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On the Nature of "Soluble" Hypericin in *Hypericum* Species

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Summary. The two red and two violet "soluble" pigments of *Hypericum* species were isolated by means of extraction, chromatography, and counter-current droplet chromatography. In contrast to authentic hypericin, they are soluble in common organic solvents and even in water. Using NMR experiments it was deduced that hypericin, pseudohypericin, protohypericin, and protopseudohypericin are present in the plant as their rapidly interconverting 3- and 4-phenolate ions. From AAS the main counter-ion of these phenolates was derived to be potassium. The potassium and N-ethyl-N,N-diisopropylammonium salts of hypericin were synthesized for comparison. A preparative procedure to isolate hypericin and pseudohypericin from plant material was developed.

Keywords. "Soluble" Hypericin; Hypericin-, Pseudohypericin-, Protohypericin-, Protopseudohypericin-, Potassium salts; ^{1}H-NMR; ^{13}C-NMR; Droplet counter-current chromatography; *Hypericum perforatum* L.

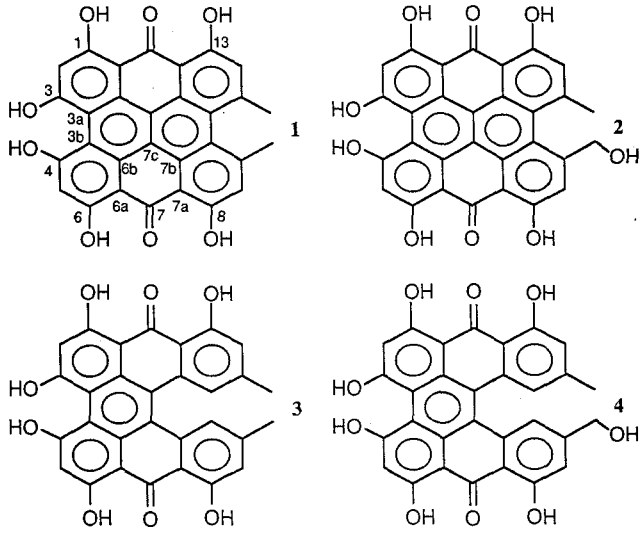
Zur Natur des „löslichen" Hypericins in *Hypericum*-Arten

Zusammenfassung. Die zwei roten und zwei violetten „löslichen" Pigmente aus *Hypericum* Arten wurde durch Extraktion, Chromatographie und Tropfen-Gegenstromchromatographie isoliert. Im Gegensatz zu authentischem Hypericin sind diese in organischen Lösungsmitteln und sogar in Wasser löslich. Aus NMR-Experimenten wurde abgeleitet, daß Hypericin, Pseudohypericin, Protohypericin und Protopseudohypericin in diesen Pflanzen als deren rasch interkonvertierende 3- und 4-Phenolationen vorliegen. Durch AAS wurde gefunden, daß diese Phenolationen Kalium als Gegenion aufweisen. Das Kalium- und N-Ethyl-N,N-diisopropylammonium-Salz des Hypericins wurden zu Vergleichszwecken dargestellt. Ein präparatives Verfahren zur Isolierung von Hypericin und Pseudohypericin aus Pflanzenmaterial wurde entwickelt.

Introduction

A renewed interest in hypericin (**1**), triggered by its antiviral and antiretroviral properties [1], makes the accessibility of this compound from natural sources a new challenge. According to literature, **1** may be isolated from plant material, as for instance from *Hypericum perforatum* L. or *Hypericum hirsutum* L. [2]. These methods start from a crude methanol extract of the plant material. The pigment fraction is precipitated with acid, and purified by chromatographic methods. Hy-

pericin itself is only very sparingly soluble in common solvents. However, as the pigment is easily extracted, it is obviously contained in the plant material in a soluble form. We now report our studies of the isolation and identification of "soluble" hypericin and its "soluble" co-pigments contained in the plant material.



Results and Discussion

As a first working hypothesis we inferred that, judged from the extensive presence of glycosidized biogenetic precursors of hypericin in plants [3], **1** might also be present in plant material as a glycoside. To test this hypothesis, freshly collected blossoms of *Hypericum perforatum* L. were frozen with liquid nitrogen, thoroughly ground, and extracted with cold 2-butanone using an "ultra turrax" device. Chromatography of the lyophilized extract on Sephadex LH-20® provided two violet and two red pigment fractions. As conventional adsorption chromatography techniques were inadequate for further purification, fractionation was carried out by means of droplet counter-current chromatography (DCCC) [4], which is a form of liquid-liquid counter current distribution chromatography. Compared with the methods of general glycoside isolation procedures described in literature [5] this workup was an extremely mild one, and should have provided the pigment glycosides in the case they were present.

However, according to the ^{13}C- and ^{1}H-NMR spectra of the isolated pigments, which were still soluble in common solvents, only hypericin type chromophores were present, i.e., hypericin (**1**), protohypericin (**3**), pseudohypericin (**2**), and protopseudohypericin (**4**) signals could be identified.

Closer examination of the ^{1}H-NMR spectra of the four fractions in dimethylsulfoxide-d_6 as the solvent revealed deuterium exchangeable signals with intensities 2:2:1 which were present in addition to the aromatic and aliphatic proton signals. These could be assigned to five hydroxyl groups in positions 1+6, 8+13, and 3 or 4 by means of chemical shift arguments and decoupling experiments together with INEPT and ^{13}C-NMR spectroscopy. Partial acetylations [6] failed to yield unambiguous assignments. Whereas the chemical shifts of the two hydroxyl proton pairs occured in the "normal" region of about 14 ppm, as was also observed for

authentic hypericin, the signal of the single hydroxyl proton was dramatically shifted to about 18 ppm. This is a rather unusual shift for phenolic or even carboxylic acid protons, and it points to a very strongly hydrogen bonded system. It is interesting to note that this signal at 18 ppm was also present in the ^{1}H-NMR spectrum of the crude plant extract. This finding provided solid evidence that the material isolated using the procedure described above was indeed the natural pigment.

The result derived from the ^{1}H-NMR measurements indicated some kind of derivatization at one of the hydroxyl groups in positions 3 or 4. Further evidence was derived from the ^{1}H-NMR spectrum of the hypericin type pigment: The number of signals for the aromatic and aliphatic carbon atoms and protons is only half the number present in the molecule indicating a symmetry of at least C_2. This symmetry is, of course, incompatible with a covalent linkage between one of the two hydroxyl groups and some derivatizing residue. Instead, there has to be a dynamic process which is fast at room temperature compared to the NMR time scale. Thus a phenolate ion was fully compatible with all experimental evidence accumulated so far.

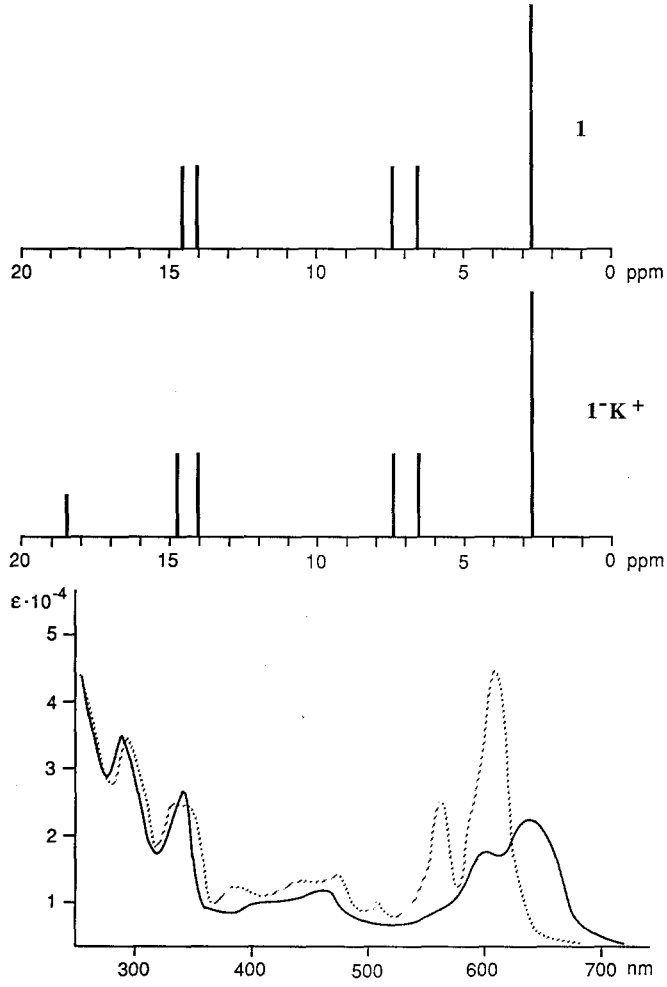
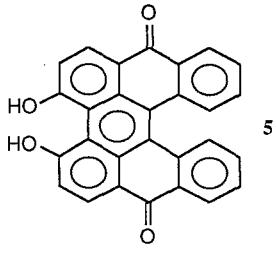


Fig. 1. ^{1}H-NMR and UV-VIS spectra of **1** (- - -) and **1**$-K^+$ (—) in *DMSO-d*$_6$; $c = 0.01\,\text{mol}\,\text{l}^{-1}$; $d_{\text{UV-VIS}} = 0.1\,\text{mm}$

Indeed, ^{1}H-NMR titration of authentic hypericin with pottassium hydroxide in dimethyl sulfoxide started with two hydroxyl signals of intensities 2:2 in the region of about 14 ppm whereas the remaining two hydroxyl proton signals were too broad to be detected. An additional one-proton signal at 18 ppm was observed upon addition of one mole of the base (Fig. 1). Upon further addition of base this signal remained unchanged, but the other hydroxyl proton signals (1 + 6 and 8 + 13) were broadened and eventually disappeared. As has been shown in a preceding paper [7], there is a significant difference in the pK_a values of the hydroxyl groups situated in the vicinity of the carbonyl groups (in positions 1, 6, 8, and 13) compared to those in the lateral positions 3 and 4; the latter being more acidic by about three pK_a units. This first deprotonation step of **1** is characterized by a pK_a value of 11 in a dimethylsulfoxide/water solvent, whereas the second one has a pK_a value of about 14 under the same conditions [7]. From the ^{1}H-NMR behavior referred to above, it now might be concluded that the second deprotonation step takes place at the 1-, 6-, 8-, or 13- and not at the second lateral hydroxyl group. The same characteristic behavior is observed using organic bases like N-ethyl-N,N-diisopropylamine or high concentrations of pyridine. As is evident from Fig. 1, concentrated solutions of the mono-anion of **1** are also characterized by a typical UV-VIS spectrum. However, it should be noted that the spectra of more diluted solutions will approach the spectrum which is characteristic of **1** (see Exp. Part). This is due to the rather high pK_a of **1** which eventually will lead to solvolysis of the salt in dilute solution.

To study the reasons of the unusual mono-deprotonation ^{1}H-NMR shift of the 3,4-hydroxyl groups of **1**, 2,2′-dihydroxybiphenyl (**6**), 2,2′-dihydroxy-1,1′-binaphthyl (**7**), and the model system **5** (which was used because the corresponding phenanthroperylene derivative is virtually insoluble, and therefore NMR spectra could not be obtained – compare Ref. [7]) were titrated with one equivalent of N-ethyl-N,N-diisopropylamine. Whereas **6** and **7** exhibited a broad signal at 9.18 and 9.21 ppm in the neutral form, this signal disappeared on the transition to the mono-deprotonated forms. A broad two proton signal ($\Delta_{1/2} \approx 400$ Hz) around 12.4 ppm was found for neutral **5** and a rather sharp signal at 17.4 ppm of the mono-deprotonation product of **5** was observed! Obviously, the steric compression of the phenolate ion onto the remaining hydroxyl group in **5** as well as in **1** yields a very distinct, strong, and efficiently stabilized hydrogen bonded system. On the contrary, in **6** and **7** the much less sterically restricted biaryl systems are not able to stabilize a certain conformation of the kind derived for **1** and **5**. This result derived from the model compounds **5**–**7** strongly corroborate the assignments and arguments arrived at above for **1** and its co-pigments **2**–**4**.



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According to the data presented above, **1** is present as its rapidly interconverting 3- and 4-phenolate ion in the plant material. To derive information about the nature

of its counter ion, the plant material, as well as the pure fractions after DCCC were worked up for basic components, but no organic bases could be detected by ^{1}H- and ^{13}C-NMR spectroscopy. This finding is in accordance with studies of natural compounds of *Hypericum sp.* in which no indications of basic materials were obtained [8]. Atomic absorption spectrometry of the crude extract as well as of the pure DCCC fractions of **1**–**4** revealed a predominant presence of potassium ions and only minor quantities of sodium, lithium, magnesium and calcium ions. Therefore it was concluded that hypericin (**1**) and its co-pigments **2**–**4** are present in the plant material mainly as their rapidly interconverting 3- and 4-potassium salts. It should be mentioned that the counter ions mentioned above do not sum up to 100%. We attribute the missing 8 to 19% to other ions not tested for, and to partial solvolysis of the salts in dilute solutions which cannot be avoided in the case of phenolates with pK_a values of about 11. This is evident from the UV-VIS spectra of concentrated versus dilute solutions (see the UV-VIS data in the experimental part).

As could also be shown, conventional preparation of the potassium and N-ethyl-N,N-diisopropylammonium salts of **1** resulted in "soluble **1**". The spectroscopic behavior of these anions was identical with the spectroscopic behavior of the corresponding natural product, thus corroborating our structural assignments.

NMR-analysis of the accessory pigment fractions of pseudohypericin (**2**), protohypericin (**3**), and protopseudohypericin (**4**), which are present together with **1** in the plant material, along the routes presented above, revealed the same kind of salt formation. Accordingly they are also present in the plant mainly as their rapidly interconverting 3- and 4-potassium salts. It should also be mentioned that the proto systems **3** and **4** are easily transformed to **1** and **2** upon irradiation thus enabling higher yields of the latter pigments to be isolated. It was also observed that plants harvested in june contained much higher relative amounts of **3** and **4** compared to **1** and **2**, whereas in the september harvest this ratio was reversed. Obviously photocyclization of the proto pigments **3** and **4** leads to accumulation of the pigments **1** and **2** in the more mature plants. A preparative procedure optimized for isolation of **1** and **2** from plant material on the basis of these results was developed and is described in the experimental section.

It should also be mentioned that although the chromophores of **1**–**4** are inherently chiral, neither the four fractions of the pure pigments, nor the crude extract showed any evidence of a chiroptical signal. Accordingly they are present as their racemates in the plant material.

Experimental Part

Melting points were taken by means of a Kofler hot stage microscope (Reichert, Vienna). ^{1}H-, ^{13}C-, IR-, and UV-VIS-spectra were recorded using the Bruker-WM-360-, Biorad-FT-IR-45-, and Hitachi-U-3210-instruments. *DMSO-d_6* (99.95% D; Uetikon) was used for the NMR experiments, *c* about 10^{-2} mol/l. Proton and carbon signal assignments were achieved using NOE measurements and the INEPT technique; these were applied using standard Bruker software. Atomic absorption spectroscopy (AAS) was carried out using a Perkin Elmer 2380 instrument. CD spectra were recorded by means of the Jobin-Yvon Circulardichrograph Mark V. Authentic **1**, **6**, and **7** were of commercial origin (Roth, Sigma, Fluka); **5** was previously described [7].

The plant material (*Hypericum perforatum* L.) was harvested at its flowering time (end of june until the end of september) in the regions of Ostermiething and Rohrbach, Upper-Austria. After collecting the whole plants, the blossoms were cut off and cooled immediately to 77 K just at the collecting site. Before extraction the plant material was frozen with liquid nitrogen and pulverized by means of a pistill. 50 g of the ground blossoms (corresponding to 5.85 g dry weight) were extracted with 300 ml 2-butanone in an Erlenmeyer-flask using an "ultra turrax" device. After 10 min the resulting red solution was filtered off and concentrated under reduced pressure with a maximum bath temperature of 35°C. The crude plant-extract was dissolved in 150 ml methanol/water (4/1), and filtered off from insoluble parts to obtain a clear red solution which was extracted four times with 50 ml of dichloromethane to remove chlorophylls and other impurities.

After lyophilization purification of the resulting 3.0 g crude extract was accomplished by gel-chromatography using Sephadex LH-20® as the stationary phase (column: 60 × 3 cm), mobile phase dichloromethane/methanol/water (4/5/1). Thereby 425 mg protopseudohypericin-, 183 mg protohypericin-, 35 mg hypericin-, and 65 mg pseudohypericin-fractions (in the order of their elution) were obtained. The chromatographic column and the fractions obtained were protected from light. The fractions were controlled by thin-layer-chromatography [silica GF_{254}, mobile phase chloroform/methanol 4/1; R_f(**1**) = 0.40, R_f(**2**) = 0.33, R_f(**3**) = 0.45, R_f(**4**) = 0.38]. To obtain pure compounds DCCC was carried out by means of a Büchi B-670 DCC chromatograph under the following conditions: DCCC-A, ascending method with the solvent system dichloromethane/methanol/water = 10/8/6 using 294 Pyrex glass tubes (40 cm, 3.0 mm i.d.) connected to each other by teflon tubings (60 cm, 0.85 mm i.d.); DCCC-B, descending method with chloroform/methanol/water = 5/5/3; DCCC-C, ascending method with chloroform/methanol/*n*-propanol/water = 9/12/1/8. These three conditions gave the same results. The properties of the four lyophilized fractions obtained by the procedure described above are as follows:

Hypericinate Fraction

Yield: 18 mg. Thermal behavior: no change up to 320°C. ^{1}H-NMR (*DMSO-d_6*, δ, 360 MHz): 18.39 (s, 1 H, OH-3 or 4), 14.70 (s, 2 H, OH-1, 6), 14.06 (s, 2 H, OH-8, 13), 7.39 (s, 2 H, CH-9, 12), 6.51 (s, 2 H, CH-2, 5), 2.70 (s, 6 H, CH_3-10, 11) ppm. NOE: CH_3-10, 11 ⇒ CH-9, 12. ^{13}C-NMR (*DMSO-d_6*, δ, 90 MHz): 183.3 (C=O-7, 14), 174.7 (C-3, 4), 166.0 (C-1, 6), 161.2 (C-8, 13), 143.5 (C-10, 11), 126.6 (C-3 a, 3 b), 126.0 (6 b, 14 b), 121.2 (7 c, 14 c), 120.7 (10 a, 10 b), 119.2 (7 b, 13 b), 118.6 (C-9, 12), 108.3 (C-6 a, 14 a), 105.4 (C-2, 5), 101.9 (7 a, 13 a), 23.6 (CH_3-10, 11) ppm; the signal assignments for 126.6/126.0 and 120.7/119.2 ppm might be interchanged. IR (KBr): ν = 1 622, 1 597 cm^{-1}. UV-VIS (CH_3OH, $c = 2.10^{-5}$ mol/l): $\lambda_{max.}$ = 589 (43 600), 546 (24 700), 509 (10 000), 472 (13 800), 446 (12 900), 384 (12 100) nm (ε). UV-VIS (CH_3OH, $c = 1.10^{-2}$ mol/l): $\lambda_{max.}$ = 630 (24 000), 588 (22 800), 467 (13 400) nm (ε). According to ^{1}H-NMR and AAS analysis its folmulae can be computed as $C_{30}H_{15}O_8^{(-)}$ $(K_{0.73}Na_{0.08}Mg_{0.01}Ca_{0.01}Li_{0.001})^{(+)}$.

Pseudohypericinate Fraction

Yield: 51 mg. Thermal behavior: no change up to 320°C. ^{1}H-NMR (*DMSO-d_6*, δ, 360 MHz): 18.46 (s, 1 H, OH-3 or 4), 14.75 (2 s, 2 H, OH-1, 6), 14.12 (2 s, 2 H, OH-8, 13), 7.71 (s, 1 H, CH-9), 7.46 (s, 1 H, CH-12), 6.59 (s, 2 H, CH-2, 5), 5.34 (s, broad, 1 H, CH_2**O**H-10), 4.67 (A-part of AB-system, J = 12.8 Hz, **CH$_2$**OH-10), 5.15 (B-part of AB-system, J = 12.8 Hz, **CH$_2$**OH), 2.68 (s, 3 H, CH_3-11) ppm. NOE: CH_3-11 ⇒ CH-12; CH_2-10 ⇒ CH-9. ^{13}C-NMR (*DMSO-d_6*, δ, 90 MHz): 184.3 (C=O-7, 14), 174.9 (C-3, 4), 168.2 (C-1, 6), 161.8 (C-8, 13), 144.8 (C-10, 11), 130.6 (C-3 a, 3 b), 128.7 (C-6 b, 14 b), 118.4 (C-7 c, 14 c), 115.0 (C-10 a, 10 b), 114.5 (C-7 b, 13 b), 101.0 (C-6 a, 14 a), 99.9 (C-7 a, 13 a), 95.1 (C-2, 5), 93.8 (C-9, 12), 66.3 (CH_2OH-10), 27.8 (CH_3-11) ppm. IR (KBr): ν = 1 623, 1 595 cm^{-1}. UV-VIS (CH_3OH, $c = 2.10^{-5}$ mol/l): $\lambda_{max.}$ = 590 (43 100), 546 (24 800), 509 (10 300), 470 (13 600), 446 (12 700), 384 (13 300) nm (ε). UV-VIS (CH_3OH, $c = 1.10^{-2}$ mol/l): $\lambda_{max.}$ = 634 (24 200), 589 (23 200), 465 (13 700) nm (ε). According to ^{1}H-NMR and AAS analysis its formulae can be computed as $C_{30}H_{15}O_9^{(-)}$ $(K_{0.81}Na_{0.10}Mg_{0.001}Ca_{0.01}Li_{0.001})^{(+)}$.

Protohypericinate Fraction

Yield: 91 mg. Thermal behavior: no change up to 320°C. ^{1}H-NMR (*DMSO-d_6*, δ, 360 MHz): 18.47 (s, 1 H, OH-3 or 4), 14.71 (2 s, 2 H, OH-1, 6), 14.41 (2 s, 2 H, OH-8, 15), 7.37 (s, 1 H, CH-14), 7.24 (s, 1 H, CH-9), 6.87 (s, 1 H, CH-12), 6.75 (s, 1 H, CH-11), 6.40 (s, 2 H, CH-2, 5), 2.03 (s, 6 H, CH_3-10, 13) ppm. NOE: CH_3-10 ⇒ CH-9, 11; CH_3-13 ⇒ CH-12, 14. ^{13}C-NMR (*DMSO-d_6*, δ, 90 MHz): 184.7 (C=O-7, 16), 172.7 (C-3, 4), 168.2 (C-1, 6), 160.1 (C-8, 15), 143.0 (C-10, 13), 136.1 (C-11, 12), 129.8 (C-3 a, 3 b), 127.8 (C-6 b, 16 b), 125.9, 122.6, 118.9, 116.4 (C-6 a, 6 c, 7 a, 11 a, 12 a, 15 a, 16 a, 16 c), 113.4 (C-9, 14), 104.2 (C-2, 5), 21.4 (CH_3-10, 13) ppm. IR (KBr): ν = 1 620, 1 593 cm^{-1}. UV-VIS (CH_3OH, $c = 2.10^{-5}$ mol/l): $\lambda_{max.}$ = 589 (32 100), 546 (25 200), 474 (11 000) nm (ε). UV-VIS (CH_3OH, $c = 1.10^{-2}$ mol/l): $\lambda_{max.}$ = 628 (24 200), 587 (23 100), 462 (13 500) nm (ε). According to ^{1}H-NMR and AAS analysis its formulae can be computed as $C_{30}H_{17}O_8^{(-)}$ $(K_{0.62}Li_{0.15}Mg_{0.01}Ca_{0.03}Li_{0.001})^{(+)}$.

Protopseudohypericinate Fraction

Yield: 255 mg. Thermal behavior: no change up to 320°C. ^{1}H-NMR (*DMSO-d_6*, δ, 360 MHz): 18.43 (s, 1 H, OH-3 or 4), 14.70 (s, 2 H, OH-1, 6), 14.21 (s, 2 H, OH-8, 15), 7.37 (s, 1 H, CH-9), 7.24 (s, 1 H, CH-14), 6.87 (s, 1 H, CH-11), 6.75 (s, 1 H, CH-12), 6.38 (s, 2 H, CH-2, 5), 4.23 (AB-system J = 5 Hz, CH_2OH-10), 2.05 (s, 3 H, CH_3-13) ppm. NOE: CH_3-13 ⇒ CH-12, 14; CH_2-10 ⇒ CH-9, 11. ^{13}C-NMR (*DMSO-d_6*, δ, 90 MHz): 184.7 (C=O-7, 16), 172.9 (C-3, 4), 168.2 (C-1, 6), 160.2 (C-8, 15), 147.6 (C-10, 13), 136.2 (C-11, 12), 129.7 (C-3 a, 3 b), 128.0 (C-6 b, 16 b), 125.9, 122.7, 118.9, 116.4 (C-6 a, 6 c, 7 a, 11 a, 12 a, 15 a, 16 a, 16 c), 113.2 (C-9, 14), 104.6 (C-2, 5), 67.2 (CH_2OH-10), 24.8 (CH_3-13) ppm. IR (KBr): ν = 1 621, 1 594 cm^{-1}. UV-VIS (CH_3OH, $c = 2.10^{-5}$ mol/l): $\lambda_{max.}$ = 589 (32 000), 547 (25 400), 476 (11 300) nm (ε). UV-VIS (CH_3OH, $c = 1.10^{-2}$ mol/l): $\lambda_{max.}$ = 630 (24 000), 588 (23 100), 465 (13 400) nm (ε). According to ^{1}H-NMR and AAS analysis its formula can be computed as $C_{30}H_{17}O_9^{(-)}$ $(K_{0.69}Na_{0.17}Mg_{0.01}Ca_{0.01}Li_{0.001})^{(+)}$.

CD spectra of the concentrated UV-VIS samples of these four fractions and the crude extract exhibited $\Delta\varepsilon < 0.01$ ($\lambda = 700 - 300$ nm).

N-Ethyl-N,N-diisopropylamino-3-hypericinate [$C_{38}H_{14}NO_8$]

To a well stirred solution of 1.3 mg (10.06 μmol) N-ethyl-N,N-diisopropylamine in 10 ml methanol 5 mg (9.92 μmol) **1** were added under an argon cushion. After stirring for 2 h at room temperature the resulting dark-red solution was evaporated and the residue chromatographed by means of dichloromethane/methanol/water = 4/5/1 on a short column of Sephadex LH-20®. Yield 5.2 mg, (83%); m.p.: not below 320°C. ^{1}H-NMR (*DMSO-d_6*, δ, 360 MHz): 18.42 (s, 1 H, OH-3 or 4), 14.71 (s, 2 H, OH-1, 6), 14.07 (s, 2 H, OH-8, 13), 7.41 (s, 2 H, CH-9, 12), 6.54 (s, 2 H, CH-2, 5), 3.60 (m, 2 H, CH−N), 3.13 (q, 2 H, J = 7 Hz, CH_2−N), 2.72 (s, 6 H, CH_3-10, 11), 1.22 (t, 15 H, J = 7 Hz, 5 alkyl-CH_3) ppm. IR (KBr): ν = 1 622, 1 596 cm^{-1}. UV-VIS (CH_3OH, $c = 2.10^{-5}$ mol/l): $\lambda_{max.}$ = 589 (43 600), 546 (27 600), 503 (9 800), 472 (13 400), 446 (12 800), 384 (12 200) nm (ε). UV-VIS (CH_3OH, $c = 1.10^{-2}$ mol/l): $\lambda_{max.}$ = 629 (24 700), 588 (23 600) 467 (13 700) nm (ε) (compare Fig. 1 for the analogous spectrum of the potassium salt with dimethylsulfoxide as the solvent).

Potassium-3-hypericinate [$C_{30}H_{15}O_8K$]

To a well stirred solution of 0.55 mg (10 μmol) KOH in 10 ml methanol 5 mg (10 μmol) **1** were added under an argon cushion. After stirring for 2 h at room temperature the resulting dark-red solution was evaporated and the residue chromatographed by means of dichloromethane/methanol/water = 4/5/1 on a short column of Sephadex LH-20®. Yield 4.8 mg, (89%); Thermal behavior: no change up to 320°C. ^{1}H-NMR (*DMSO-d_6*, δ, 360 MHz): 18.27 (s, 1 H, OH-3 or 4), 14.64 (s, 2 H, OH-1, 6), 14.20 (s, 2 H, OH-8, 13), 7.41 (s, 2 H, CH-9, 12), 6.51 (s, 2 H, CH-2, 5), 2.70 (s, 6 H, CH_3-10, 11) ppm.

NOE: CH_3-10, 11 ⇒ CH-9, 12. ^{13}C-NMR (*DMSO-d_6*, δ, 90 MHz): 183.3 (C=O-7, 14), 174.7 (C-3, 4), 166.0 (C-1, 6), 161.2 (C-8, 13) 143.5 (C-10, 11), 126.6 (C-3a, 3b), 126.0 (6b, 14b), 121.2 (7c, 14c), 120.7 (10a, 10b), 119.2 (7b, 13b), 118.6 (C-9, 12), 108.3 (C-6a, 14a), 105.4 (C-2, 5), 101.9 (7a, 13a), 23.6 (CH_3-10, 11) ppm; the signal assignments for 126.6/126.0 and 120.7/119.2 ppm might be interchanged. ppm. IR (KBr): $\nu = 1622$, $1597\,cm^{-1}$. UV-VIS (CH_3OH, $c = 2.10^{-5}$ mol/l): $\lambda_{max.} = 589$ (44100), 546 (24900), 512 (10300), 472 (13800), 446 (12900), 384 (12100) nm (ε). UV-VIS (CH_3OH, $c = 1.10^{-2}$ mol/l): $\lambda_{max.} = 638$ (24500), 589 (23900), 470 (13900) nm (ε), compare Fig. 1 for the UV-VIS spectrum in dimethylsulfoxide. According to ^{1}H-NMR and AAS analysis its formulae can be computed as $C_{30}H_{15}O_8^{(-)}$ $(K)^{(+)}$.

Preparative Procedure to Isolate **1** *and* **2** *from Plant Material*

500 g blossoms of *Hypericum perforatum* L. were frozen with liquid nitrogen, ground, and extracted for 30 min with 1300 ml dichloromethane using an "ultra turrax" device. The mixture was filtered, the residue was washed with an additional portion of 500 ml dichloromethane and the organic phases containing carotenoids, chlorophyll, and other pigments, was discarded. The resulting filter cake was extracted with 1200 ml 2-butanone containing 30 ml N-ethyl-N,N-diisopropylamine using an "ultra-turrax" device. After filtration and washing with 500 ml 2-butanone the extract was evaporated on a rotavapor. The resulting residue was dissolved in 600 ml methanol/water = 4/1, filtered off from some insoluble mass and evaporated to dryness (10.5 g). During this time the evaporation flask was irradiated with a 500 W tungsten lamp to achieve photo-transformation of the protopigments. Chromatography of the residue on Sephadex LH-20® (dichloromethane/methanol/water = 4/5/1) afforded after precipitation of the fractions with trifluoroacetic acid and careful drying at 10^{-2} torr 1.1 g **1** and 2.9 g **2**, which were identical with authentic material.

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