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Concerning the Enantiomerization Barrier of Hypericin

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Summary. The Syntheses of ω-(*R*)-menthyl and ω,ω′-*bis*-(*R*)-menthyl derivatives **2** and **3** of hypericin were achieved, and the corresponding diastereomers could be separated. The equilibria between the respective diastereomers are slightly displaced in favor of the chromatographically faster moving ones. Kinetic measurements on these easily equilibrating diastereomers of **2** and **3** provided an *Arrhenius* activation energy for the interconversion barrier between the two propeller conformers of 83 and 89 kJ/mol. It could be shown that the ω-menthyl residues are of minor relevance to the height of this barrier, as is also the case for the *bay* hydroxyl ionization and quinone tautomerization equilibria. It was thus concluded that the intrinsic barrier for the propeller conformer enantiomerization of hypericin is in the order of 80 kJ/mol. These results are in accord with those obtained from semiempirical calculations.

Keywords. ω-Menthyl-hypericin derivatives; Equilibrium; Activation energy.

Zur Enantiomerisierungsbarriere von Hypericin

Zusammenfassung. Nach Synthese der ω-(*R*)-Menthyl- und ω,ω′-*bis*-(*R*)-Menthylderivate **2** und **3** des Hypericins konnten die entsprechenden Diastereomerenpaare getrennt werden. Die Gleichgewichte sind etwas zugunsten der chromatographisch rascher wandernden Diastereomeren verschoben. Kinetische Messungen an diesen leicht äquilibrierenden Diastereomeren von **2** und **3** führten zu einer *Arrhenius*schen Aktivierungsenergie für die Interkonversionsbarriere zwischen den beiden Propellerkonformeren von 83 und 89 kJ/mol. Es konnte gezeigt werden, daß die ω-Menthylreste für die Höhe dieser Barriere nur geringfügige Bedeutung haben, ebenso wie das *bay*-Hydroxyl-Ionisierungsgleichgewicht und das Tautomeriegleichgewicht. Daraus wurde geschlossen, daß die intrinsische Barriere für die Enantiomerisierung der beiden Hypericinpropellerkonformeren in der Größenordung von 80 kJ/mol liegt. Dieses Resultat stimmt mit den Ergebnissen semiempirischer Rechnungen überein.

Introduction

Due to its physiological properties [1], the chemistry of hypericin (**1**) and its derivatives has been intensely investigated in the last decade. Besides various studies concerning its synthesis [2] and photochemistry [3], it has been demonstrated by means of X-ray crystallography [4, 5] and molecular modeling [4] that **1** and its *bay* phenolate ion $\mathbf{1}^-$ adopt a propeller conformation of C_2 symmetry. Due to this dissymmetry **1** can be thought to consist of a racemate of

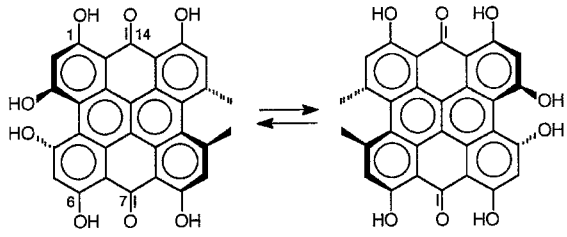


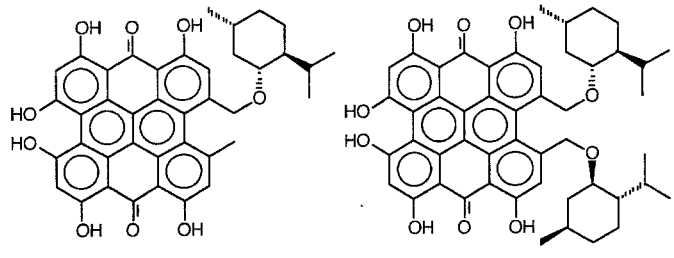
Fig. 1. Interconversion of the two enantiomeric propeller conformations (*P*)-**1** and (*M*)-**1**

(interconverting) enantiomeric propeller conformers (*P*)-**1** and (*M*)-**1** as shown in Fig. 1.

The other type of possible conformers, which are of the 'butterfly' type, have been shown to be energetically somewhat destabilized compared to the propeller conformers [4]. With respect to an upper limit of the height of the enantiomerization barrier, an estimation from force field calculations resulted in 113 kJ/mol [4]. It has also been tried to deduce a lower limit of the barrier height from the absence of a coalescence phenomenon in the NMR spectra of pseudohypericin, which yielded 80 kJ/mol [4]. However, to our knowledge, a satisfactory experimental value for this barrier using several techniques could not be advanced up to now. Thus, *e.g.* chromatography on chiral adsorbents or NMR studies on **1** or $\mathbf{1}^-$ dissolved in chiral solvents as well as employing chiral shift reagents and salts with chiral bases failed so far. The only experimental indication of the existence of such a barrier has been found for highly brominated derivatives, the so called gymnochromes [6]. Unfortunately, the isomerization barrier height could not be measured due to decomposition tendencies of these materials [6]. Moreover, model calculations in the latter case revealed that this barrier concerns conformers of a distorted butterfly type [7], and therefore such a figure could in principle not be used to estimate the propeller interconversion barriers of **1** or $\mathbf{1}^-$. Since the height of the propeller inversion barrier is one of the fundamental properties of the chemistry of hypericin, the present paper is devoted to this problem.

Results and Discussion

To solve the problem indicated above, the investigation of hypericin derivatives substituted with bulky asymmetric residues at the hypericin methyl groups seemed to be most promising. Therefore, the diastereomeric ω-(*R*)-(−)-menthyl derivatives **2** and **3** were envisaged for synthesis. After separation of the respective diastereomers they could be studied with respect to their relative thermodynamic

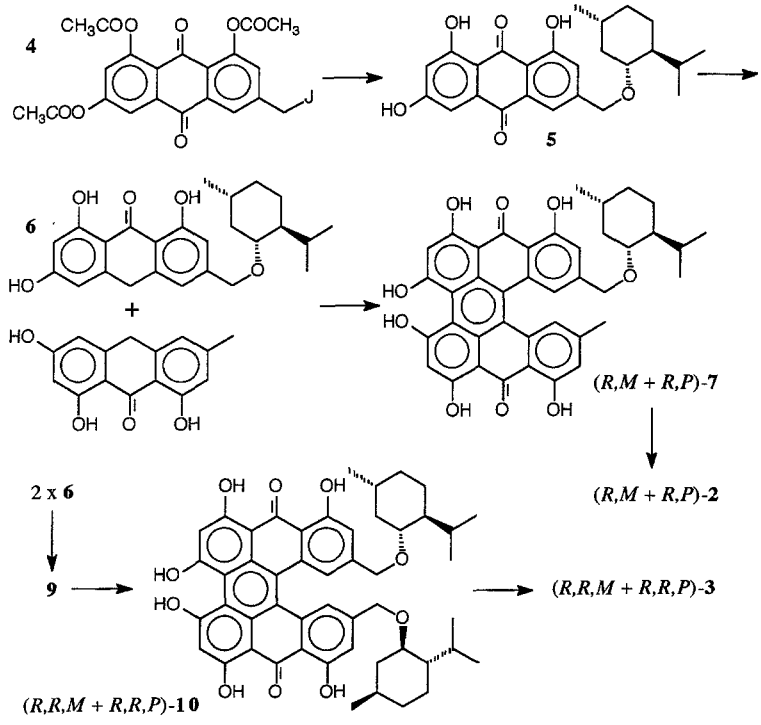


2 **3**

stability and interconversion activation energies. These data could then be compared with results obtained from force field calculations, thus allowing to estimate a reliable propeller isomerization barrier for hypericin itself.

Synthetic aspects

The synthesis strategy for compounds **2** and **3** was based on the oxidative dimerization used so far successfully for ω,ω'-appended hypericin derivatives [8]. Accordingly, the starting material had to be emodin derivatized at the methyl position with an (*R*)-(−)-menthyl residue. However, it turned out that nucleophilic substitution of ω-bromo-triacetoxy-emodin, which had been used as a convenient starting material before [8], proceeded only slowly and in very low yields in the case of (*R*)-(−)-menthol. Therefore, the much more reactive iodine derivative **4** was used instead. When silver perchlorate served as promoter, the yield of the ω-menthyl-emodin derivative **5** was about 70%, and it could be even improved to 95% by using silver triflate. The derivative **5** could then be easily reduced with $SnCl_2$ under standard conditions [2] to yield more than 80% of the anthrone derivative **6**. The mixed oxidative dimerization of **6** with an excess of emodin anthrone resulted – after separation from the symmetrical dimers – in a 28% yield of the helianthrone diastereomeric mixture (*R,M*+*R,P*)-**7**. This mixture was then photocyclized quantitatively to the (1:1) diastereomeric mixture (*R,M*+*R,P*)-**2**. Using a Sephadex column cooled to 10°C, the two diastereomers **2a** (the faster moving one) and **2b** could be separated chromatographically.



Scheme 1

Preparation of the intended *bis*-ω-menthyl derivative **3** could be achieved starting with the self dimerization of **6** to yield the bianthraquinoyl derivative **9**. This material proved to be extremely difficult to purify without extensive material losses. Therefore, it was used in a crude form in the next step to provide the helianthrone derivative **10** in 22% yield based on **6**. **9** was isolated, yields of **10** were considerably lower. Photocyclization of **10**, as in the case of **2**, proceeded quantitatively to yield the (1:1) diastereomeric mixture (*R*,*R*,*M*+*R*,*R*,*P*)-**3**. Chromatographic separation of the diastereomers **3a** (the faster moving one) and **3b** was achieved using a Sephadex column cooled to about 10°C.

Thermodynamic and kinetic aspects of the diastereomer **2a/2b** *and* **3a/3b** *interconversions*

The diastereomeric pairs of the mono-menthyl (**2a/2b**) and *bis*-menthyl (**3a/3b**) hypericin derivatives were found to equilibrate fast and easily at temperatures above room temperature – independent of the solvent (acetone, dimethylsulfoxide, tetrahydrofuran) and the presence of acid or base. Within the temperature range from 20 to 70°C, the equilibrium concentrations of the two diastereomers proved to be temperature independent within experimental error limits. The ratio between the two diastereomers was determined as 58.5:41.5 for **2a** $\rightleftharpoons$ **2b**, yielding a free enthalpy difference $\Delta G_0 = 0.9 \pm 0.05$ kJ/mol. In the case of **3a** $\rightleftharpoons$ **3b**, the relative equilibrium diastereomer ratio was observed to amount to 64.0:36.0 from which a free enthalpy difference $\Delta G_0 = 1.5 \pm 0.05$ kJ/mol was calculated.

These rather small experimental stabilization energies of the chromatographically faster moving diastereomers **2a** and **3a** could be compared with the difference in the heats of formation obtained for these two pairs from a semiempirical AM1 calculation which yielded values of 3.3 and 10.0 kJ/mol in favor of the (*R*,*P*)-**2** and (*R*,*R*,*P*)-**3** diastereomers. However, such a comparison would have involved the assignment of an absolute configuration of the propeller conformer to a certain diastereomer of the pairs **2a/2b** and **3a/3b**. We refrained from such an assignment because it would have been based solely on calculated energy differences. However, this issue will be addressed in a forthcoming paper on the chiroptical properties of **2** and **3**.

It should be stressed that, due to the ionization of **1** and its derivatives in protic and aprotic dipolar solvents and upon addition of base [2], the data derived so far refer to the *bay*-phenolates. However, calculations and experimental evidence (addition of trifluoroacetic acid) demonstrated that the thermodynamic energy differences of the dissociated and undissociated species were not significantly different. Moreover, these data were found to hold also for solutions of **2** and **3** in tetrahydrofuran. Since it has been shown that in solutions of **1** in this solvent the tautomeric equilibrium is shifted from the 7,14-dioxo tautomer **1** in favor of the 1,6-tautomer **1′** [9], the thermodynamic results obtained for **2**, $\mathbf{2}^-$, **3**, and $\mathbf{3}^-$ are also valid for the corresponding 1,6-dioxo tautomers **2′** and **3′**.

The *Arrhenius* plots for **2a** → **2b** and **2b** → **2a** as well as for **3a** → **3b** and **3b** → **3a** as illustrated in Fig. 2 allowed to calculate activation energies for the corresponding propeller inversion barriers of 83 ± 3 and 89 ± 3 kJ/mol. According to the solvents and additives used in the kinetic measurements, these activation

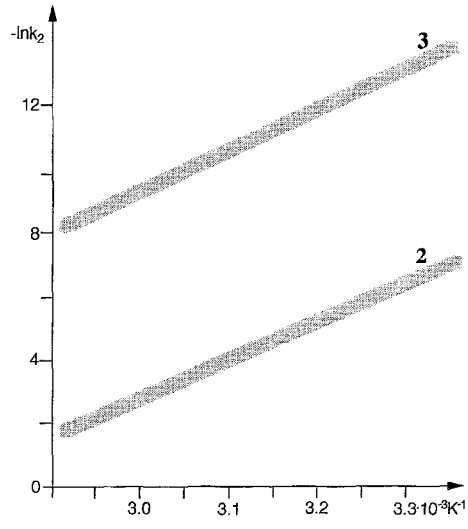


Fig. 2. *Arrhenius* plots for the interconversions of **2** and **3** for solutions in dimethylsulfoxide, acetone, tetrahydrofuran, dimethylsulfoxide + trifluoroacetic acid, and dimethylsulfoxide + *Hünig*'s base

barriers correspond to those of the undissociated (**2**, **3**), the dissociated ($\mathbf{2}^-$, $\mathbf{3}^-$), and the 1,6-dioxo-tautomeric (**2′**, **3′**) species. From the comparison of the two diastereomeric pairs it became immediately evident that for the inversion barrier of the hypericin propeller system the presence of the menthyl moieties were of only minor importance. Accordingly, it was concluded that the intrinsic propeller inversion barrier of hypericin (**1**) as well as of its dissociated ($\mathbf{1}^-$) and tautomerized forms (**1′**) (Fig. 1) would not be significantly different from those of the two diastereomeric pairs **2** and **3**, and was thus assigned to be in the order of 80 kJ/mol. This value was found to be quite close to the lower limit of this barrier which had been estimated to amount to 80 kJ/mol from NMR data of pseudohypericin [4].

Force field calculations concerning the interconversion barriers between (*R*, *M*)-**2** and (*R*, *P*)-**2** on the one hand and (*R*, *R*, *M*)-**3** and (*R*, *R*, *P*)-**3** on the other hand, following the principles developed for hypericin in Ref. [4], resulted in upper limits for this barrier of 119.0 and 119.5 kJ/mol, values which are quite close to the 113 kJ/mol advanced in the calculations for **1** [4] and thus corroborating the conclusion drawn above from the experimental results with respect to the independence of the height of the inversion barrier of hypericin to the presence or absence of ω-menthyl residues. Calculations on the corresponding *bay* phenolates $\mathbf{2}^-$ and $\mathbf{3}^-$ as well as on their 1,6-dioxo tautomers **2′** and **3′** resulted in barriers which did not deviate significantly from those obtained for **2** and **3**. This result was also found to be in accordance with calculations obtained for the tautomeric systems of **1** [9]. Of course, the behavior of this system can be easily understood from the fact that the isomerization barrier is mainly due to the intrinsic steric strain of the phenanthroperylene system and the steric requirements of the methyl or methylene groups attached to the *bay* region in positions 10 and 11. The sterically much less demanding groups attached to the *bay* region of positions **3** and **4**, or the tautomerization from the 7,14- to the 1,6-dioxo-tautomer, were found to provide only an almost negligible contribution to the activation barrier. Therefore, the values derived experimentally for the two menthyl derivatives **2** and **3** (and at the same time their deprotonated and tautomeric species) can indeed be

safely accepted as the characteristic values of the hypericin propeller inversion system.

Experimental

Melting points were taken by means of a Kofler hot stage microscope (Reichert, Vienna). ^{1}H, ^{13}C, IR, UV/Vis, M, and fluorescence spectra were recorded using Bruker DRX 500 and DPX 200, Biorad FT-IR 45, Perkin-Elmer IR-710B, Hitachi U-3210, MAT 95, 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 standard. Concentration measurements for thermodynamic and kinetic data were performed by integration of the corresponding ω-CH_2-O- signals in the ^{1}H NMR spectra of **2a** or **2b** (31.5, 38.4, 47.6, 53.0, 68.0°C) and **3a** or **3b** (32.6, 39.3, 46.5, 54.3, 69.5°C) dissolved in acetone-d_6 (at the three lower temperatures), *DMSO*-d_6 (at all temperatures given), *DMSO*-d_6 + 1 drop trifluoroacetic acid or *Hünig*'s base (N,N-diisopropyl-N-ethyl-amine; two intermediate temperatures), and tetrahydrofuran-d_8 (two intermediate temperatures) in an NMR sample tube thermostatted to ±0.1°C. The equilibrium constants K and thus ΔG_0 were calculated in the common way from the concentrations of the **a** and **b** diastereomers after equilibrium was reached in each case. Using the measured velocity constants k_1 or k_2 at the temperatures given above, the activation energy E_a was calculated from $K = k_1/k_2$ and the *Arrhenius* equation, $\ln k_2 = \ln A - E_a/RT$. Force field and semiempirical AM1 calculations were performed by means of the MM2+ and MOPAC+ program packages [10, 11] extended to handle molecules up to the required number of atoms. The input geometries for the calculations were derived from the X-ray data of hypericinate [4, 5] and (*R*)-(−)-menthol [12].

1,3,4,6,8,13-Hexahydroxy-10-(R)-(−)-menthyloxymethyl-11-methyl-phenanthro[1,10,9,8-o,p,q,r,a]-perylene-7,14-dione (**2**; $C_{40}H_{34}O_9$)

10 mg **7** were dissolved in 250 ml acetone and irradiated at 20°C for 2 h using a 700 W tungsten lamp. After evaporation of the solvent, 10 mg (100%) **2** were obtained as a 1:1 diastereomeric mixture. In solutions at room temperature, the two diastereomers equilibrated at a ratio of 58.5:41.5 m.p.: > 340°C. The two diastereomers were separated by chromatography on a Sephadex LH 20 column operated at 10°C using methanol as the eluent.

Fraction **2a**: ^{1}H NMR (acetone-d_6, δ, 500 MHz): 18.5 (s, OH), 14.79 (s, 2OH), 14,23 (s, OH), 14.21 (s, OH), 7.59 (H-ar), 7.35(H-ar), 6.61(H-ar), 6.58(H-ar), 5.02(AB-system, $J = 12.0$ Hz, HCH), 3.17 (dt, $J_d = 3.9$ Hz, $J_t = 10.1$ Hz, OCH=), 2.75 (s, CH_3), 1.4 (m, 9-menthyl-H), 0.69 (d, $J = 6.1$ Hz, CH_3), 0.59 (d, $J = 7.1$ Hz, CH_3), 0.09 (d, $J = 6.9$ Hz, CH_3) ppm.

Fraction **2b**: ^{1}H NMR (acetone-d_6, δ, 500 MHz): 18.5 (s, OH), 14.71 (s, 2OH), 14.09 (s, OH), 14.07 (s, OH), 7.58 (H-ar), 7.46 (H-ar), 6.60 (H-ar), 6.58 (H-ar), 4.97 (AB-system, $J = 12.0$ Hz, HCH), 2.70 (s, CH_3), 2.30 (m, OCH=), 1.3 (m, 9-menthyl-H), 0.80 (s, CH_3), 0.72 (d, $J = 6.7$ Hz, CH_3), 0.62 (d, $J = 6.5$ Hz, CH_3) ppm.

The ^{13}C NMR spectrum could be obtained only for the diastereomeric mixture because during the necessary acquisition times diastereomerization took place. As no significant differences in the IR, UV/Vis, fluorescence, and mass data were observed for the two diastereomers, they are given below also only once.

^{13}C NMR (acetone-d_6, δ, 50 MHz; if not indicated otherwise, each peak corresponds to 2 carbon atoms): 185.6 (4C, C=O), 177.4, 177.0, 170.8, 170.7, 163.8, 163.3, 145.2, 145.1, 129.1 (4C), 128.3, 127.3, 123.5 (4C), 121.9 (4C), 120.3, 120.1, 119.8 (4C), 111.7, 110.6, 107.3 (4C), 104.1, 104.0, 80.9 (1C), 78.3 (1C, O-CH=), 71.3 (1C), 69.8 (1C, CH_2-O), 49.8 (1C), 49.6 (1C), 35.8 (1C), 35.6 (1C), 32.7 (1C), 32.6 (1C), 26.8 (1C), 26.4 (1C), 24.4 (1C), 24.2 (2C + CH_3), 23.1 (2C), 22.0 (1C), 21.8

(1C), 17.1 (1C), 16.3 (1C) ppm; UV/Vis (ethanol; $c = 3.2 \cdot 10^{-6}$ mol/l): $\lambda_{max} = 592$ (43040), 549 (20140), 511 (7070), 478 (10990), 383 (10420), 329 (26000), 286 (34425) nm (ε); fluorescence (ethanol): $\lambda_{em} = 600$ (1), 648 (0.3) nm (rel. intensity); $\Phi_f = 0.19$; IR (KBr): $\nu = 3437$, 2945, 2926, 2869, 1592 cm^{-1}; MS (FAB pos/Noba, scan from 300 to 1000): *m/z* (%) = 681.2 (3; $M^+ + Na^+$), 659.4 (14; $M^+ + 1$), 658.4 (14; M^+), 551.7 (38), 550.7 (100), 548.7 (20), 519.2 (5), 505.2(6), 489.2 (6), 460.2 (7), 413.4 (20), 392.4 (18), 391.4 (64), 329.2 (20), 308.2 (14), 307.1 (59).

1,3,4,6,8,13-Hexahydroxy-10,11-bis-(−)-(R)-menthyloxymethyl-phenanthro[1,10,9,8-o,p,q,r,a]perylene-7,14-dione (**3**; $C_{50}H_{52}O_{10}$)

10 mg **10** were dissolved in 250 ml acetone and irradiated at 20°C for 2h using a 700 W tungsten lamp. After evaporation of the solvent, 10 mg (100%) **3** were obtained as a 1:1 diastereomeric mixture, m. p.: > 340°C. At room temperature the diastereomers equilibrated at a mixture of 64.0:36.0. The two diastereomers were separated by chromatography on Sephadex LH 20 column operated at 10°C using methanol as the eluent.

Fraction **3a**: 4.5 mg (45%); ^{1}H NMR (acetone-d_6, δ, 500 MHz): 18.5 (s, OH), 14.80, 14.24 (2s, OH-1, 6, 8, 13), 7.64 (s, H-9+12), 6.61 (s, H-2+5), 5.17 and 4.73 (AX-system, J=12.0 Hz, CH_2–O), 2.50 (m, 2O–CH=), 1.90 (m, menthyl-H8, 8′, 2, 2′), 1.0 (m, 14-menthyl-H), 0.75 (s, $2CH_3$), 0.68 (d, J = 6.9 Hz, $2CH_3$), 0.21 (d, J = 6.9 Hz, $2CH_3$) ppm; ^{13}C NMR (acetone-d_6, δ, 50 MHz; the peaks correspond to 2 carbon atoms): 185.5 (C=O), 177.4 (C-3+4), 170.8 (C-1+6), 164.1 (C-8+13), 145.5 (C-10+11), 129.2 (C-3a+3b), 127.7 (C-6b+14b), 123.4 (C-6c+13c), 121.9 (C-10a+10b), 120.9 (C-7b+13b),119.9 (C-9+12), 111.7 (C-6a+4a), 107.3 (C-2+5),104.2 (C-7a+13a), 78.8 (-OCH=), 69.6 (CH_2-O), 49.7 (menthyl-C-4), 41.2 (menthyl-C-2), 35.8 (menthyl-C-6), 32.7 (menthyl-C-1), 26.5 (menthyl-C-8), 24.3 (menthyl-C-5), 23.2 (CH_3), 21.8 (CH_3), 16.5 (CH_3) ppm.

Fraction **3b**: 4.5 mg (45%); ^{1}H NMR (acetone-d_6, δ, 500 MHz): 18.5 (s, OH), 14.79 (s, 2OH), 14.27 (s, 2OH), 7.62 (s, ar-H), 6.59 (s, ar-H), 4.96 (AB-system, J = 12.0 Hz, CH_2-O), 3.17 (dt J_d = 4.07 Hz, J_t = 10.5 Hz, 2OCH=), 2.25 (m, menthyl-H-8, 8′, 2, 2′), 1.8 (m, menthyl-H-6, 6′, 5, 5′), 1.2 (m, 10-menthyl-H), 0.69 (d, J = 6.5 Hz, $2CH_3$), 0.78 (d, J = 7.1 Hz, $2CH_3$), 0.82 (d, J = 7.2 Hz, $2CH_3$) ppm; ^{13}C NMR (acetone-d_6, δ, 50 MHz; the peaks correspond to 2 carbon atoms): 185.6 (C=O), 177.1 (C-3+4), 170.8 (C-1+6), 163.7 (C-8+13), 146.0 (C-10+11), 129.1 (C-3a+3b), 128.0 (C-6b+14b), 123.4 (C-6c+13c), 121.7 (C-10a+10b), 120.6 (C-7b+13b), 119.6 (C-9+12), 111.7 (C-6a+4a), 107.3 (C-2+5), 104.2 (C-7a+13a), 80.9 (OCH=), 70.8 (CH_2O), 49.9 (menthyl-C-4), 41.8 (menthyl-C-2), 35.8 (menthyl-C 6), 32.8 (menthyl-C-1), 26.9 (menthyl-C-8), 24.5 (menthyl-C-5), 23.1 (CH_3), 22.0 (CH_3), 17.2 (CH_3) ppm.

As no significant differences were observed for the two diastereomers, IR, UV/Vis, fluorescence, and mass data are given only once. UV/Vis (ethanol, $c = 5.7 \cdot 10^{-6}$ mol/l): $\lambda_{max} = 593$ (39820), 550 (19410), 511 (7470), 478 (18960), 388 (10010), 329 (25600) nm (ε); fluorescence (ethanol): $\lambda_{em} = 600$ (1), 648 (0.3) nm (rel. intensities); $\Phi_f = 0.17$; fluorescence (dimethyl-sulfoxide): $\lambda_{em} = 611$ (1), 658 (0.3) nm (rel. intensities); $\Phi_f = 0.16$; IR (KBr): ν = 3448, 2955, 2923, 2868, 1591 cm^{-1}; MS (FAB pos/Noba, scan from 300 to 1000): *m/z* (%) = 836.51 (10; M+Na^+), 814.52 (65; M+H^+), 656.37 (8), 537.09 (8), 520.06 (24), 505.94 (35), 391.38 (40), 329.29 (100).

1,3,8-Triacetoxy-6-iodomethyl-anthraquinone (**4**; $C_{21}H_{15}O_8J$)

1500 mg (3.16 mmol) 1,6,8-Triacetoxy-3-bromomethylanthraquinone (prepared according to Ref. [13]) and 950 mg (6.32 mmol) NaJ were dissolved in 300 ml dry acetone and refluxed under stirring for 1.5 h. The solvent was evaporated and the residue dissolved in 200 ml $CHCl_3$ and washed once with 50 ml std. $Na_2S_2O_7$ solution and twice with 50 ml H_2O. The organic layer was dried over Na_2SO_4 and evaporated.

Yield: 1533 mg (93%); m.p.: 214–217°C; ^{1}H NMR ($CDCl_3$, δ, 200 MHz): 8.17, 7.40 (2d, J = 2.0 Hz, 2H-ar), 7.95, 7.25 (2d, J = 2.4 Hz, 2H-ar), 4.47 (s, CH_2J), 2.44 (s, $OCOCH_3$), 2.43 (s,

$OCOCH_3$), 2.36 (s, $OCOCH_3$) ppm; ^{13}C NMR ($CDCl_3$, δ, 50 MHz): 180.8 (C=O), 179.4 (C=O), 169.2 (COO), 169.0 (COO), 167.9 (COO), 154.8, 151.5, 150.6, 146.9, 135.4, 134.7, 130.25, 125.4, 124.6, 123.6, 123.2, 118.4 (12C-ar), 21.1 ($2CH_3COO$), 21.0 (CH_3COO), 1.2 (CH_2J) ppm; UV/Vis (ethanol): $\lambda_{max} = 344$ (6200), 262 (30420), 213 (27960) nm (ε); IR (KBr): ν=1767, 1678, 1667, 1602 cm^{-1}.

1,3,8-Trihydroxy-6-(R)-(−)-menthyloxymethyl-anthracen-9,10-dione (**5**; $C_{25}H_{28}O_6$)

1000 mg (1.91 mmol) **4**, 980 mg (3.8 mmol)$Ag^+CF_3SO_3^-$, and 10 g (64 mmol) (*R*)-(−)-menthol were refluxed in 20 ml dry CH_2Cl_2 for 20 h. The reaction mixture was filtered over filter paper, washed with water, the organic solvent was evaporated and the excess of menthol removed by means of steam distillation. The residue was dissolved in 150 ml methanol and refluxed after addition of a mixture of 2 ml conc. H_2SO_4 and 5 ml water for 2 h. The reaction mixture was concentrated to 20 ml, and 1000 ml brine was added. The precipitate was filtered, washed with water, and chromatographed with $CHCl_3$:CH_3OH = 20:1 over a silica column.

Yield: 770 mg (95%); m.p.: 144–145°C; ^1H NMR ($CDCl_3$, δ, 200 MHz): 12.24 (s, OH), 12.18 (s, OH), 7.73 (s, H-ar), 7.32 (s, H-ar), 7.29, 6.62 (2d, 2H, J = 2.5 Hz, H-ar), 4.73, 4.47 (AB-system, J = 13.0 Hz, HCH), 3.24 (dt, J_t = 10.5 Hz, J_d = 4.1 Hz, O-CH=), 2.32 (m, menthyl-H-8), 2.20 (m, menthyl-H-2), 1.6 (m, menthyl-H-5+6), 1.30 (m, menthyl-H-1+4), 1.1 (m, menthyl-H-2+5+6), 0.96 (d, J = 6.5 Hz, CH_3-7), 0.95 (d, J = 7.0 Hz, CH_3), 0.76 (d, J = 7.0 Hz, CH_3) ppm; ^{13}C NMR (CD_3OD, δ, 50 MHz): 190.9 (C=O), 182.4 (C=O), 167.8, 166.4, 163.1, 150.3, 136.2, 134.15, 115.5, 110.6 (8C-ar), 122.7, 118.8, 109.9, 109.0 (4CH-ar), 80.9 (O-CH=), 70.0 (CH_2-O), 50.3 (menthyl-C-4), 41.4 (menthyl-C-2), 35.7 (menthyl-C-6), 32.7 (menthyl-C-1), 26.8 (menthyl-C-8), 24.4 (menthyl-C-5), 22.8 (CH_3), 21.5(CH_3), 16.7 (CH_3) ppm; UV/Vis (ethanol): λ_{max} = 459 (5310), 292 (9670), 255 (12900), 218 (19700) nm (ε); IR (KBr): ν = 3400, 2956, 2925, 2870, 1627 cm^{-1}.

1,3,8-Trihydroxy-6-(R)-(−)-menthyloxymethyl-10H-anthracen-9-one (**6**; $C_{25}H_{30}O_5$)

86 mg (0.20 mmol) **5** were dissolved under heating in 20 ml glacial acetic acid, 450 mg (2.0 mmol) $SnCl_2 \cdot 2H_2O$ in 2.5 ml conc. HCl were added, and the reaction mixture was refluxed until the color disapeared (about 20 min). The reaction mixture was diluted with 250 ml water, cooled with ice, and filtered after 1h. After washings with water, the precipitate was chromatographed with $CHCl_3$: CH_3OH = 20:1 over a silica column.

Yield: 67 mg (82%); m.p.: 177–178°C; ^1H NMR ($CDCl_3$: CD_3OD = 20:1, δ, 200 MHz): 6.75 (s, CH-ar), 6.73 (s, CH-ar), 6.27 (s, CH-ar), 6.19 (s, (*H*-ar), 4.44 (AB-system, J = 12.7 Hz, CH_2-O), 4.11 (s, ar-CH_2-ar), 3.13 (dt, J_d = 4.1 Hz J_t = 10.5 Hz, OCH=), 2.25 (m, menthyl-H-2+8), 1.6 (m, menthyl-H-5+6), 1.2 (m, menthyl-H-1+4), 0.9 (m, menthyl-H-2+5+6), 0.87 (d, J = 6.4 Hz, CH_3), 0.86 (d, J = 7.0 Hz, CH_3), 0.69 (d, J = 7.0 Hz, CH_3) ppm; ^{13}C NMR (CD_3OD:$CDCl_3$=1:1, δ, 50 MHz): 192.2 (C=O), 165.5, 165.3, 162..7, 147.9, 144.9, 142.1, 114.2, 109.7 (8C-ar), 118.0, 115.4, 107.7, 101.8 (4CH-ar), 79.9 (O-CH=), 70.1 (–CH_2–O–), 48.8 (menthyl-C-4), 40.7 (menthyl-C-2), 34.9 (menthyl-C-6), 32.0 (menthyl-C-1), 26.1 (menthyl-C-8), 23.6 (menthyl-C-5), 22.5 (CH_3), 21.2 (CH_3), 16.3 (CH_3), 33.3 (ar-CH_2-ar) ppm; UV/Vis (ethanol): λ_{max} = 361 (14300), 270 (8800), 221 (22000) nm (ε); IR (KBr): ν = 3340, 2955, 2923, 2870, 1626, 1602 cm^{-1}.

1,3,8,1′,3′,8′-Hexahydroxy-6-(R)-(−)-menthyloxymethyl-6′-methyl-helianthrone (**7**; $C_{40}H_{36}O_9$)

129 mg (0.31 mmol) **6** and 500 mg (1.85 mmol) emodine anthrone were added to 40 mg $FeSO_4 \cdot 7H_2O$ and 600 mg (6.6 mmol) pyridine-N-oxide in a mixture of 3 ml pyridine and 2 ml piperidine and stirred at 100°C for 1 h under Ar and protection from light. Then the reaction mixture was acidified with 10% HCl; the precipitate was filtered and washed with water. It was dissolved in a minimum

amount of acetone and chromatographed on silica plates using $CHCl_3$:CH_3OH =20:1 as the eluent. The first fraction was twice rechromatographed with methanol on a Sephadex LH 20 column.

Yield: 50 mg (28%); m.p.: > 340°C, the two (at room temperature) inseparable diastereomers are present in a ratio of 1:1; ^{1}H NMR (acetone-d_6, δ, 200 MHz; 1:1 mixture of the diastereomers): 14.51 (s, 4OH), 13.13 (s, OH), 13.12 (s, OH), 13.06 (s, OH), 13.04 (s, OH), 7.51, 7.29, 6.83, 6.69 (4s, 2H-ar), 6.35 (s, 4H-ar), 4.33 (AB-system, J = 13.0 Hz, CH_2-O), 4.30 (AB-system, J =12.4 Hz, CH_2-O), 3.09 (dt, J_d = 4.1 Hz, J_t = 10.6Hz, 2O-CH=), 2.13 (s, $2CH_3$), 2.0 (m, 4-mentyl-H), 0.8-1.6 (m, 26-menthyl-H + -$(CH_3)_4$), 0.68, 0.62 (2d, 3H, J = 7.0, CH_3) ppm; ^{13}C NMR (acetone-d_6, δ, 50 MHz): 186.6 (2C, C=O), 186.3 (2C, C=O), 176.5 (2C), 176.1 (2C), 170.8 (2C), 162.4 (2C), 162.3 (2C), 145.5, 145.4, 144.2 (2C), 138.3 (2C), 131.8, 129.6, 127.5, 125.5, 124.9, 121.9, 117.7, 116.4, 115.2, 115.0, 106.0, 101.8, 101.7, 80.2 (O-CH=), 79.7 (O-CH=), 70.7 (CH_2-O), 70.2 (CH_2O-), 49.8 (2C), 41.4, 41.3, 35.9 (2C), 32.7 (2C), 26.9 (2C), 24.5 (2C), 23.3 (2C), 22.6 (2C), 21.9 (2C), 17.1 (2C) ppm; UV/Vis (ethanol): λ_{max} = 591 (13000), 546 (13700), 374 (11700), 241 (29600) nm (ε); IR (KBr): ν = 3475, 2954, 2920, 2867, 1583 cm^{-1}.

1,3,8,1′,3′,8′-Hexahydroxy-6,6′-bis-(R)-(−)-menthyloxymethyl-bianthraquinoyl (**9**; $C_{50}H_{58}O_{10}$)

44 mg (0.11 mmol) **6** were dissolved in 20 ml hot ethanol p.a., and 29.7 mg (0.11 mmol) $FeCl_3.6H_2O$ dissolved in 5 ml ethanol p.a. were slowly added dropwise. After refluxing for 3 h, 250 ml 5% HCl were added after cooling, and the reaction mixture was extracted three times with 100 ml ether. The organic layer was washed with water and dried over Na_2SO_4, evaporated, and filtered over a short silica column using $CHCl_3$:CH_3OH= 10:1 as the eluent. Yield: 40 mg; the product was extremely difficult to purify without severe losses in yield. Therefore, it was used in the next step without further characterization. Nevertheless, it proved to be more convenient to proceed from **8** to **10** *via* a crude isolate of **9**.

1,3,8,1′,3′,8′-Hexahydroxy-6,6′-bis-(R)-(−)-menthyloxymethyl-helianthrone (**10**; $C_{50}H_{54}O_{10}$)

40 mg (0.049 mmol) **9** and 70 mg (0.8 mmol) pyridine-N-oxide were dissolved in a mixture of 1 ml pyridine and 1 ml piperidine and stirred for 1 h at 100°C under exclusion of light. The reaction mixture was acidified with 10% HCl, the precipitate collected, washed with water, dissolved in a minimum amount of acetone, and chromatographed on a silica plate using $CHCl_3$:CH_3OH = 10:1 as the eluent.

Yield of the inseparable diastereomeric mixture (1:1): 10 mg (22% based on **6**); m.p.: >340 C; ^{1}H NMR (acetone-d_6, δ, 500 MHz): 14.55 (s, OH), 14.54 (s, OH), 13.12 (s, OH), 13.10 (s, OH), 7.53 (s, ar-H), 7.49 (s, ar-H), 6.82 (s, ar-H), 6.36 (s, ar-H), 4.34 (AB-system, J = 12.2 Hz, CH_2-O), 4.21, (AB-system, J = 12.2 Hz, CH_2-O), 3.06 (dt, J_d = 3.94 Hz, J_t = 10.26 Hz, 4O-CH=), 2.10 (m, 2 × menthyl-H-2, 2′,8,8′), 1.6 (m, 2x menthyl-H5,5′,6,6′), 1.1 (m, 20 menthyl-H), 0.83 (d, J = 6.1 Hz, $4CH_3$), 0.68 (d, J = 6.8 Hz, $4CH_3$), 0.62 (d, J = 7.0 Hz, $4CH_3$) ppm; ^{13}C NMR (acetone-d_6, δ, 50 MHz): 186.4 (2C, C=O), 176.9 (2C), 171,1 (2C), 162.5 (2C), 145.8, 145.7, 138.4, 138.3, 131.9 (2C), 129.8, 125.5, 125.0, 122.2 (2C), 116.5 (1C), 115.5, 115.1, 105.9 (2C), 101.8 (2C), 80.2 (O-CH=), 80.0 (O-CH=) 70.9 (CH_2-O), 70.4 (CH_2-O), 49.9 (2C, menthyl-C-4), 41.5 (menthyl-C-2), 41.4 (menthyl-C-2), 36.0 (menthyl-C6), 36.0 (menthyl-C-6), 32.8 (menthyl-C-1), 32.8 (menthyl-C-1), 27.0 (2C, menthyl-C-8), 24.6 (menthyl-C-5), 24.5 (menthyl-C-5), 23.3, 23.3, 17.1, 17.1 ($8CH_3$), 22.0 ($2CH_3$) ppm; UV/Vis (ethanol): λ_{max} = 578 (13830), 546 (15120), 373 (11510), 257 (37980), 220 (36060) nm (ε); IR (KBr): ν = 3448, 2955, 2923, 2869, 1591 cm^{-1}.

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