Monatshefte für Chemie 124, 77–81 (1993)

Monatshefte für Chemie
Chemical Monthly

On the Bromination of Hypericin: The Gymnochrome Chromophores

Heinz Falk* and **Wolfgang Schmitzberger**

Institut für Chemie, Johannes Kepler Universität Linz, A-4040 Linz, Austria

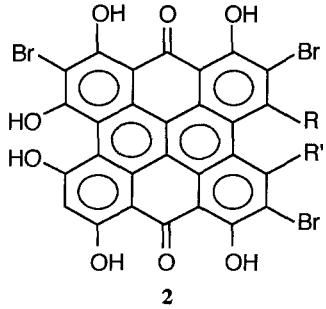
Summary. Stepwise electrophilic bromination of hypericin in pyridine as the solvent yields regioselectively 2,5-dibromohypericin, 2,5,9-tribromohypericin, and 2,5,9,12-tetrabromohypericin. The compounds were characterized by means of NMR-, UV-Vis-, and fluorescence-spectra, and pK_a and pK_a^* measurements. These properties were compared with those of hypericin on the one hand and of the recently isolated gymnochromes on the other hand.

Keywords. Bromo-hypericines; Gymnochromes; ^{1}H-NMR; ^{13}C-NMR; UV-Vis; pK_a values; Fluorescence.

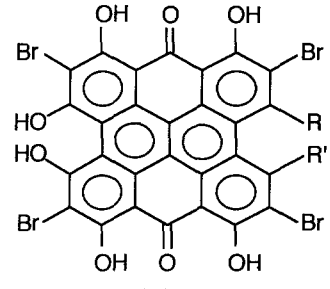
Zur Bromierung des Hypericins: Die Gymnochrom-Chromophore

Zusammenfassung. Schrittweise elektrophile Bromierung von Hypericin in Pyridin als Lösungsmittel liefert regioselektiv 2,5-Dibromhypericin, 2,5,9-Tribromhypericin und 2,5,9,12-Tetrabromhypericin. Diese Verbindungen wurden durch NMR-, UV-Vis- und Fluoreszenz-Spektrometrie sowie durch pK_a- und pk_a^* -Messungen charakterisiert. Diese Eigenschaften wurden mit jenen des Hypericins und der kürzlich isolierten Gymnochrome verglichen.

Introduction



2

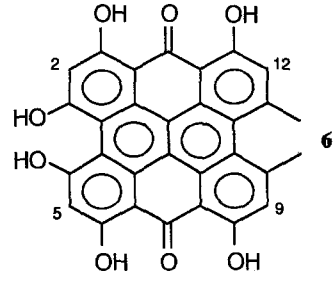


1, 3, 4

Five brominated hydroxy-phenanthroperylenequinones, the gymnochromes A-D, and isogymnochrome D, which is an atropisomer of gymnochrome D [**1**: R = (*S*)-$CHOHCH_3$, R′ = (*R*)-$CHOH(CH_2)_2CH_3$; **2**: R = (*S*)-$CHOHCH_3$, R′ = (*R*)-$CHOH(CH_2)_2CH_3$ or R′ = (*S*)-$CHOHCH_3$, R = (*R*)-$CHOH(CH_2)_2CH_3$; **3**: R = (*S*)-$CHOHCH_3$, R′ = (*S*)-$CHOSO_3HCH_3$; **4**: R = R′ = (*R*)-$CHOSO_3H(CH_2)_2CH_3$);

5 = atropisomer of **4**] were recently isolated from the marine deep-water stalked crinoid *Gymnocrinus richeri* [1]. Their structures and stereochemistries were derived from spectroscopic measurements. The chromophores of these compounds consist of tri- or tetrabromophenanthroperylenequinones, which in addition are substituted by six hydroxyl groups and two alkyl residues. The substitution pattern of these latter substituents is characteristic of the dimerized polyketide systems present in a variety of animals and plants [2].

Hypericin (**6**) which is found in plants and certain microorganisms also belongs to this polyketide type of compounds. As it can be rather easily prepared by chemical synthesis [3–5], or isolated from *Hypericum* species [6] it seemed to be interesting to investigate its electrophilic bromination with respect to a synthetic or semi-synthetic access of this kind of novel bromophenanthroperylenequinone chromophores.



Results and Discussion

To probe **6** for an electrophilic bromination, it was dissolved in pyridine-d_5 and titrated with a solution of bromine in the same solvent (see Fig. 1). Upon addition of two moles of bromine the proton signal of H-2 + 5 had completely disappeared.

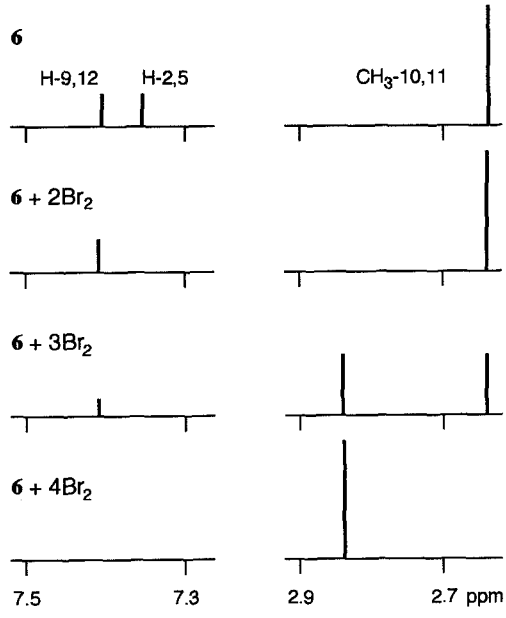


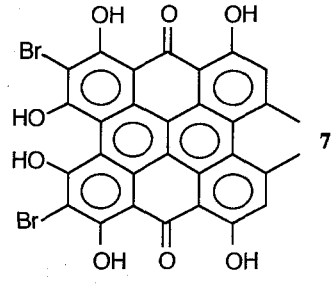
Fig. 1. ^{1}H-NMR spectrometric titration of **6** with bromine in pyridine-d_5 solution (schematic spectra)

It should also be mentioned that before the two moles had been added all signals were split with a spacing of about 2 Hz, afterwards they appeared as singlets again. Further addition of one mole of bromine resulted in the gradual disappearance of half the intensity of H-9 + 12 together with the development of a new methyl signal at 2.82 ppm (assigned to CH_3-10) at the expense of the methyl-10 + 11 (then becoming the CH_3-11 signal) signal intensity. Upon addition of a fourth mole of bromine all aromatic proton signals had disappeared and all the intensity of the methyl protons occurred at 2.82 ppm (= methyl-10 + 11). Thus, hypericin (**6**) was stepwise regioselectively brominated only depending on the relative amounts of bromine used – the most susceptible positions being 2 and 5. This regioselectivity is in accord with what one would have guessed from the comparison of the two aromatic positions under electrophilic attack, one between two hydroxyl groups (2, 5) and one between a hydroxyl and a methyl group (9, 12).

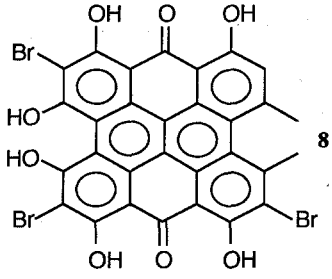
According to this experiment **6** could be stepwise brominated to preparatively yield 2,5-dibromohypericin (**7**), 2,5,9-tribromohypericin (**8**), and 2,5,9,12-tetrabromohypericin (**9**). Following this strategy **7**, **8**, and **9** were prepared in nearly quantitative yields by simply adding appropriate amounts of a bromine solution in pyridine to a solution of **6** in pyridine, followed by conventional work-up.

Comparing the gymnochromes **1**–**5** with the brominated hypericins **7**–**9**, only the tetrabromo derivative **9** appeared to be a chromophore of the tetrabrominated gymnochromes **1**, **3**, **4**, and **5**. On the contrary, gymnochrome B (**2**) contains a chromophoric positional isomer of the tribrominated system **8**. As the positions 2 and 5 of the hexahydroxy-dimethyl-phenanthroperylenequinones are the ones which are most easily brominated, one might speculate that with respect to biogenesis of the tribrominated system **2**, this could only be the result of a debromination of the tetrabromo compounds. Therefore **2** cannot be the product of a stepwise bromination sequence during its biosynthesis.

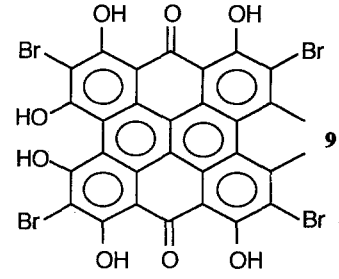
The deprotonation pK_a and pK_a^* values of hypericin (**6**; $pK_a = 11.0$, $pK_a^* = 9.8$ [7]) are lowered with successive bromination. The tetrabromosystem **9** is a rather



7



8



9

strongly acidic phenol with a $pK_a = 8.8$ and $pK_a^* = 5.7$. It should be mentioned that the addition of one mole of Hünig's base to dimethylsulfoxide-d_6 solutions of **7–9** yielded a strongly shifted OH ^{1}H-NMR signal at about 18 ppm which is comparable to the one observed with **6** [7]. However, in contrast to the sharp signal observed with **6**, the signals of **7–9** are very broad. The carbonyl group basicity of **9** ($pK_a = 1.2$, $pK_a^* = 4.1$) is strongly enhanced compared with **6** ($pK_a = -6.0$, $pK_a^* = -3.2$ [7]). This phenomenon is due to the $+M$ effect of the bromine substituents. Although the excited state pK_a^* values for protonation and deprotonation are quite close to each other, the relative acidities and basicities of hydroxyl and carbonyl groups in ground and excited states are retained.

The absorption spectra of **7–9** were bathochromically shifted by 4, 5, and 6 nm compared with the one of **6** [6]. This shift is similar to the one observed for the gymnochromes [1]. The fluorescence quantum yields of the brominated systems **7–9** were reduced comparing them to the one of hypericin ($\Phi_f = 0.27$ [4, 8]) by nearly one order of magnitude. This is what could be expected from an enhancement of intersystem crossing due to the heavy atom effect of the bromine atoms [9].

Experimental Part

Melting points were taken by means of a Kofler hot stage microscope (Reichert, Vienna). ^{1}H-, ^{13}C-, IR-, UV-VIS-, and fluorescence spectra were recorded using the Bruker-WM-360-, Biorad-FT-IR-45-, Hitachi-U-3210-, and Hitachi-F-4010-instruments. *DMSO*-d_6 (99.95% D; Uetikon) and pyridine-d_5 (99.8% D, Uetikon) were used for the NMR experiments, c about 10^{-2} mol/l; signal assignments were achieved according to the details given in [6, 7], they are in accord with the assignments derived for the gymnochromes [1]. The solvent used for the fluorescence measurements (ethanol) was of fluorescence quality, Merck. The solutions were degassed by bubbling them with argon for 1 h. **6** was isolated from *Hypericum perforatum* L. according to [6]. Spectrophotometric titrations and Förster cycle calculations for evaluating the deprotonation and protonation equilibria were performed according to the details given in [7].

Bromination Procedure

15 mg **6** (0.03 mmol) were dissolved in 7 ml pyridine and the appropriate amount of a freshly prepared solution of bromine in pyridine ($c = 0.4$ mmol/ml) was added at once. After stirring for 15 min at room temperature under argon protection the solution was evaporated to dryness under vacuum. The residue was chromatographed over a short column of Sephadex® LH-20 with methanol/pyridine = 9/1 as the eluent.

2,5-Dibromo-hypericin [**7**; $C_{30}H_{14}O_8Br_2$]

Yield 97%; m.p. > 350 °C. ^{1}H-NMR(360 MHz, δ, *DMSO*-d_6): 15.39(s, broad, 2H, OH-1,6), 13.56(s, broad, 2H, OH-8,13), 7.26(s, 2H, CH-9,12), 2.55(s, 6H, CH_3-10, 11) ppm. ^{1}H-NMR(360 MHz, δ, pyridine-d_5): 7.45(s, 2H, CH-9,12), 2.65(s, 6H, CH_3-10,11) ppm. ^{13}C-NMR (90 MHz, δ, *DMSO*-d_6): 184.8(C=O-7,14), 175.1(C-3,4), 168.2(C-1,6), 163.0(C-8,13), 144.7(C-10,11), 127.3(C-3a, 3b), 126.3(C-6b,14b), 122.3(C-7c,14c), 121.1(C-10a, 10b), 120.0(C-7b,13b), 117.4(C-9,12), 109.1(C-6a,14a), 106.3(C-2,5), 102.2(C-7a, 13a), 24.1[CH_3-10,11) ppm. IR(KBr): $\nu = 1635\,cm^{-1}$. UV-Vis(ethanol, $c = 2 \cdot 10^{-5}$ mol/l): $\lambda_{max} = 290(40\,800), 332(31\,200), 391(8\,500), 485(11\,700), 549(19\,300), 593\,(37\,200)$ nm(ε). Fluorescence(ethanol, room temp.): $\lambda = 601$, 648 nm, $\Phi_f = 0.07$ ($\lambda_{ex} = 550$ nm). Spectrophotometric titration (*DMSO*/water, 80%, [7]) $pK_a = 10.5$, $pK_a^* = 7.4$, $\lambda_7 = 598$ nm, $\lambda_{7^-} = 656$ nm $\varepsilon_\lambda/\varepsilon_{\lambda^-} = 1.74$.

2,5,9-Tribromo-hypericin [**8**; $C_{30}H_{13}O_8Br_3$]

Yield 96%; m.p. > 350 °C. ^{1}H-NMR(360 MHz, δ, *DMSO-d_6*): 15.67(s, broad, 2H, OH-1,6), 13.77(s, broad, 2H, OH-8,13), 7.31(s, 1H, CH-12), 2.78(s, 3H, CH_3-10), 2.57(s, 3H, CH_3-11) ppm. ^{1}H-NMR(360 MHz, δ, pyridine-d_5): 7.45(s, 1H, CH-12), 2.84(s, 3H, CH_3-10), 2.65(s, 3H, CH_3-11) ppm. ^{13}C-NMR(90 MHz, δ, *DMSO-d_6*): 183.8(C=O-7,14), 174.6(C-3,4), 167.8(C-1,6), 163.5(C-8,13), 145.3(C-10,11), 126.7(C-3a,3b), 125.8(C-6b,14b), 123.3(C-7c,14c), 122.1(C-10a,10b), 121.4(C-7b,13b), 118.2(C-9,12), 109.9(C-6a,14a), 107.7(C-2,5), 102.9(C-7a,13a), 25.2(CH_3-10,11) ppm. IR(KBr): $\nu = 1628\,cm^{-1}$. UV-Vis(ethanol, $c = 2 \cdot 10^{-5}$ mol/l): $\lambda_{max} = 290(39\,700)$, 333(31 500), 390(8 700), 485(12 000), 550(18 900), 594(36 100) nm(ε). Fluorescence(ethanol, room temp.): $\lambda = 598$, 646 nm, $\Phi_f = 0{,}04$ ($\lambda_{ex} = 550$ nm). Spectrophotometric titration (*DMSO*/water, 80%, [7]): $pK_a = 9.6$, $pK_a^* = 6.4$, $\lambda_8 = 598$ nm, $\lambda_{8^-} = 658$ nm, $\varepsilon_\lambda/\varepsilon_{\lambda^-} = 1.86$.

2,5,9,12-Tetrabromo-hypericin [**9**; $C_{30}H_{12}O_8Br_4$]

Yield 96%; m.p. > 350 °C. ^{1}H-NMR(360 MHz, δ, *DMSO-d_6*): 15.81(s, broad, 2H, OH-1,6), 14.12(s, broad, 2H, OH-8,13), 2.80(s, 6H, CH_3-10,11) ppm. ^{1}H-NMR (360 MHz, δ, pyridine-d_5): 2.83(s, 6H, CH_3-10,11) ppm. ^{13}C-NMR (90 MHz, δ, *DMSO-d_6*): 184.1(C=O-7,14), 175.2(C-3,4), 167.9(C-1,6), 163.2(C-8,13), 144.6(C-10,11), 126.1(C-3a,3b), 125.0(C-6b,14b), 124.4(C-7c,14c), 122.9(C-10a,10b), 120.1(C-7b,13b), 116.2(C-9,12), 111.3(C-6a,14a), 105.7(C-2,5), 103.5(C-7a,13a), 26.6(CH_3-10,11) ppm. IR(KBr): $\nu = 1631\,cm^{-1}$. UV-Vis(ethanol, $c = 2 \cdot 10^{-5}$ mol/l): $\lambda_{max} = 290(39\,000)$, 332(31 100), 390(8 200), 484(11 200), 550(18 100), 595(35 600) nm(ε). Fluorescence(ethanol, room temp.): $\lambda = 600$, 647 nm, $\Phi_f = 0.04$ ($\lambda_{ex} = 550$ nm). Spectrophotometric titration. Deprotonation (*DMSO*/water, 80%, [7]): $pK_a = 8.8$, $pK_a^* = 5.7$, $\lambda_9 = 598$ nm, $\lambda_{9^-} = 654$ nm $\varepsilon_\lambda/\varepsilon_{\lambda^-} = 1.78$. Protonation(aqueous sulfuric acid [7]): $pK_a = 1.2$, $pK_a^* = 4.1$, $\lambda_9 = 598$ nm, $\lambda_{9H^+} = 648$ nm, $\varepsilon_\lambda/\varepsilon_{\lambda^+} = 0.37$.

References

[1] De Riccardis F., Iorizzi M., Minale L., Riccio R., Richer de Froges R., Debitus C. (1991) J. Org. Chem. **56**: 6781. One should be aware that the position numbering scheme of these authors is "clockwise" and is in contrast to the one used in this paper. We decided to use a "counterclockwise" numbering scheme, which according to the rules of nomenclature yields the lowest numbers for the higher priority substituents (OH).

[2] Thomson R. H. (1971) Naturally Occurring Quinones, 2nd Ed. Acad. Press, London; 3rd Ed. (1987) Recent Advances. Chapman & Hall, New York; Weiss U., Merlini L., Nasini G. (1987) Fortschr. Chem. Org. Naturstoffe **52**: 50; Roth L. (1990) Hypericum-Hypericin, Botanik · Inhaltsstoffe · Wirkung; ecomed, Landsberg; Sherman D. H., Bibb M. J., Simpson T. J., Johnson D., Malpartida F., FernandezMoreno M., Martinez E., Hutchinson C. R., Hopwood D. A. (1991) Tetrahedron **47**: 6029

[3] Falk H., Schoppel G. (1991) Monatsh. Chem. **122**: 739

[4] Falk H., Schoppel G. (1992) Monatsh. Chem. (in press)

[5] Mazur Y., Bock H., Lavie D. (1992) Can. Pat. Appl. CA2,029,993, Chem. Abstr. **116**: 6343z

[6] Falk H., Schmitzberger W. (1992) Monatsh. Chem. **123**: 731

[7] Falk H., Meyer J., Oberreiter M. (1992) Monatsh. Chem. **123**: 277

[8] Falk H., Meyer J., hitherto unpublished results

[9] Barltrop J. A., Coyle J. D. (1975) Excited States in Organic Chemistry. Wiley, London, p 82

Received April 28, 1992. Accepted May 19, 1992