Monatshefte für Chemie 133, 717–721 (2002)

Monatshefte für Chemie
Chemical Monthly

Concerning the Diastereomerization of Stilbenoid Hypericin Derivatives[a]

Bernd Lackner and **Heinz Falk***

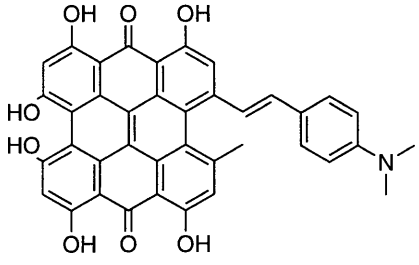
Institute of Chemistry, Johannes Kepler University, A-4040 Linz, Austria

Summary. Experiments on a newly prepared (*E*)-configured ω-benzal-hypericin derivative using TLC and ^{1}H NMR together with quantum chemical calculations revealed that in stilbenoid hypericin derivatives photodiastereomerization between the (*E*)- and (*Z*)-diastereomers occurs in principle. However, due to its low diastereomerization quantum yield and photo and thermal equilibria, which reside mostly on the side of the (*E*)-diastereomer, this photoreaction is only of marginal importance to the photochemistry of stilbenoid hypericin derivatives. Thus, photodiastereomerization does not appreciably interfere with the photoreactions important for photodynamic therapy. This was demonstrated by comparing the sensitized bilirubin photodestruction of hypericin and the ω-benzal-hypericin derivative.

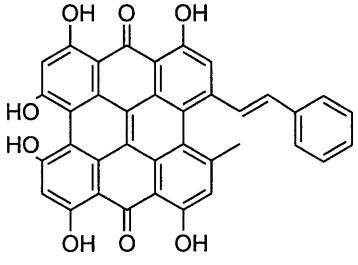
Keywords. Photodynamic therapy; Bilirubin photooxidation; (*Z*)/(*E*)-Diastereomers; ω-Benzal-hypericin.

Introduction

In continuation of our quest for novel hypericin derivatives useful in photodynamic therapy [1] we have recently prepared the promising molecule **1** [2]. It nicely displayed most of the properties desirable for that purpose, like a bathochromic shift of its long wavelength band and a very low fluorescence quantum yield. However, it failed to sensitize the production of singlet oxygen or corresponding reactive oxidative species, which are *inter alia* important for its use in photodynamic therapy [3].



1



2

[a] Dedicated to Prof. *Silvia Braslavsky* on the occasion of her 60th birthday

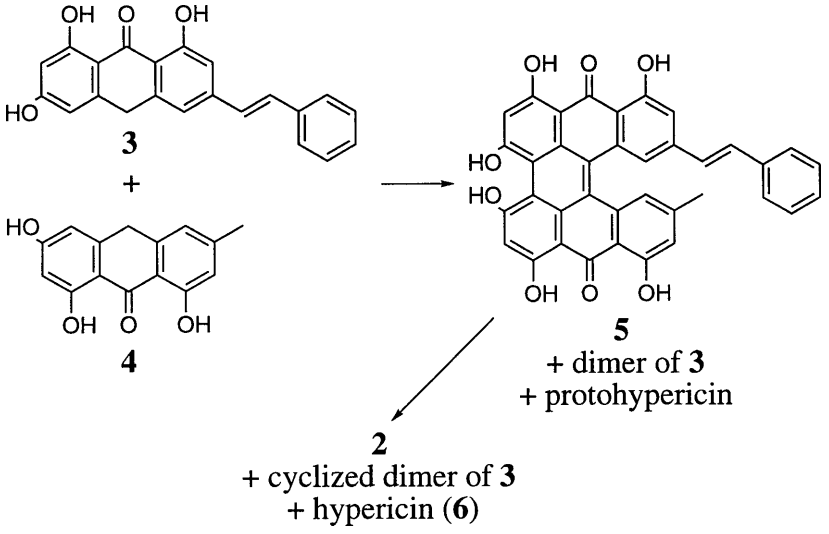
* Corresponding author. E-mail: heinz.falk@jku.at

One reason for this failure was suspected to be based in a photodiastereomerization reaction involving the stilbenoid double bond of **1**, but no indication of such a process has been obtained so far. This could have been due to either a rapid thermal equilibration reaction or the absence of such a photodiastereomerization reaction. To clarify this problem, we now report on investigations of the unsubstituted parent compound, the ω-benzal-hypericin derivative **2**.

Results and Discussions

Synthesis

To prepare **2**, we followed the path established for the synthesis of **1** [2]. When emodin anthrone (**4**) was reacted in six-fold molar excess with the stilbene analogue **3** under the conditions of protohypericin synthesis [4], the heterodimer **5** was formed in optimal relative yield with respect to the dimer of **3** and protohypericin. Photocyclization of the reaction mixture gave a mixture of the target compound **2** with the corresponding cyclized symmetrical dimer of **3** described earlier [1a] and the dimer of **4**, *i.e.* hypericin (**6**). A 9% yield of **2** could be isolated by chromatography. From the rather large coupling constant observed for the two protons of the ethylene fragment ($J \cong 16$ Hz) this diastereomer was assigned the (*E*)-configuration.



Photochemical and thermal properties of **2**

A first indication that photodiastereomerization of (*E*)-**2** is indeed an option of stilbenoid hypericin photochemistry came from a TLC experiment. Using the solvent system petrol ether:methanol = 1:1 on silica, (*E*)-**2** moved with an R_f-value of 0.66. Upon irradiation of a spot of **2** on silica a second, much smaller spot was observed to develop with irradiation time at $R_f = 0.57$. This phenomenon could

also be substantiated for irradiation of homogeneous solutions of **2** in ethanol, and further by using ^{1}H NMR spectroscopy. Thus, (*E*)-**2** was dissolved in ethanol-d_6, and its ^{1}H NMR spectrum was recorded. Upon irradiation, the intensity of the AX system of the ethylene group was rather slowly reduced (as compared with the photodiastereomerization of the (*E*)-diastereomer of stilbene conducted under comparable conditions), and concomitantly a new signal set of low intensity overlapping severely with the signals of the aromatic moiety developed. The relative intensity of the signals did not further change after about 40 minutes irradiation time. Due to severe signal overlap neither the relative intensities (an estimated 10% of (*Z*)-**2**) nor the coupling constant could be measured (an estimated value of about 12 Hz). However, the nature of this new isomer was corroborated to be the photodiastereomer (*Z*)-**2** by its thermal reversion: after warming the photostationary reaction mixture to 55°C for 15 minutes, virtually all of the signals pertaining to the new material were reverted to those of the educt (*E*)-**2**. It should be noted that ES-MS controls attested that the molecular mass signal of **2** remained unchanged in the course of these photochemical and thermal transformations.

Taken together these observations we concluded that the diastereomer (*E*)-**2** undergoes photodiastereomerization to (*Z*)-**2** in rather low quantum yield, resulting in a photoequilibrium mixture which is mostly on the side of the (*E*)-diastereomer. The photodiastereomer (*Z*)-**2** easily reverts back thermally to the educt diastereomer (*E*)-**2**, which thus is obviously strongly favoured from the thermodynamical point of view.

To account for the observed relative thermodynamic stabilities, the two diastereomers of **2** were investigated using the AM1 method [5, 6]. This method has proven to yield results with respect to the steric situation of phenanthroperylene quinones which favourably agreed with experimental findings [7]. Thus, it was calculated that the (*E*)-configured diastereomer of **2** is more stable by $27\,\mathrm{kJ}\cdot\mathrm{mol}^{-1}$ than the corresponding (*Z*)-diastereomer, thereby nicely corroborating the experimental results. The destabilization of the (*Z*)-diastereomer was derived to originate from a higher torsion of the single bonds joining the ethylene double bond ($\theta_{\mathrm{hyp},=} = 51°$, $\theta_{\mathrm{benz},=} = 47°$) due to a higher steric congestion as compared to the (*E*)-diastereomer ($\theta_{\mathrm{hyp},=} = 55°$, $\theta_{\mathrm{benz},=} = 56°$).

The stilbenoid hypericin derivative (*E*)-**2** was quite effective in the hypericin sensitized destruction of bilirubin, which has been established as a rapid means to assess sensitized production of singlet oxygen or reactive oxygen species [8]. As can be derived from Fig. 1, the stilbenoid derivative **2** is nearly as effective as hypericin (**6**) itself. Thus, the low rate of photodiastereomerization only marginally interferes with the singlet oxygen sensitization due to the rather low quantum yields of this photodiastereomerization process. These observations are also in accordance with the fluorescence quantum yield of **2**, which was found to be in the same order of magnitude as that observed for hypericin (**6**).

In conclusion, it turned out that in stilbenoid hypericin derivatives photodiastereomerization is an optional photoreaction. However, due to its low diastereomerization quantum yield and a photo and thermal equilibrium, which resides mostly on the side of the (*E*)-diastereomer, this photoreaction is only of marginal importance in the photochemistry of stilbenoid hypericin derivatives and thus does not appreciably interfere with the photoreactions important for

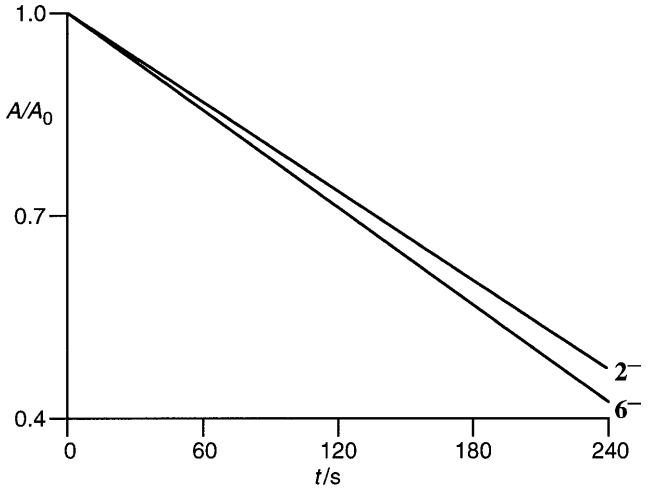


Fig. 1. Hypericin derivative sensitized photooxidation of bilirubin IXα: normalized absorption (A/A_0) *vs.* time curves of solutions of disodium bilirubinate IXα together with sodium hypericinate ($^{(3-)}$**6**) and the sodium salt of the stilbenoid hypericin derivative $^{(3-)}$**2** in aereated 80% EtOH upon irradiation at $\lambda > 570$ nm

photodynamic therapy. Accordingly, stilbenoid or vinylogous homologization would still constitute a proper lead in search of improved hypericin derived phototherapeutic agents. With respect to the dimethylaminobenzal derivative **1** mentioned in the introduction we might conclude that, besides a rather small contribution from photodiastereomerization, a very effective process like intramolecular charge transfer in the excited state prohibits singlet oxygen sensitization in this case.

Experimental

Solvents were of *p.a.* quality. ^{1}H NMR, UV/Vis, fluorescence, and ES mass spectra were recorded using Bruker DRX 500, Hewlett Packard 8453, Hitachi 4010F, and Hewlett Packard 59987 quadrupole instruments. The fluorescence quantum yield was determined as described previously [9]. Hypericin sensitized photooxidation of bilirubinate IXα was executed as described in Ref. [8]. AM1 calculations [5] were performed at the SGI Origin 2000 of the LIZENS using the MOPAC package [6]. The anthrone derivatives **3** and **4** were prepared according to Refs. [1a] and [10].

(E)-10-(2-phenylethen-1-yl)-1,3,4,6,8,13-hexahydroxy-11-methyl-phenanthro[1,10,9,8-opqra]perylen-7,14-dione (**2**; $C_{37}H_{20}O_8$)

A solution of 103 mg **3** (0.3 mmol), 461 mg **4** (1.8 mmol), 30 mg $Fe(SO_4)_2 \cdot 7H_2O$ (0.1 mmol), and 1.076 g pyridine-*N*-oxide (11.3 mmol) in 10.5 cm^3 absolute pyridine was stirred under Ar at 105°C for 1 h. After cooling to room temperature the violet reaction mixture was poured on 75 cm^3 2*M* HCl and stirred for 30 min. After centrifugation the residue was washed twice with 3% HCl, three times with distilled H_2O, and dried overnight *in vacuo* over P_4O_{10} resulting in 976 mg of a mixture of the protodimers and unidentified contaminants. This mixture was dissolved under sonication in 12 dm^3 acetone and irradiated for 15 min by means of a 700 W Hg high pressure lamp with fluorescence

screen (Philips) under stirring and air admission. After evaporation of the solvent the residue was chromatographed on preparative silica TLC plates (*THF*:petrol ether:glacial acetic acid = 7:1:1) and then on a Sephadex LH 20 column (35 × 3 cm) using MeOH as the eluent.

Yield: 16 mg (9%); m.p.: > 310°C; ^{1}H NMR (500 MHz, δ, *DMSO*-d_6): 18.47 (s, OH-3/4), 14.78 (s, OH-1,6 or OH-8,13), 14.17 (s, OH-8,13 or OH-1,6), 8.02 (s, H-9), 7.75 (d, J = 16.5 Hz, C=CH), 7.47 (s, H-12), 7.44 (t, J = 7.3 Hz, MM′ part of AA′MM′X, H_{ph}-3,5), 7.35 (d, J = 7.3 Hz, X-part of AA′MM′X, H_{ph}-4), 7.32 (d, J = 16.5 Hz, HC=C), 7.32 (d, J = 7.6 Hz, AA′-part of AA′MM′X, H_{ph}-2,6), 6.60 (s, H-2 or H-5), 6.59 AA′MM′X (s, H-5 or H-2), 2.74 (s, CH_3) ppm; ^{1}H NMR (500 MHz, δ, EtOH-d_6): 7.82 (s, H-9), 7.58 (d, J = 7.7 Hz, AA′-part of AA′MM′X, H_{ph}-2,6), 7.55 (d, J = 16.3 Hz, C=CH), 7.42 (t, J = 7.7 Hz, MM′-part of AA′MM′X, H_{ph}-3,5), 7.33 (d, J = 8.6 Hz, X-part of AA′MM′X, H_{ph}-4), 7.32 (d, J = 16.3 Hz, HC=C), 7.32 (s, H-12), 6.77 (s, H-2 or H-5), 6.76 (s, H-5 or H-2), 2.69 (s, CH_3) ppm; UV/Vis (80% EtOH): λ_{max} = 609 (6500), 598 (6600), 565 (4000), 329 (7100), 287 (7490) nm (ε); fluorescence (80% EtOH, λ_{exc} = 550 nm): λ_{em} = 617 nm, Φ_f = 0.32; ES-MS (MeOH:H_2O = 4:1 + 1% NH_3, negative ion mode): m/z = 591 ([M–H]$^-$).

Acknowledgments

We gratefully acknowledge the recording of mass spectra by Doz. Dr. *C. Klampfl*.

References

[1] a) Obermüller RA, Hohenthanner K, Falk H (2001) Photochem Photobiol **74**: 211; b) Obermüller RA, Dax TG, Falk H (2001) Monatsh Chem **132**: 1057; c) Obermüller RA, Etzlstorfer C, Falk H (2002) Monatsh Chem **133**: 89
[2] Obermüller RA, Falk H (2001) Monatsh Chem **132**: 1519
[3] For a recent overview of hypericin chemistry see: Falk H (1999) Angew Chem **111**: 3306; Angew Chem Int Ed **38**: 3116
[4] Mazur Y, Bock H, Lavie D (1992) Can Pat Appl CA 2.029,993
[5] Dewar MJS, Zoebisch EG, Healy EF, Stewart JJP (1985) J Am Chem Soc **107**: 3902; Dewar MJS, Dieter KM (1986) J Am Chem Soc **108**: 8075; Stewart JJP (1990) J Comp Aided Mol Design **4**: 1
[6] MOPAC 6.0 DEC-3100 Ed 1990, FJ Seiler Res Lab, USAF Acad CO80840
[7] For AM1 calculations on phenanthroperylene quinones see: Gutman I, Markovic Z, Solujic S, Sukdolak S (1998) Monatsh Chem **129**: 481; Etzlstorfer C, Falk H (1998) Monatsh Chem **129**: 855; Gutman I, Markovic Z (1998) Monatsh Chem **129**: 1019; Etzlstorfer C, Gutman I, Falk H (1999) Monatsh Chem **130**: 1333
[8] Hagenbuchner K, Falk H (1999) Monatsh Chem **130**: 1075
[9] Altmann R, Falk H, Gruber HJ (1998) Monatsh Chem **129**: 235
[10] Falk H, Meyer J, Oberreiter M (1993) Monatsh Chem **124**: 339

Received December 6, 2001. Accepted December 21, 2001