9-hydroxy-anthracene (**6**) [16,17] were prepared from chrysazine (Merck). 10-Ethylanthralin (**3**) [18] and 10,10-di(*n*-propyl)anthralin (**4**) [19] were obtained from anthralin. All anthralin derivatives were purified by column chromatography (SiO_2/CH_2Cl_2) and recrystallization from CH_2Cl_2/hexane. DMF (Uvasol®, Merck), methanol (Baker, HPLC-grade) and methylene chloride (Merck) were additionally dried according to general procedures. All aqueous buffer solutions (pH 4.0: citric acid 0.1 M, Na_2HPO_4 0.2 M; pH 7.0–8.0: KH_2PO_4 0.15 M, Na_2HPO_4 0.15 M; pH 9.0–11.0: Na_2CO_3 0.1 M, $NaHCO_3$ 0.1 M; pH 12.0: Glycin 0.1 M; NaCl 0.1 M; NaOH 0.1 M) were prepared in double distilled water, degassed and stored under nitrogen. In our study, we used methanol–aqueous buffers (1:1) containing 1% of methylene chloride resulting from the solvent of the stock solutions.

The solutions for the absorption and emission measurements were prepared as follows: 50.0 μl (in case of absorption measurements) or 100 μl (in case of emission measurements) of a stock solution (2×10^{-3} M) in meth-

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ylene chloride (which is soluble enough under these conditions) were diluted with 5.00 ml of the respective solvent or buffer system (MeOH/buffer 1:1). The pH values of the aqueous buffer systems were measured with a WTW 526 pH meter. pH values of the resulting buffer/organic solvent mixtures are given as measured for the buffer systems. The absorption spectra from 200 to 550 nm were obtained with a U-3000 Hitachi UV spectrometer at room temperature using UV cells with 10 mm light paths. Fluorescence measurements were performed with a SLM-Amico Bowman (AB 2) spectrofluorometer. The spectra are not corrected. The respective excitation and emission bandpasses used in the measurements were as follows: (**1**): 0.5/4 nm; (**3**): 4/4 nm; (**5**): 4/4 nm; (**6**): 1/1 nm.

3. Results

3.1. Optical absorption spectroscopy of anthralin and related compounds

Fig. 1 shows the absorption spectra of anthralin (**1**) in methylene chloride and methanol which are comparable to that of 2,2′-dihydroxybenzophenone [20] (**2**) in ethanol (Fig. 2). In accordance with Retzow [3] shoulders at 382 and 438 nm in dry methanol according to Refs. [6,7] were not observed. Switching from methylene chloride to DMF leads to a dramatic change in the absorption of the spectrum of **1** in that the maximum at 356 nm disappears, while new bands occur with a maximum at 385 nm and the typical vibrational structure of anthracenes (Fig. 3). In buffer systems of varying pH, two well defined isosbestic points are observable at 279 and 361 nm (Fig. 4). The change in the absorption spectra of compounds **3** and **5**, characterised by CH-acidity at C-10, on switching from methanol or methylene chloride to DMF or to aqueous basic conditions, is the same as for anthralin **1** itself (Table 1). In the pH-dependent spectra of **3** and **5**, two isosbestic points can be observed.

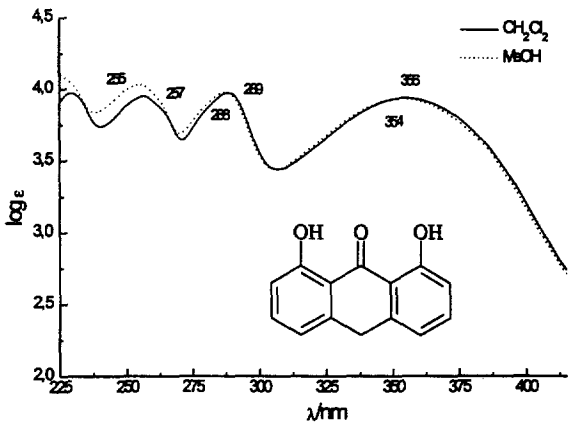


Fig. 1. Absorption spectra of anthralin (**1**) in CH_2Cl_2 (———) and methanol (···).

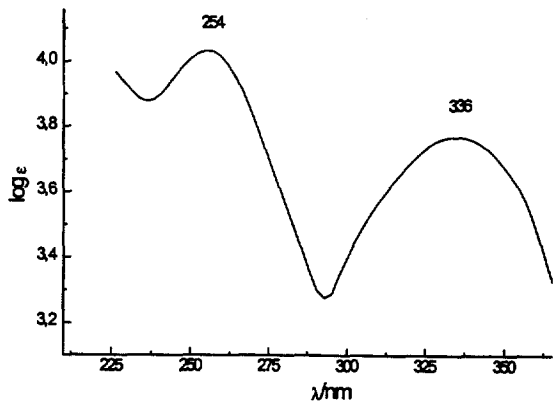


Fig. 2. Absorption spectrum of 2,2′-dihydroxybenzophenone (**2**) in ethanol (———).

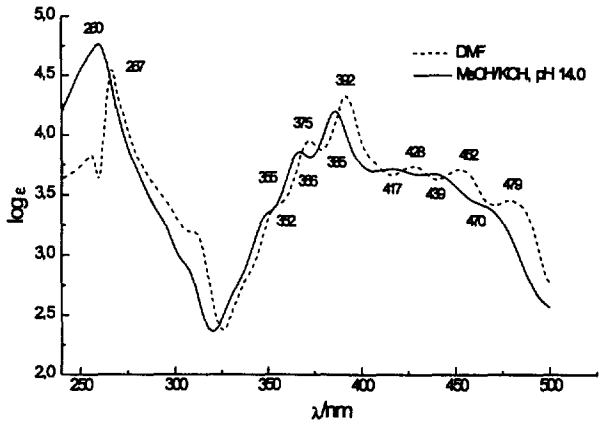


Fig. 3. Absorption spectrum of anthralin (**1**) in DMF (···) and alkaline methanol (———) (methanol/KOH 1 N).

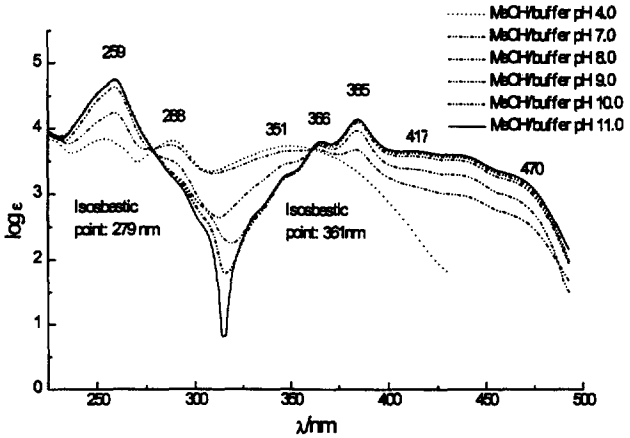


Fig. 4. Absorption spectrum of anthralin (**1**) in methanol containing 50% buffer of pH 4.0 (···) to pH 11.0 (———).

The spectrum of **6** is characteristic of a fully aromatic anthracene (Fig. 5). On the other side, the spectrum of **4**, C-10 of which is dialkylated (so that no CH-acidity can occur), shows no vibrational structure of the anionic form (Fig. 6).

Table 1
Absorption and emission maxima ($\lambda > 300$ nm only) of anthralin and some of its alkylated derivatives

Compound	Solvent	Absorption maxima		Emission maxima		
		λ (nm)	log ε	λ_{exc} (nm)	λ_{flu} (nm)	Stokes' shift (10^3 cm^{-1})
1	CH_2Cl_2	356	3.94	385	–	
	MeOH	354	3.93	385	495	–
					527	–
	DMF	375	3.92	392	497	6.55
		392	4.33		532	6.71
		428	3.74		567	5.73
		452	3.71			
		479	3.46			
	MeOH/KOH 1 N	352	3.83	385		
		366	3.84		494	7.08
		386	4.20		525	6.86
		417	3.72		558	6.06
		439	3.68			
		470	3.37			
3	CH_2Cl_2 or MeOH	362	3.94	468	–	
	DMF	378	3.95	468	516	7.08
		399	4.30		548	6.19
		449	3.71		591	5.35
		464	3.70			
		495	2.89			
	MeOH/KOH 1 N	373	3.77	468		
		392	4.12		512	5.98
		426	3.81		540	4.96
		452	3.70		583	4.97
		483	3.26			
4	CH_2Cl_2 or MeOH or DMF	366	3.97	367	–	
	MeOH/KOH 1 N	413	3.86	367	–	
5	CH_2Cl_2 or MeOH			397	451	–
		334	3.92		472	–
					495	in MeOH only
	DMF	376	3.86	397	536	7.94
		396	4.15		554	7.20
		433	3.53			
		457	3.52			
		493	3.24			
	MeOH/KOH 1 N	365	3.85	397		
		384	4.18		500	6.04
		409	3.72		528	5.51
		438	3.64			
		464	3.33			
6	CH_2Cl_2	359	3.75	378		
		377	4.03		446	4.10
		402	3.70		471	3.64
		425	3.41		496	3.37
	DMF	359	3.75	378		
		377	4.03		446	4.10
		402	3.70		471	3.64
		425	3.41		496	3.37
	MeOH; or MeOH/KOH 1 N	357	3.73	378		
		375	4.01		441	3.99
		396	3.70		466	3.79
		418	3.44		496	3.76

3.2. Determination of pK_a values

The pK_a values were determined by measurement of the absorbance at different pH values and plotting the pH vs. absorbance. The turning point of the resulting graph was determined by differentiating the plot and extrapolation of the resulting maximum [21] to $d(dE) = 0$, which corresponds to the pK_a value of the compound. In each case the analytical wavelengths were adjusted to the longwave maximum of the anionic form of the respective compound.

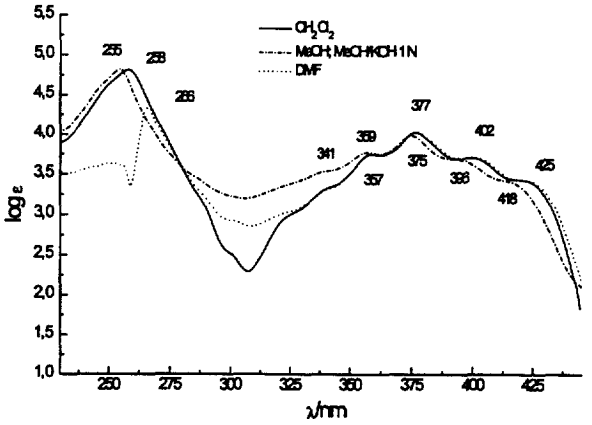


Fig. 5. Absorption spectrum of **6** in CH_2Cl_2 (———) methanol/KOH at pH 14 (- - -) and DMF (···).

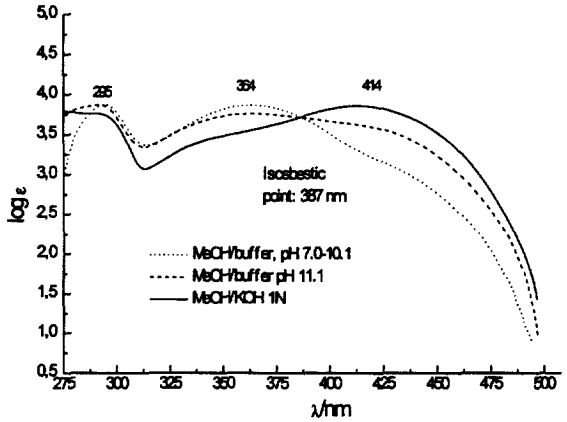


Fig. 6. pH dependent absorption spectrum of **4** in methanol/buffer.

The pK_a value of anthralin was also derived from the appropriate form of the Henderson–Hasselbach equation [21] and is, therefore, referred as pK_{aH}:

$$pK_{aH} = pH + \log\left(\frac{A_i - A}{A - A_m}\right) \quad (1)$$

where A_i is the absorption of the fully ionized species, A that of the observed species at an intermediate pH and A_m the absorption of the pure molecular species. Rearrangement of Eq. (1) gives Eq. (2):

$$-\log Q = pH - pK_{aH} \quad (2)$$

with

$$Q = \left(\frac{A_i - A}{A - A_m}\right) \quad (3)$$

for $Q = 0$ the plot $-\log Q$ vs. pH gives $pH = pK_{aH}$. The pK_a values obtained for anthralin by both methods were in good agreement with each other ($pK_a = 8.3$; $pK_{aH} = 8.2$). All pK_a values are compiled in Table 2.

3.3. Fluorescence of anthralin and related compounds

Raab and Gmeiner [11] found a solution of anthralin in DMF to act as a more potent inhibitor of glucose-6-phosphate dehydrogenase after than before irradiation. The combined action of anthralin and UV light, known as the Ingram method [22], suggests that not only the photosensitizing potency of the anthralin anion [6,7] may play a role in the pharmacological profile of anthralin, but also the formation of a phototautomeric form. Therefore, the pH dependence of the fluorescence spectra was investigated because it can reveal the presence of phototautomers.

Table 2
pK_a values of anthralin and its derivatives, along with data from the literature

Compound	pK_a (this study)	pK_a from literature (Reference)
1	8.3	9.06 [4]; 9.4 [2]
3	9.6	–
4	11.3	–
5	8.6	–
6	>14	–

The fluorescence spectra of anthralin **1** in buffers of varying pH value and in DMF are shown in Fig. 7. The vibrational structure is similar to that of the absorption spectrum of the anionic form **1a** (Fig. 3). Even in pure methanol a weak fluorescence with identical vibrational structure and wavelengths to that in alkaline solution can be measured. The

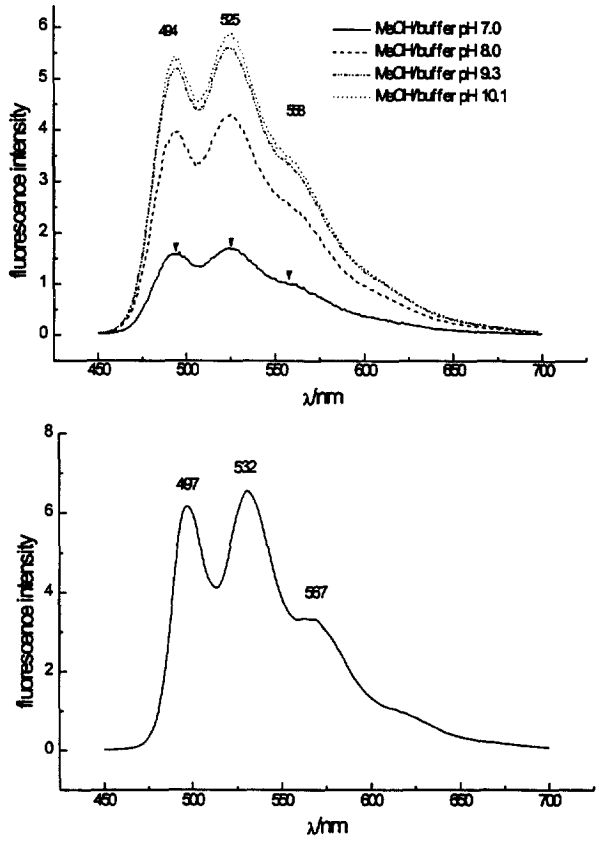


Fig. 7. Top: Fluorescence spectra of anthralin (**1**) at pH 7.0 (———), pH 8.0 (- - -), pH 9.3 (·—·—) and pH 10.1 (···). Bottom: Fluorescence spectrum of anthralin (**1**) in DMF. The fluorescence quantum yield is much higher under these conditions.

fluorescence quantum yields of the main important compound **1a**, upon excitation at 450 nm, which selectively excites the monoanion, reaches 0.1 in alkaline solution and 0.7 in DMF solution, respectively [2]. As in the absorption spectrum, there is also a small longwave shift in DMF solution (Fig. 7). An extraordinarily high Stokes' shift of 120 to 140 nm can be observed. Measurements in methylene chloride, nitromethane and carbon tetrachloride revealed no fluorescence at $\lambda_{exc} = 385$ nm. The only pH dependent effect observable is the increase in fluorescence with the fraction of deprotonated species **1a**.

The monoalkylated derivative **3** shows fluorescence after deprotonation in alkaline medium as well as by deprotonation in DMF. The dimethyl ether **6** shows strong fluorescence under all conditions. There is only a small solvent-dependent shift in the spectrum. The fluorescence was quenched in methanol or strongly alkaline solution.

The monomethyl ether **5**, however, behaves differently. It shows fluorescence already at pH 7.0 with a maximum at 490 nm. On going to pH 8.0, the intensity increases and the maximum is shifted to 528 nm (Fig. 8). This shift of 38 nm is comparable to that in absorption ($\Delta\lambda = 45$ nm). Fluorescence is also observable in methylene chloride or methanol solution. In methylene chloride, the shoulder at 495 nm is absent. In DMF, a very broad band is present with an unexpectedly large Stokes' shift of about 160 nm (Table 1).

4. Discussion

The similarity in the absorption spectra of **1** in methylene chloride or MeOH compared to that of **2** (Fig. 1) suggests a predominantly benzophenone type of structure in these solvents. The additional peaks at 382 and 438 nm reported by Müller et al. [6,7], were not observed in our study. They may have been caused by traces of water in the methanol used, for no additional drying procedure (as in our study) is described. Surprisingly, even in pure methanol a weak fluorescence of identical vibrational structure and wavelengths comparable to those in alkaline solution (Table 1) could be measured, indicating that a small fraction of **1** is deprotonated to form **1a**.

The presence of isosbestic points at 279 and 361 nm [4] is in agreement with the equilibrium of **1** and **1a**. Furthermore, the similarity in the spectra of alkaline solutions and DMF solutions indicates that the aprotic dipolar character of DMF is sufficient for deprotonation of anthralin **1** even in the absence of a base. Further addition of NaOH is reported [3] to have no effect on the spectrum in DMF. The small shift (from 385 nm in methanol to 392 nm in DMF) is due to solvatochromism [6,7].

In order to confirm that C–H acidity at C-10 is unambiguously necessary for the fully aromatic character of the anion, analogous studies with alkylated derivates shown in Scheme 1 were performed. Due to this acidity at C-10, the spectra of compounds **3** and **5** show a benzophenone structure in methylene chloride, but an anthracene character of the anionic forms in DMF or in a strong base. Isosbestic points point toward an equilibrium between **1** and **1a**, including its tautomers. The bathochromic shift in the spectrum of **1a** (392 nm, Scheme 2) in comparison to **6** (377 nm, both in DMF) are further evidence for the anionic structure of **1a**. Compound **4**, which is dialkylated at C-10 and consequently lacks CH-acidity, cannot rearrange. Therefore, it shows no additional splitting in the bathochromically shifted spectra of the anion. The anthracene character of the 1,8-dimethyl ether **6**

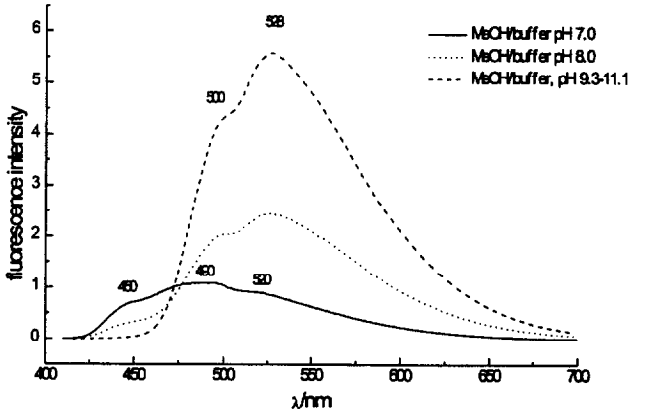
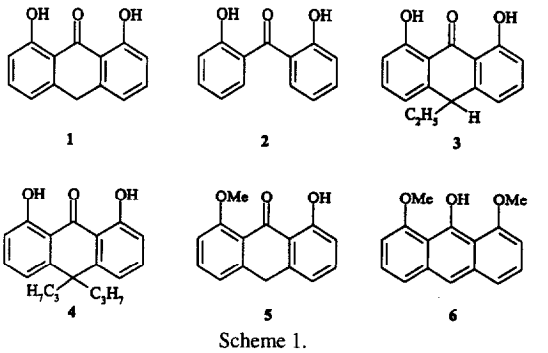
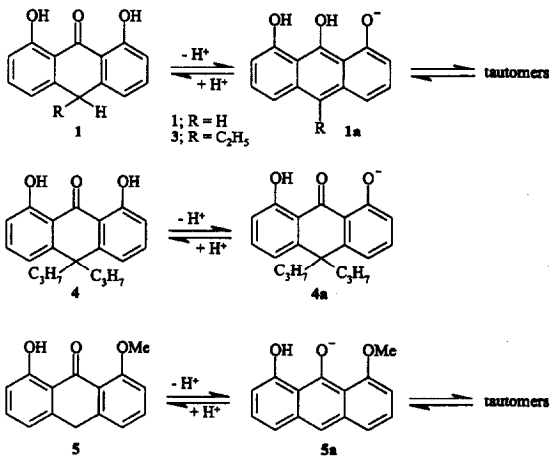


Fig. 8. Emission spectrum of **5** at pH 7.0 (———), pH 8.0 (···) and pH 9.3–11.1 (- - -) when excited at 397 nm. The maximum shifts from 490 to 528 nm. This is accompanied by an increase in emission intensity.



Scheme 1.



Scheme 2.

in any solvent can be explained by the lack of an intramolecular hydrogen bond that would stabilize the corresponding anthrone form.

Due to the presence of two protons in position 10, **1** and **5** are more acidic than the monoalkylated **3**. A substantial decrease in acidity can be observed on dialkylation as in compound **4** (Table 2). Therefore, it is reasonable to assume that the proton at C-10 is responsible for the extraordinary high acidity of **1** and **5**, since intramolecular hydrogen bonding of hydroxy groups normally results in a rather low acidity of phenols [23]. Consequently the pK_a value of compound **4** was determined to be 11.3.

There is a discrepancy in the aqueous pK_a values for anthralin **1**. Others have reported 9.06 [4] and 9.4 [2], respectively. However, the entirely different medium conditions do not allow direct comparison between these values.

As shown in Fig. 7, the emission spectra of anion **1a** are similar, in terms of vibrational structure, to the absorption spectra in alkaline solution. A pH-dependent increase in emission intensity without shift in the fluorescence spectrum indicates that fluorescence arises only from the anionic form **1a** and is not due to excitation of the neutral species **1** [24]. Measurements in methylene chloride, nitromethane, and carbon tetrachloride ($\lambda_{exc} = 385$ nm), however, showed no fluorescence, as should be expected from an aromatic 1,8,9-trihydroxyanthracene phototautomer, which may be formed by intramolecular proton transfer [24]. The large Stokes' shift of 120 to 140 nm (Table 1) in DMF and alkaline solution implicate similar excited state energies for **1a** in these solvents.

Tautomerization of anthralin **1** to the trihydroxyanthracene form might occur in the electronically excited singlet or triplet state. If the excited tautomer triplet has a sufficiently long lifetime, it could be the active species in the light enhanced treatment of psoriasis [22]. Tautomerization in a deuterated protic solvent should lead to efficient H/D exchange. We followed the H/D exchange of **1** in a mixture of deuterated methanol and acetone by NMR spectroscopy. One sample was kept in the dark, whereas another sample was irradiated between the NMR measurements with a high pressure Hg lamp (G 11 Filter, $\lambda < 260$ nm). The amount of light absorbed was measured by an actinometer irradiated for the same time interval and in the same position as the NMR tube. Slow H/D exchange at the C-10 CH_2 occurred in both samples, with no significant difference, a fact that again points to the absence of phototautomerization.

Despite the lack of a shoulder at 382 nm in the absorption spectrum (Fig. 1), a small fraction of deprotonated species is still present in methanol as revealed by the emission spectrum.

Experiments with dialkylated derivative **4** confirm this result. Dialkylation at C-10 suppresses fluorescence, for no aromatization is possible. Compound **6**, which exists in an anthracene structure in any solvent, shows high fluorescence and only a small solvent-dependent shift in its spectra. The fluorescence quenching in alkaline media is in analogy to the fluorescence quenching observed for anthralin monoanion **1a** due to its strong interactions in the first excited singlet state with hydroxy ions leading to non-radiative deactivation [2].

In the mono-methylated form **5** both the protonated and the deprotonated species show fluorescence. The increase in intensity and a bathochromic shift ($\Delta\lambda = 38$ nm) of the fluorescence spectrum of **5** changing the pH from 7.0 to 9.3 (Fig. 8), indicates the presence of the fluorescent neutral and of the anionic form.

5. Conclusion

The earlier proposed pharmacologically active species of the antipsoriatic anthralin in fact is the anthralin anion **1a** [6,7]. Phototautomeric forms of anthralin were not observed. This can be explained by the stabilization of the anthrone form by the hydroxy groups in positions 1 and 8. In the case of **5**, both the neutral and the anionic form are fluorescent, and can be distinguished by their emission maxima. In the cases of **1** and **3** the only fluorescent forms that can be observed are the anionic aromatic forms **1a** and **3a**, respectively. Furthermore, for an rearrangement to the anthracene structure to occur, CH-acidity at C-10 is necessary. The proton at C-10 also seems to be responsible for the relatively high acidity of anthralin derivatives in comparison to those of analogous hydrogen bond chelated phenols such as 2-hydroxybenzophenone or 2,2′-dihydroxybenzophenone (**2**).

Acknowledgements

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