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Syntheses, Constitutions and Properties of Stentorin and Isostentorin

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Summary. Stentorin and Isostentorin were synthesized from 2-isopropyl-1,3,6,8-tetrahydroxyanthrone by dimerization and chromatographic separation of the resulting regioisomers. The anthrone was prepared in four steps starting from easily available properly substituted benzene derivatives; the overall yield of the stentorins was 11%. The constitutions of stentorin and isostentorin could be unequivocally assigned from the ^{1}H NMR spectra of their potassium salts and were found to be in agreement with those derived recently by means of a rational synthesis. The spectroscopic, dissociation, and acid-base properties in ground and excited states as well as the chiroptical properties of the human serum albumin complexes were investigated and discussed comparing them with respective data of hypericin, fringelite D, and the natural *Stentor* pigment.

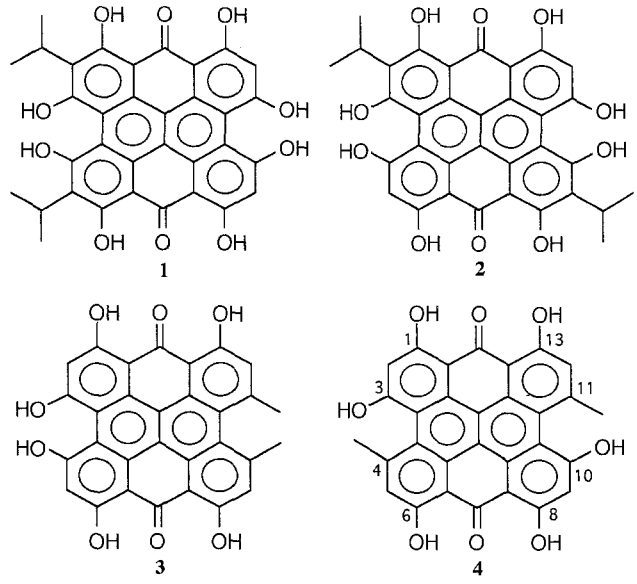
Keywords. Stentorin; Isostentorin; Synthesis; Dissociation; Protonation; Deprotonation; Spectroscopic properties; Association.

Synthese, Konstitution und Eigenschaften von Stentorin und Isostentorin

Zusammenfassung. Stentorin und Isostentorin wurden ausgehend von 2-Isopropyl-1,3,6,8-tetrahydroxyanthron durch Dimerisierung und anschließende Chromatographie der Regioisomeren dargestellt. Das Anthron wurde in vier Schritten aus einfach zugänglichen, entprechend funktionalisierten Benzolderivaten erhalten; die Gesamtausbeute der Stentorine betrug dabei 11%. Die Konstitutionen von Stentorin und Isostentorin wurden zweifelsfrei aus den ^{1}H NMR Spektren der Kaliumsalze abgeleitet und waren in Übereinstimmung mit einem früher erzielten Ergebnis einer Synthesestudie. Die spektroskopischen, Dissoziations- und Säure-Basen-Eigenschaften in Grund- und angeregtem Zustand, sowie die chiroptischen Eigenschaften des Human-Serumalbuminkomplexes wurden untersucht und diskutiert und mit den entsprechenden Daten von Hypericin, Fringelit D und dem natürlichen *Stentor*-Pigment verglichen.

Introduction

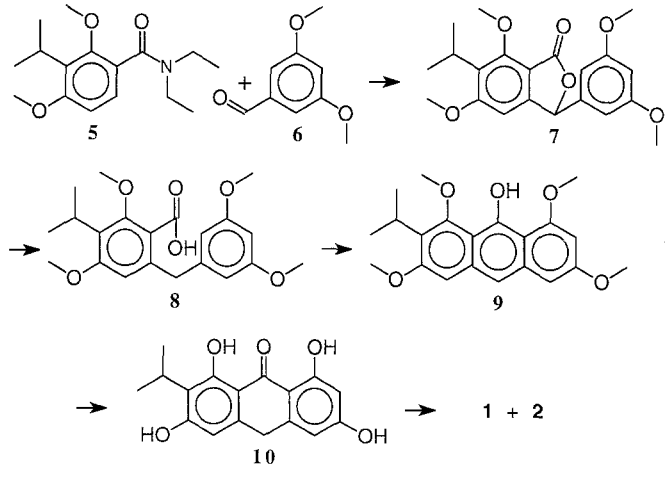
The photoreceptor pigment of the step-up photophobic and negative phototactic responses of the heterotrich protozoan *Stentor coeruleus* has been isolated in pure form [1]. It has been assigned the constitution of stentorin (**1**) or isostentorin (**2**) by means of ^{1}H NMR and mass spectrometry [1]. A differentiation between the two isomers **1** and **2** has been reached recently in favor of **1** by means of ingenious regioselective syntheses [2].



At the time the mentioned synthesis [2] has been published we just had accomplished an approach along the lines developed for efficient syntheses of hypericin (**3**), isohypericin (**4**) [3], and the non-alkylated parent system of **1** and **2**, fringelite D [4]. As several interesting physical and chemical properties of **1** and **2** still awaited a thorough study, we thought it to be worthwhile to report on our respective investigations.

Results and Discussion

Synthesis of Stentorin and Isostentorin



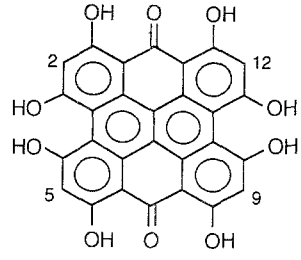
Following the lines of the previously reported syntheses of **3**, **4**, and fringelite D [3, 4], the benzamide **5** was regioselectively *ortho*-lithiated and reacted with the

commercially available aldehyde **6** to provide the lactone **7** in 40% yield. This reaction proved to be more efficient using *tert*-butyl lithium instead of the recently used *sec*-butyl lithium. The lactone was reductively ring-opened to the acid **8** (86%) by means of catalytic hydrogenation over palladium on charcoal. Upon treatment with trifluoroacetic anhydride in trifluoroacetic acid, **8** was smoothly cyclyzed in 78% yield to the anthranole **9** which proved to be the more stable tautomer – for analogous derivatives compare Refs. [3, 4]. The latter was demethylated by means of hydroiodic acid in acetic acid to yield almost quantitatively the anthrone **10**. Oxidative coupling of **10** and photocyclization in the usual manner [3, 4] resulted in a mixture of **1** and **2** with an overall yield of 11% **1** + **2** (about 1:1) based on **5**. This mixture could be easily separated by preparative thin layer chromatography yielding pure **1** and **2** in about equal amounts.

The monocyclic starting material **5** of this synthesis could be prepared along established procedures. Thus, 2,6-dimethoxy-isopropylbenzene, which was accessible by a three step synthesis from the commerically available 2,6-dimethoxybenzaldehyde [5], provided by means of a *Vilsmeier* formylation almost quantitatively 2,4-dimethoxy-3-isopropyl-benzaldehyde. The latter was oxidized to the corresponding 2,4-dimethoxy-3-isopropyl-benzoic acid in 68% yield using potassium permanganate. Conversion of this acid into its acid chloride and reaction with diethylamine finally provided **5** in 88% yield.

Properties of Stentorin and Isostentorin

The constitutions of **1** and **2** followed from the synthesis path and their FAB mass spectra as those of two fringelites D (**11**) substituted with isopropyl groups in positions 2,5 or 2,9.



11

The ^{1}H NMR spectra of these two compounds were found to be similar but displayed significantly different chemical shifts of the two aromatic protons (6.89 and 6.80 ppm). The constitutional regioisomers **1** and **2** could be assigned unequivocally from the ^{1}H NMR spectra of their potassium salts. As indicated in Fig. 1, the two protons (**H** and ***H***) of the anionic bay region in **1**·2 K^+ were found to be non identical resonating at 17.59 and 16.69 ppm. This is due to the averaged localization of the two protons *within* the formal C_2 symmetry axis of the molecule. In **2**·2 K^+, these two protons (ℍ) became identical (17.3 ppm) due to their homotopic positions *off* the formal C_2 symmetry axis. It should be noted that in the corresponding hypericin and fringelite D salts the exchange between the two positions of a bay region (*e.g.* 3,4) has been found to be fast compared to the NMR time scale [4].

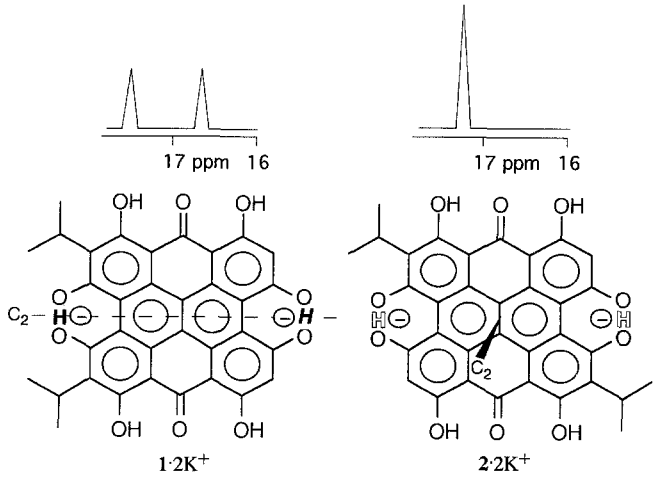


Fig. 1. Schematic partial ^1H NMR spectra (*DMSO*-d_6) and formulae of the potassium salts of **1** and **2**

The spectroscopic properties of **1** synthesized along the route described above matched those reported for natural stentorin [1], and both constitutional isomers **1** and **2** corresponded to those prepared recently on selective routes [2]. Accordingly, an independent proof of the constitution of **1** has now been provided.

The preponderant presence of the 7,14-dioxo tautomers out of the 15 possible ones of **1** and **2** followed from the symmetry derived from the ^1H NMR spectra and a comparison with the results on **3**, **4** and **11** [4]. This finding was in agreement with the results of a recent molecular modeling investigation [6], from which it has been deduced that the 7,14- tautomers of **1** and **2** should be more stable than the next stable ones by about 50 kJ/mol.

The absorption spectra of **1** and **2** (or more precisely, for dilute solutions of their dissociated forms) were found to be similar with long wavelength bands at 600 nm (ethanol). Thus, they are shifted slightly bathochromically with respect to their parent compound **11** (596 nm, ethanol [4]). This small shift between **1** and **2** on the one hand and **11** on the other hand pointed to very tiny differences in the torsional deformation of the chromophoric systems. Moreover, the virtually identical spectra of **1** and **2** indicated that the isopropyl groups exert only a very limited influence on the torsional deformation of the condensed ring system. Indeed, it has been found from molecular modeling studies that the two "bay sides" of **1** are twisted to much the same extent, regardless of the presence of a neighboring isopropyl group ($\Theta_{3,3a,3b,4} = 32.8°$ and $\Theta_{10,10a,10b,11} = 31.1°$ [6]). The spectra of **1** and **2** were found to be only slightly solvent shifted, as were the emission data. It should be kept in mind, however, that similar to the situation found for **11** [4] the absorption spectra are concentration dependent due to dissociation equilibria. Comparable to the parent compound **11**, **1** and **2** displayed *Stokes* shifts of about 9 nm with fluorescence quantum yields in the order of 0.6. These data varied only slightly with different solvents.

The dissociation, protonation, and deprotonation behavior of **1** and **2** resembled those of the fringelite parent system **11**. As summarized in Fig. 2, the protonation of the carbonyl systems was achieved using rather concentrated sulfuric acids in the region of about $pK_a = -5$ for all three compounds. This indicated that the carbonyl protonation was independent of the alkyl substitution. The first deprotonation step

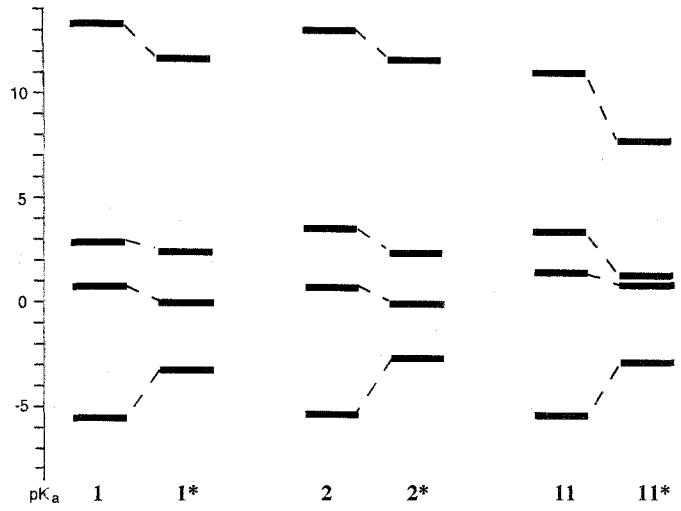
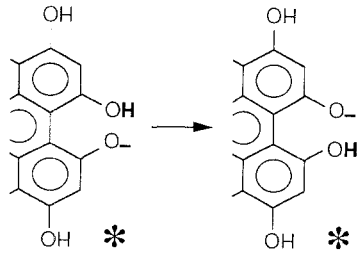


Fig. 2. Protonation and deprotonation pK_a values in ground (**1**, **2**, **11**) and excited (**1***, **2***, **11***) states

was found to be the same for **1** and **2**, but **11** has been found to be a little less acidic. The second deprotonation step was found to be different in all three compounds with **2** as the weakest acid. Although these differences were found to be significant, due to the rather small absolute difference values we refrain from an interpretation. The third deprotonation step was found to be considerably retarded in the stentorins.

With respect to the natural pigment system in *Stentor* it may be inferred from these results that, due to its low pK_a values, **1** would be partially dissociated in the native state. Due to its higher acidity in its excited states, it could easily transfer a proton to an external acceptor system upon photoexcitation as has been discussed by *Song* [7]. Intramolecular excited state proton transfer to the carbonyl system, as has been discussed for **3** [8], would be highly improbable due to the low basicity of the carbonyl group even in the excited state – for the similar base properties of the parent compound, the unsubstituted phenanthroperylene-7,14-dione, see Ref. [9]. Note, however, that excited state intramolecular proton transfer would be a proper path for the dissociated species of **1**–**4** and **11** between the strongly acidic -**OH** and the strongly basic -O^- in the bay regions as indicated in Scheme 1! This type of transfer could provide an explanation for the fast excited state processes observed for hypericin solutions, instead of attributing them to an improbable photo-tautomerization process in the *peri* region [8, 10]. This type of excited state bay proton transfer has also been proposed recently as the mechanism of photochemical hole burning [11].



Scheme 1

With respect to association, the anions of **1** and **2** were found to homoassociate comparable to those of **3** and **11** [4, 12] which of course, was interesting with respect to the non-covalent association of stentorin to an apoprotein in the natural pigment system [7]. Heteroassociation of **1** and **2** to human serum albumin was found to be feasible as has been studied in the case of **3** [11, 12]. They were readily displaced by addition of **3** and thus shown to be weaker bound to the subdomaine *IIIA* of the albumin. The two pigments displayed the absorption and emission characteristics of the doubly ionized species. Ambient temperature fluorescence excitation polarization spectra of the complexes of **1** and **2** comparable to the one observed for the human serum albumin complex of **3** [12] could be recorded in both cases, indicating strong binding between pigments and protein. Whereas the serum albumin complex of **1** exhibited chiroptical signals ($\Delta\varepsilon_{605} = -1.8 \pm 0.1$) of the long wavelength band, that of **2** displayed only marginal optical activity ($\Delta\varepsilon_{599} = -0.2 \pm 0.2$). Compared to the CD of the complex of **3**, this meant a tenfold decrease in optical activity in the case of the complex of **1** ($\Delta\varepsilon_{600} = +19$) and is thus hardly amenable to an interpretation with respect to inherently chiral chromophores. As for the complexes of **2** and **11** [4], this result could either point to a nearly racemic mixture of the two enantiomeric propeller conformers [6] associated to the protein or a chirally perturbed inherently achiral double butterfly conformer. With respect to the natural stentorin system, rather strong CDs have been observed [7, 13], favouring the first argumentation, *i.e.* the stabilization of a nearly racemic mixture of the enantiomeric propeller conformers.

Note added in proof: An additional regioselective synthesis of stentorin has just been published [Iio H, Zenfukul K, Tokoyorama T (1995). Tetrahedron Lett **36**: 5921]. Up to compound **9**, the authors mainly use the strategy given by us recently [3] and in the present paper. Regioselectivity was achieved *via* bromination and *Ullmann* coupling.

Experimental

Melting points were measured by means of a Kofler hot stage microscope (Reichert, Vienna). ^1H, ^{13}C, IR, UV/Vis, and fluorescence spectra were recorded using Bruker-WM-360 and AC-200, Biorad-FT-IR-45, Perkin-Elmer IR-710B, Hitachi-U-3210, and F-4010 instruments. 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 ($\Phi_f = 0.69$) was used as a standard. CD spectra were run on an ISA Mark VI instrument. The pK_a and pK_a^* values of **1** were determined spectro-photometrically using *DMSO*/H_2O mixtures and tetrabutylammonium hydroxide as the base [14] and by means of *Förster* cycle calculations [15]. A series of aqueous sulfuric acids of known H_0 values was used to determine the protonation pK_a and pK_a^* values [16].

1,3,4,6,8,10,11,13-Octahydroxy-2,5-diisopropyl-phenanthro[1,10,9,8,o,p,q,r,a]perylene-7,14-dione (*Stentorin*, **1**; $C_{34}H_{24}O_{10}$) and *1,3,4,6,8,10,11,13-Octahydroxy-2,9-diisopropyl-phenanthro[1,10,9,8, o,p,q,r,a]perylene-7,14-dione* (*Isostentorin*, **2**; $C_{34}H_{24}O_{10}$)

106 mg of **10** (353 μmol) were mixed with 2 ml pyridine p.a., 0.2 ml piperidine, 200 mg pyridine-N-oxide, and 5 mg $FeSO_4 \cdot 7H_2O$. After stirring this mixture for 90 min at 110 °C under argon and exclusion of light it was cooled down to ambient temperature and acidified with 10 ml 3% HCl. After settling for 1 h at 4 °C, the black precipitate was centrifuged and washed twice with water. Then the precipitate was dissolved in 400 ml acetone and irradiated for 3 h with a Philips type-G/7412 (700 W) UV lamp. After

filtration and evaporation of the solvent, the residue was first chromatographed on silica with MeOH as the solvent and afterwards for a second time on silica with $CHCl_3$/MeOH = 10/1 to 0/1. This procedure provided 42 mg of a mixture of **1** + **2** (40%). Based on their R_f values of 0.34 (**1**) and 0.43 (**2**) on silica with $CHCl_3$/MeOH = 2/1, they could be separated by preparative TLC yielding 20 mg of each compound (19%).

1·2 K^+ (prepared by titration of **1** with 2 equivalents KOH in MeOH and evaporation of the solvent): m.p.: not below 320 °C; ^{1}H NMR (200 MHz, δ, *DMSO*-d_6): 17.59, 16.69 (2 br s, OH-3 or 4 and OH-10 or 11), 15.46, 14.93 (2 br s, OH-1, 6 and OH-8, 13), 6.57 (s, H-9, 12), 4.03 (septet, J = 6.9 Hz, 2CH), 1.48 (d, J = 6.2 Hz, 4CH_3) ppm; ^{13}C spectra could not be obtained due to solubility limitations.

1 (by ether extraction of the potassium salt with 3% HCl): m.p.: not below 320 °C; ^{1}H NMR (360 MHz, δ, *DMSO*-d_6): 15.35, 14.70 (2 br s, OH-1, 6 and OH-8, 13), 6.89 (s, H-9, 12), 4.02 (septet, J = 6.9 Hz, 2CH), 1.48 (d, J = 6.9 Hz, 4CH_3) ppm; UV/Vis (EtOH, $c = 10^{-5}$ mol/l): λ_{max} = 600 (23730), 556 (11360), 519 (4220), 453 (8080), 345 (20640), 293 (22910) nm (ε); UV/Vis (MeOH, $c = 10^{-5}$ mol/l): λ_{max} = 598 (24200), 554 (11740), 516 (4410), 452 (8270), 344 (20950), 293 (23120) nm (ε); UV/Vis (MeCN, $c = 10^{-5}$ mol/l): λ_{max} = 610 (21180), 564 (10500), 526 (4000), 444 (7040) nm (ε); UV/Vis (acetone, $c = 10^{-5}$ mol/l): λ_{max} = 614 (21040), 567 (10270), 529 (3980), 442 (6900) nm (ε); UV/Vis (*DMF*, $c = 10^{-5}$ mol/l): λ_{max} = 616 (23030), 570 (10960), 531 (4150), 445 (7310) nm (ε); UV/Vis (*DMSO*, $c = 10^{-5}$ mol/l): λ_{max} = 616 (19920), 570 (9500), 531 (3600), 448 (6650) nm (ε); UV/Vis (pyridine, $c = 10^{-5}$ mol/l): λ_{max} = 679 (770), 617 (22900), 570 (10740), 448 (7320) nm (ε); UV/Vis (*THF*, $c = 10^{-5}$ mol/l): λ_{max} = 674 (8690), 582 (12790), 541 (6830), 504 (3650), 459 (8230), 384 (9070), 334 (17020) nm (ε). Fluorescence (MeOH, 298 K, λ_{exc} = 550 nm): λ_{em} = 607 (1), 652 (0.33) nm (rel. intensity); Φ_f = 0.58; Fluorescence (EtOH, 298 K, λ_{exc} = 550 nm): λ_{em} = 609 (1), 654 (0.32) nm (rel. intensity); Φ_f = 0.58; Fluorescence (MeCN, 298 K, λ_{exc} = 550 nm): λ_{em} = 620 (1), 665 (0.33) nm (rel. intensity); Φ_f = 0.57; Fluorescence (acetone, 298 K, λ_{exc} = 550 nm): λ_{em} = 622 (1), 671 (0.29) nm (rel. intensity); Φ_f = 0.63; Fluorescence (*DMF*, 298 K, λ_{exc} = 550 nm): λ_{em} = 624 (1), 674 (0.29) nm (rel. intensity); Φ_f = 0.65; Fluorescence (*THF*, 298 K, λ_{exc} = 550 nm): λ_{em} = 606 (1), 650 (0.33) nm (rel. intensity); Φ_f = 0.49; Fluorescence (pyridine, 298 K, λ_{exc} = 550 nm): λ_{em} = 626 (1), 677 (0.28) nm (rel. intensity); Φ_f = 0.61; Fluorescence (*DMSO*, 298 K, λ_{exc} = 550 nm): λ_{em} = 625 (1), 675 (0.29) nm (rel. intensity); Φ_f = 0.65. pK_a-Determinations: protonation: λ_1 = 596 nm, $\lambda_{1.H^+}$ = 635 nm, $\varepsilon_\lambda/\varepsilon_{\lambda H^+}$ = 0.12, pK_a(p) = -5.5 ± 0.2, pK_a^*(p) = -3.3 ± 0.2; deprotonation (80% *DMSO*): λ_1 = 588 nm, λ_{1^-} = 604 nm, $\varepsilon_\lambda/\varepsilon_\lambda$ = 0.74; $pK_a(d^1) = 0.8 \pm 0.5$, $pK_a^*(d^1) = -0.1 \pm 0.5$; $\lambda_{1^{2-}}$ = 614 nm, $\varepsilon_\lambda/\varepsilon_{\lambda^{2-}}$ = 0.50; $pK_a(d^2) = 2.9 \pm 0.4$; $pK_a^*(d^2) = 2.04 \pm 0.4$. Using the H_2O-*DMSO*-tetrabutylammonium hydroxide system [14]: $\lambda_{1^{2-}}$ = 616 nm, $\lambda_{1^{3-}}$ = 650 nm, $pK_a(d^3) = 13.5 \pm 0.2$, $pK_a^*(d^3) = 11.7 \pm 0.2$. Fluorescence (80% *DMSO*): **1**: 593 (1), 638 (0.32), Φ_f = 0.57; **1**$^-$: 614 (1), 656 (0.41), Φ_f = 0.46; **1**$^{2-}$: 624 (1), 674 (0.29) Φ_f = 0.58. Using the H_2O-*DMSO*- tetrabutylammonium hydroxide system [14]: **1**$^{3-}$: 692, Φ_f = 0.33. MS (FAB; glycerol): *m/e* (%) = 594.15 (65), 593.14 (100), 592.14 (30; M^+), 591.14 (14), 577.14 (31), 576.14 (12), 561.12 (9).

The human serum albumin complex of **1** was prepared in analogy to that of **3** [12] in 0.1 *M* aqueous phosphate buffer of *pH* 7.0, $c = 1.6.10^{-5}$ *M* by mixing solutions of HSA (Sigma) and **1**·2 K^+. UV/Vis: 605 (1), 560 (0.87), 466 (1.27), 446 (1.33) nm (rel. intensity). Fluorescence (λ_{exc} = 550 nm): 613 (1), 657 (0.37) nm (rel. intensity); Fluorescence-excitation (λ_{em} = 650 nm): 607 (1), 563 (0.41), 526 (0.15), 465 (0.14), 349 (0.16) nm (rel. intensity); Fluorescence polarization spectrum: 650 − 520 (+1), 450 (−0.2), 380 (+0.1) 355 (−0.1) nm (relative intensity). CD: 605 (−1.8), 591 (+0.4), 564 (−0.2), 487 (−1.4), 452 (−2.4), 349 (−2.4) nm ($\Delta\varepsilon$). Addition of one equivalent of a **3**. K^+ solution to the solution of the HSA-**1** complex [12] resulted in the complete replacement of the chiroptical signals of **1** by those of the HSA-**3** complex.

2·2 K^+ (prepared by titration of **1** with 2 equivalents KOH in MeOH and evaporation of the solvent): m.p.: not below 320 °C; ^{1}H NMR (200 MHz, δ, *DMSO*-d_6): 17.13 (br s, OH-3 or 4 and OH-10 or 11), 15.48, 14.91 (2 br s, OH-1, 8 and OH-6, 13), 6.60 (s, H-5, 12), 4.02 (septet, J = 6.4 Hz, 2CH), 1.47 (d, J = 6.4 Hz, 4CH_3) ppm; ^{13}C spectra could not be obtained due to solubility limitations.

2 (by ether extraction of the potassium salt with 3% HCl): m.p.: not below 320 °C; ^{1}H NMR (360 MHz, δ, *DMSO*-d_6): 15.33, 14.73 (2 br s, OH-1, 8 and OH-6, 13), 6.80 (s, H-5, 12), 4.01 (septet, $J = 7.1$ Hz, 2CH), 1.48 (d, $J = 7.1$ Hz, $4CH_3$) ppm; UV/Vis (EtOH, $c = 5.10^{-6}$ mol/l): $\lambda_{max} = 600$ (24280), 556 (12540), 518 (5540), 456 (10100), 345 (23280), 293 (29120) nm (ε); UV/Vis (MeOH, $c = 5.10^{-6}$ mol/l): $\lambda_{max} = 598$ (20000), 547 (11180), 506 (5600), 457 (8940), 334 (19640), 290 (28460) nm (ε); UV/Vis (MeCN, $c = 5.10^{-6}$ mol/l): $\lambda_{max} = 609$ (21280), 565 (11200), 527 (4800), 451 (7920) nm (ε); UV/Vis (acetone, $c = 5.10^{-6}$ mol/l): $\lambda_{max} = 671$ (2960), 600 (17920), 556 (9980), 514 (5280), 438 (7740) nm (ε); UV/Vis (*DMF*, $c = 5.10^{-6}$ mol/l): $\lambda_{max} = 616$ (22960), 570 (11780), 532 (5180), 449 (8860) nm (ε); UV/Vis (*DMSO*, $c = 10^{-5}$ mol/l): $\lambda_{max} = 616$ (20360), 570 (9910), 530 (3940), 450 (6940) nm (ε); UV/Vis (pyridine, $c = 5.10^{-6}$ mol/l): $\lambda_{max} = 618$ (19360), 571 (9560), 533 (3960), 452 (7020) nm (ε); UV/Vis (*THF*, $c = 5.16^{-6}$ mol/l): $\lambda_{max} = 584$ (18820), 541 (9860), 504 (4700), 464 (8020), 334 (19800) nm (ε). Fluorescence (EtOH, 298 K, $\lambda_{exc} = 550$ nm): $\lambda_{em} = 610$ (1), 655 (0.31) nm (rel. intensity); $\Phi_f = 0.61$; Fluorescence (MeOH, 298 K, $\lambda_{exc} = 550$ nm): $\lambda_{em} = 598$ (1), 643 (0.33) nm (rel. intensity); $\Phi_f = 0.58$; Fluorescence (MeCN, 298 K, $\lambda_{exc} = 550$ nm): $\lambda_{em} = 619$ (1), 665 (0.33) nm (rel. intensity); $\Phi_f = 0.57$; Fluorescence (acetone, 298 K, $\lambda_{exc} = 550$ nm): $\lambda_{em} = 622$ (1), 671 (0.30) nm (rel. intensity); $\Phi_f = 0.61$; Fluorescence (*DMF*, 298 K, $\lambda_{exc} = 550$ nm): $\lambda_{em} = 624$ (1), 674 (0.29) nm (rel. intensity); $\Phi_f = 64$; Fluorescence (*THF*, 298 K, $\lambda_{exc} = 550$ nm): $\lambda_{em} = 611$ (1), 658 (0.32) nm (rel. intensity); $\Phi_f = 0.59$; Fluorescence (pyridine, 298 K, $\lambda_{exc} = 550$ nm): $\lambda_{em} = 626$ (1), 676 (0.28) nm (rel. intensity); $\Phi_f = 0.62$; Fluorescence (*DMSO*, 298 K, $\lambda_{exc} = 550$ nm): $\lambda_{em} = 625$ (1), 675 (0.29) nm (rel. intensity); $\Phi_f = 0.64$. pK_a-Determinations: protonation: $\lambda_1 = 595$ nm, $\lambda_{1\cdot H^+} = 638$ nm, $\varepsilon_\lambda/\varepsilon_{\lambda H^+} = 0.20$, $pK_a(p) = -5.1 \pm 0.6$, $pK_a^*(p) = -2.7 \pm 0.6$; deprotonation (80% *DMSO*): $\lambda_1 = 584$ nm, $\lambda_1^- = 600$ nm, $\varepsilon_\lambda/\varepsilon_\lambda^- = 0.78$; $pK_a(d^1) = 0.8 \pm 0.1$, $pK_a^*(d^1) = -0.2 \pm 0.1$; $\lambda_1^{2-} = 615$ nm, $\varepsilon_\lambda/\varepsilon_\lambda^{2-} = 0.73$; $pK_a(d^2) = 3.5 \pm 0.4$, $pK_a^*(d^2) = 2.7 \pm 0.4$. Using the H_2O-*DMSO*-tetrabutylammonium hydroxide system [14]: $\lambda_1^{2-} = 622$ nm, $\lambda_1^{3-} = 649$ nm, $pK_a(d^3) = 13.0 \pm 0.2$, $pK_a^*(d^3) = 11.6 \pm 0.2$. Fluorescence (80% *DMSO*) **1**: 608 (1), $\Phi_f = 0.51$; $\mathbf{1}^-$: 611 (1), 654 (0.36), $\Phi_f = 0.52$; $\mathbf{1}^{2-}$: 623 (1), 672 (0.30), $\Phi_f = 0.63$. Using the H_2O-*DMSO*-tetrabutylammonium hydroxide system [14]: $\mathbf{1}^{3-}$: 670, $\Phi_f = 0.28$. MS (FAB; glycerol): *m/e* (%) = 594.15 (65), 593.15 (100), 592.15 (42; M^+), 591.13 (25), 577.09 (17), 561.11 (14).

The human serum albumin complex of **2** was prepared in analogy to that of **3** [12] in 0.1 *M* aqeuous phosphate buffer of *pH* 7.0, $c = 1.6.10^{-5}$ *M* by mixing solutions of HSA (Sigma) and $\mathbf{2}\cdot 2\,K^+$. UV/Vis: 606 (1), 562 (0.64), 517 (0.45), 466 (0.77), 446 (0.77) nm (rel. intensity); Fluorescence ($\lambda_{exc} = 550$ nm): 612 (1), 658 (0.32) nm (rel. intensity); Fluorescence-excitation ($\lambda_{em} = 650$ nm): 606 (1), 563 (0.38), 526 (0.11), 467 (0.14), 350 (0.15) nm (rel. intensity); Fluorescence polarization spectrum: 650 – 520 (+1), 450 (−0.2), 380 (+0.1) 355 (−0.1) nm (relative intensity). CD: 599 (−0.2), 480 (−1.6), 456 (−1.7), 349 (−0.8) nm ($\Delta\varepsilon$). Addition of one equivalent of a $\mathbf{3}\cdot K^+$ solution to the solution of the HSA-**2** complex [12] resulted in the complete replacement of the chiroptical signals of **2** by those of the HSA-**3** complex.

2,4-Dimethoxy-3-isopropyl-benzaldehyde ($C_{12}H_{16}O_3$)

1.52 ml N-Methyl-formanilide (1.67 g, 12.4 mmol) and 1.13 ml $POCl_3$ (1.90 g, 12.4 mmol) were stirred for 45 min at ambient temperature and exclusion of moisture. Then, 2.23 g 2,6-dimenthoxy- isopropyl-benzene (prepared according to Ref. [5]) were dropped into this mixture at a velocity which allowed for a temperature rise up to maximal 35 °C. The reaction mixture was stirred for 1 h at ambient temperature, 2 h at 60 °C, and then set aside overnight at room temperature. The reaction mixture was poured into iced water and brought to *pH* 9 by means of 5% NaOH. Extraction with ether, washings with 1% HCl, H_2O, and lime, and drying with Na_2SO_4 provided a dark yellow oil after evaporation of the solvent. Chromatography over a silica column with ethyl acetate: *n*-hexane = 1:9 as the eluent yielded 2.465 g (96%) of a light yellow oil. ^{1}H NMR (200 MHz, δ, $CDCl_3$): 10.20 (s, CHO), 7.73 (d, $J = 8.7$ Hz, H-6), 6.75 (d, $J = 8.7$ Hz, H-5), 3.89 (s, OCH_3), 3.85 (s, OCH_3), 3.51 (septet, $J = 7.1$ Hz, CH), 1.32 (d, $J = 7.1$ Hz, $2CH_3$) ppm; ^{13}C NMR (50 MHz, δ, $CDCl_3$): 188.8 (CHO), 164.5, 161.7, 129.2 (C-1,2,4), 126.1 (C-6), 122.6 (C-3), 107.4 (C-5), 64.2, 55.2 ($2OCH_3$), 24.5 (CH), 20.3 ($2CH_3$) ppm; IR (KBr): $\nu = 1670$, 1590, 1460 cm^{-1}; MS (70 eV, 40 °C): *m/e* (%) = 209 (30), 208 (40; M^+), 194 (13), 193 (100), 180

(10), 175 (11), 165 (36), 163 (14), 150 (8), 147 (6), 135 (11), 121 (6), 107 (9), 105 (19), 103 (10), 91 (20), 89 (9), 83 (9), 79 (14), 77 (21), 65 (10), 53 (8), 51 (11).

2,4-Dimethoxy-3-isopropylbenzoic acid ($C_{12}H_{16}O_4$)

2.92 g of the above aldehyde (14 mmol) were dissolved in 15 ml acetone (p.a.); after addition of 2.2 g $KMnO_4$ the mixture was stirred for 16 h at ambient temperature. After addition of 1 g $KMnO_4$ (a total of 20 mmol), the mixture was stirred for an additional h, filtered, and acidified with 5% HCl. Extraction with ether, washings with H_2O and brine, and drying with Na_2SO_4 afforded after evaporation of the solvent the crude product. It was crystallized from $EtOH/H_2O$ to yield 2.14 g (68%) white crystals, m.p.: 153–154 °C. ^1H NMR (200 MHz, δ, $CDCl_3$): 11.20 (br s, COOH), 7.99 (d, $J = 9.0$ Hz, H-6), 6.78 (d, $J = 9.0$ Hz, H-5), 3.89 (s, OCH_3), 3.87 (s, OCH_3), 3.44 (septet, $J = 7.1$ Hz, CH), 1.34 (d, $J = 7.1$ Hz, $2CH_3$) ppm; ^{13}C NMR (50 MHz, δ, $CDCl_3$): 168.4 (COOH), 163.9, 158.9, 131.6, 114.6 (C-1,2,3,4), 129.7 (C-6), 107.5 (C-5), 63.3, 55.4 ($2OCH_3$), 25.0 (CH), 20.5 ($2CH_3$) ppm; IR (KBr): 2990, 1690, 1670, 1590 cm^{-1}. MS (70 eV, 55 °C): *m/e* (%) = 224 (25; M^+), 209 (25), 191 (100), 149 (9), 135 (31), 105 (42), 91 (42), 83 (14), 79 (28), 77 (32), 71 (25), 69 (21), 57 (32), 55 (45).

2,4-Dimethoxy-3-isopropylbenzoic chloride ($C_{12}H_{15}ClO_3$)

1.0 g of the above acid (4.5 mmol) was dissolved in 1.3 ml $SOCl_2$ and 10 ml benzene and refluxed for 2.5 h. Benzene and $SOCl_2$ were evaporated and left a lightly yellow oil, which was used as it was in the preparation of the following amide. ^1H NMR (200 MHz, δ, $CDCl_3$): 8.06 (d, $J = 9.1$ Hz, H-6), 6.72 (d, $J = 9.1$ Hz, H-5), 3.91 (s, OCH_3), 3.78 (s, OCH_3), 3.56 (septet, $J = 7.1$ Hz, CH), 1.30 (d, $J = 7.1$ Hz, $2CH_3$) ppm; ^{13}C NMR (50 MHz, δ, $CDCl_3$): 165.2 (C=O), 163.6, 159.7, 134.9, 119.3 (C-1,2,3,4), 130.8 (C-6), 106.8 (C-5), 62.7, 55.7 ($2OCH_3$), 25.0 (CH), 20.6 ($2CH_3$) ppm; MS (70 eV, 40 °C): *m/e* (%) = 244 (2), 243 (1), 242 (7, M^+), 239 (1), 238 (4), 224 (1), 223 (3), 209 (2), 208 (1), 207 (100), 205 (1), 192 (3), 191 (2), 179 (4), 178 (1), 177 (1), 175 (1), 163 (3), 161 (2), 151 (3), 149 (6), 148 (2), 147 (3), 135 (5), 134 (2), 133 (3), 121 (6), 119 (3), 115 (2), 105 (7), 103 (5), 91 (13), 89 (4), 79 (4), 78 (5), 77 (10), 65 (5), 63 (6), 53 (5), 51 (6).

2,4-Dimethoxy-3-isopropyl-N,N-diethylbenzamide (**5**; $C_{16}H_{24}NO_3$)

The above acid chloride was dissolved in 10 ml absol. diethyl ether and reacted with 10 g Et_2NH for 15 min. Then, 50 ml iced H_2O were added, the organic layer was separated and extracted with 50 ml diethyl ether. Washings with 2 × 2% HCl, H_2O, 2 × 2% NaOH, H_2O, and drying over Na_2SO_4 gave after evaporation of the solvent the crude product which was purified by means of column chromatography over silica using $CHCl_3$/MeOH = 10/1 as the eluent. Yield 1.1 g (88%; based on the respective acid) of a light yellow oil. ^1H NMR (200 MHz, δ, $CDCl_3$): 7.04 (d, $J = 8.4$ Hz, H-6), 6.65 (d, $J = 8.4$ Hz, H-5), 3.81 (s, OCH_3), 3.76 (s, OCH_3), 3.45 (m, $CH + NCH_2$), 3.17 (m, NCH_2), 1.31 (d, $J = 7.1$ Hz, $CH(CH_3)_2$), 1.25 (t, $J = 7.1$ Hz, CH_2CH_3). 1.02 (t, $J = 7.1$ Hz, CH_2CH_3) ppm; ^{13}C NMR (50 MHz, δ, $CDCl_3$): 169.4 (C=O), 159.8, 154.4, 129.5, 124.4 (C-1, 2, 3, 4), 125.3 (C-6), 107.2 (C-5), 62.4, 55.4 ($2OCH_3$), 42.9, 38.8 ($2CH_2$), 25.1 (CH), 20.9 ($CH(CH_3)_2$), 13.9, 12.7 ($2CH_2CH_3$) ppm; IR(KBr) $\nu = 1610$, 1460, 1440 cm^{-1}; MS (70 eV, 35 °C): *m/e* (%) = 280 (16), 279 (21), 278 (12; M^+), 248 (5), 208 (14), 207 (100), 191 (11), 177 (13), 165 (5), 149 (6), 121 (7), 105 (5), 103 (5), 91 (14), 73 (12), 72 (14), 58 (12), 42 (11).

5,7-Dimethoxy-6-isopropyl-3-(3,5-dimethoxyphenyl)-3H-isobenzofuranone (**7**; $C_{21}H_{24}O_6$)

A thoroughly flamed reaction vessel was charged with 60 ml absol. *THF*, 4.8 ml *TMEDA* (Fluka) (15.3 mmol), and 2.00 g of the amide **5** (7.2 mmol) under an argon atmosphere. After cooling to −80 °C, 9.3 ml of a 1.7 *M tert*-butyl lithium solution (15.8 mmol) were added dropwise within 15 min. After stirring for additional 15 min, the solution was colored dark yellow. Within 30 min 2.5 g (15.0 mmol) of

3,5-dimethoxybenzaldehyde (**6**; Aldrich) were added in portions; the solution became first dark orange and, after stirring the reaction mixture for two additional hours at −80 °C, it became lightly yellow. The reaction mixture was then brought to room temperature, quenched with 60 ml 3 *M* HCl and stirred overnight under an argon atmosphere. The solution was extracted twice with 50 ml ether and then 50 ml $CHCl_3$. The organic layer was washed twice with 50 ml 2% $NaHCO_3$, 70 ml H_2O, and finally with 70 ml sat. NaCl solution. After drying with Na_2SO_4, the solvent was evaporated on a rotary evaporator, and the residue crystallized from toluene/*n*-hexane. The yield was 1.0 g (40%) of white crystals; m.p.: 137–138 °C. ^{1}H NMR (200 MHz, δ, $CDCl_3$): 6.44 (m, H-2′, 4′, 6′, 4), 6.11 (s, H-3), 4.07 (s, OCH_3-7), 3.80 (s, OCH_3-5), 3.78 (s, OCH_3-3′,5′), 3.62 (m, CH), 1.28 (dd, J = 7.1 Hz, J = 1.9 Hz, $2CH_3$) ppm; ^{13}C NMR (50 MHz, δ, $CDCl_3$): 168.3 (C=O), 161.1 (C-3′, 5′), 164.8, 157.8, 151.3, 139.2, 130.3, 109.6 (C-3a, 5, 6, 7, 7a, 1′), 104.9 (C-2′, 6′), 100.5, 99.9 (C-4, 4′), 81.3 (C-3), 62.9 (OCH_3-7), 55.9 (OCH_3-5), 55.4 (OCH_3-3′, 5′), 24.6 (CH), 20.8, 20.7 ($2CH_3$) ppm; IR (KBr): ν = 1750, 1700, 1600, 1470 cm^{-1}; UV/Vis (EtOH, $c = 2.10^{-5}$ mol/l): λ_{max} = 262 (12600), 224 (36200) nm (ε); MS (70 eV), 100 °C): *m/e* (%) = 373 (8), 372 (100; M^+), 357 (26), 354 (53), 339 (27), 219 (60), 207 (32), 178 (17), 165 (42), 151 (39), 149 (54), 135 (47), 105 (48), 91 (97), 79 (88), 70 (96), 59 (84), 57 (89).

2,4-Dimethoxy-3-isopropyl-6-[(3,5-dimethoxyphenyl)-methyl]-benzoic acid (**8**; $C_{21}H_{26}O_6$)

345 mg **7** (0.92 mmol) were dissolved in 25 ml acetic acid. 70 mg Pd(10%)/C were added and the reaction mixture hydrogenated at 1 bar at 65–70 °C for 3 h. The solution was filtered from the catalyst. The catalyst was then washed with hot acetic acid, and the united solutions evaporated on a rotatory evaporator. Crystallization from EtOH/H_2O provided 298 mg (86% yield) of white crystals; m.p.: 124–125 °C. ^{1}H NMR (200 MHz, δ, $CDCl_3$): 6.45 (s, H-5), 6.37 (d, J = 2.1 Hz, H-2′,6′), 6.31 (t, J = 2.1 Hz, H-4′), 4.06 (s, CH_2), 3.80, 3.73 (s, OCH_3-2, 4), 3.75 (s, OCH_3-3′, 5′), 3.41 (septet, J = 7.0 Hz, CH), 1.30 (d, J = 7.0 Hz, $2CH_3$) ppm; ^{1}H NMR (200 MHz, δ, *DMSO*-d_6): 12.95 (br s, COOH), 6.65 (s, H-5), 6.40 (d, J = 2.3 Hz, H-2′, 6′), 6.30 (t, J = 2.3 Hz, H-4′), 3.80 (s, CH_2), 3.72, 3.66 (s, OCH_3-2, 4), 3.68 (s, OCH_3-3′, 5′), 3.40 (CH covered by solvent peak), 1.23 (d, J = 7.2 Hz, $2CH_3$) ppm; ^{13}C NMR (50 MHz, δ, $CDCl_3$): 170.0 (C=O), 160.7 (C-3′, 5′), 161.3, 156.7, 142.6, 140.6, 127.6, 117.9 (C-1, 2, 3, 4, 6, 1′), 110.0 (C-5), 107.3 (C-2′, 6′), 98.1 (C-4′), 63.5, 55.3, 55.2 ($4OCH_3$), 39.7 (CH_2), 25.3 (CH), 20.7 ($2CH_3$) ppm; ^{13}C NMR (50 MHz, δ, *DMSO*-d_6): 169.3 (C=O), 160.3 (C-3′, 5′), 159.3, 154.7, 142.5, 136.6, 126.3, 122.7 (C-1, 2, 3, 4, 6, 1′), 108.9 (C-5), 106.9 (C-2′, 6′), 97.6 (C-4′), 62.6, 55.6, 55.0 ($4OCH_3$), 40.0 (CH_2, covered by solvent signal), 24.5 (CH), 20.8 ($2CH_3$) ppm; IR (KBr): ν = 1700, 1600, 1570 cm^{-1}; UV/Vis (EtOH; $c = 2.10^{-5}$ mol/l): λ_{max} = 281 (2700), 276 (2800) nm (ε); MS (70 eV, 110 °C): *m/e*(%) = 374 (18; M^+), 356 (12), 341 (25), 332 (6), 285 (6), 247 (23), 238 (23), 205 (20), 191 (28), 151 (21), 111 (82), 105 (31), 91 (48), 87 (30), 81 (100), 79 (54), 77 (48).

1,3,6,8-Tetramethoxy-2-isopropyl-anthracen-9-ol (**9**; $C_{21}H_{24}O_5$)

450 mg **8** (1.20 mmol) were dissolved in 16 ml trifluoroacetic acid and cooled to −12 °C. 10 ml of precooled trifluoroacetic acid anhydride were added at once and the mixture stirred for 2 h at −10 °C under an argon atmosphere. The mixture was then evaporated on a rotatory evaporator and the residue crystallized from MeOH, which resulted in 343 mg (78% yield) of yellow crystals; m.p.: 108–109 °C. ^{1}H NMR (200 MHz, δ, $CDCl_3$): 10.67 (br s, OH-9), 7.47 (br s, H-10), 6.81 (s, H-4), 6.63 (s, H-5 or 7), 6.31 (s, H-7 or H-5), 4.07, 3.89 (OCH_3-1, 3), 3.91 (s, OCH_3-6, 8), 3.80 (septet, J = 7.0 Hz, CH) 1.40 (d, J = 7.0 Hz, $2CH_3$) ppm; ^{13}C NMR (50 MHz, δ, $CDCl_3$): 158.6, 158.4, 157.2, 154.3 (C-1, 3, 6, 8), 152.2 (C-9), 135.0, 134.8 (C-4a, 10a), 127.5 (C-2), 112.9 (C-10), 109.5, 107.1 (C-8a, 9a), 100.5 (C-4), 96.9, 96.3 (C-5, 7), 63.0, 56.2 (OCH_3-1, 3), 55.2, 54.9 (OCH_3-6, 8), 25.4 (CH), 21.4 ($2CH_3$) ppm; IR (KBr): ν = 1620, 1580, 1540 cm^{-1}; UV/Vis (EtOH; $c = 2.10^{-5}$ mol/l): λ_{max} = 372 (5340), 280 (61130) 273 (60200), 244 (19250) nm (ε); MS (70 eV, 100 °C): *m/e* (%) = 356 (13; M^+), 341 (6), 191 (16), 149 (6), 127 (4), 119 (4), 105 (12), 83 (14), 77 (17), 69 (100).

1,3,6,8-Tetrahydroxy-2-isopropyl-anthrone (**10**; $C_{17}H_{16}O_5$)

100 mg **9** (0.29 mmol) were dissolved in 10 ml acetic acid. After addition of 4.1 ml HJ ($d = 1.70$ g/ml), the mixture was refluxed for 90 min at 120 °C. The reaction mixture was then cooled with an ice bath, and after addition of a few drops of H_2O, 85.5 mg (99%) slightly yellow microcystalline product separated; m.p.: 207–209 °C. ^{1}H NMR (200 MHz, δ, $CDCl_3$): 13.00, 12.51 (br 2s, OH-1,8), 10.66, 10.54 (br 2s, OH-3, 6), 6.42 (s, H-4), 6.38 (d, $J = 1.8$ Hz, H-5), 6.20 (d, $J = 1.8$ Hz, H-7), 4.21 (s, CH_2-10), 3.50 (septet, $J = 7.0$ Hz, CH), 1.28 (d, $J = 7.0$ Hz, $2CH_3$) ppm; ^{13}C NMR (50 MHz, δ, $CDCl_3$): 190.2 (C=O), 164.3, 164.2, 162.4, 162.3 (C-1, 3, 6, 8), 144.3, 141.0 (C-4a, 10a), 118.1 (C-2), 108.3, 108.0 (C-8a, 9a), 107.1, 106.9 (C-4, 5), 101.0 (C-7), 32.2 (CH_2-10), 23.1 (CH), 20.2 ($2CH_3$) ppm; The assignments were advanced by means of an INEPT experiment and the comparison with the respective fringelite D precursor [4]. IR (KBr): $\nu = 1610$, 1500 cm^{-1}; UV/Vis (EtOH; $c = 2.10^{-5}$ mol/l): $\lambda_{max} = 361$ (18150), 280 (9360) nm (ε), MS (70 eV, 135 °C): m/e (%) = 300 (30; M^+), 286 (10), 285 (100), 271 (24), 152 (5), 142 (10), 129 (6), 115 (10), 110 (9), 105 (23), 91 (26), 69 (43).

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