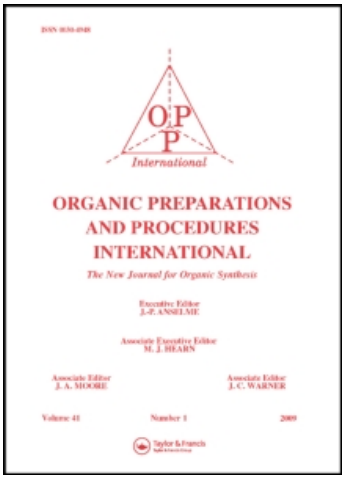

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AN IMPROVED SYNTHESIS OF HYPERICIN AND RELATED COMPOUNDS †

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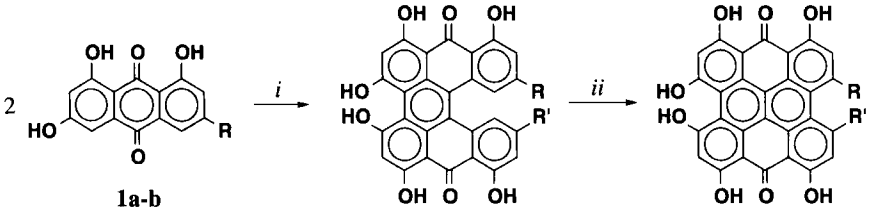
Hypericin (**3a**), the natural hydroxylated polycyclic quinone from the traditional medicinal plant St. John's wort (*Hypericum* genus) has been known for decades.[1] In recent years, the use of hypericin experienced a renaissance and much attention has been directed to it as a photodynamic agent possessing light-induced antiviral activity against several enveloped viruses, including human immunodeficiency virus (HIV), herpes simplex (HSV), Sindbis virus, murine cytomegalovirus (MCMV), equine infectious anemia virus (EIAV).[2] Hypericin has been demonstrated to photoinactivate protein kinase C (PKC),[3a] succinoxidase,[3b] tyrosine protein kinase (TPK)[3c] and was identified as a phosphatidylinositol-3-kinase (PtdIns-3-kinase) inhibitor.[3d] The photochemistry, photophysics, stereochemistry, tautomerization, photobiological activities and biomedical applications of hypericin have been intensively investigated by a broad spectrum of researchers.[4] The compound was shown to inhibit the growth of a variety of neoplastic cell types.[5] Hypericin is used as a novel photosensitizer for both photodynamic therapy (PDT) and for diagnostic applications.[6]

In 1957 Brockmann et al.[7] published the first multistep synthesis of hypericin (**3a**) starting with 3,5-dimethoxyphthalic anhydride and m-cresol. One year later, the synthesis of **3a** was accomplished from emodin anthrone, which was used in the oxidative dimerization with ferric chloride to give emodin bianthrone followed by a base-catalyzed oxidation to protohypericin (**2a**) and finally exposure to bright sunlight to yield **3a**.[8a] This synthetic strategy was later adopted by others.[8b-c] Another oxidative dimerization of emodin anthrone in pyridine, in the presence of pyridine N-oxide, piperidine and ferrous sulfate followed by photocyclization was reported in the patent literature.[9] The synthesis of hypericin directly from emodin (**1a**) by reductive coupling was described by Cameron et al. [8b] and Steglich et al.[10] This dimerization was conducted in a sealed tube, in a nitrogen atmosphere, in a 3 week long treatment with aqueous alkali and hydroquinone at 110°. After workup, irradiation in bright sunlight and purification, **3a** was obtained in 29% yield.

We were attracted to the latter synthetic methodology (see Scheme), which does not start from emodin anthrone, available through a tedious, multistep procedure starting with 3-methylsalicylic acid [11] or by reduction of emodin.[8] It is also not necessary to separate and purify a mixture of meso and racemic bianthrones, intermediates in the oxidative coupling of emodin anthrone. Emodin, a

commercially available starting material for reductive dimerization, is also easily isolated in good yields from the commercial bark *Cortex frangulae*.[8c]

In order to find an efficient method of improving hypericin accessibility, we proceeded to systematically investigate a combination of variable parameters. For the purpose of excluding oxidative coupling, aneorobic reaction conditions were maintained in each case. The following parameters were investigated: base and its concentration, solvent, concentration of inhibitor of free radical reactions, the molar ratios of emodin and base, reaction times and temperatures, applications of catalysts, use of different reaction vessels, and the reaction scale.



1a-b

a) R = CH_3
b) R = CO_2H

2a-c

3a-c

a) R = R' = CH_3
b) R = R' = CO_2H
c) R = CH_3, R' = CO_2H

i) 1. KOH/H_2O, N_2, hydroquinone, 155°, 6 days, 2. aq. HCl *ii*) bright sunlight, acetone, 20 min.

The convertion of emodin (**1a**) into protohypericin (**2a**), initially formed in the dimerization reaction, under aneorobic conditions (in a nitrogen atmosphere) requires the application of a strong base, high concentration of base and emodin. The yield depends greatly on the temperature of the reaction, higher temperatures resulting in higher yields. Application of a catalyst (piperidine) also increased the yield, but at temperatures lower than 140° (100-130°). Above that temperature, the catalyst showed no effect on improving the yield of coupling. Results showed that it is necessary to carry out the reaction in the presence of a free radical inhibitor. Hydroquinone was used in a molar ratio 2:1 vs. emodin. By the use of equimolar amounts of emodin and hydroquinone the reaction was completed in 2 days, but lower yields of desired product was obtained. The alkaline dimerization of **1a** is best carried out in a sealed system consisting of a capped heavy wall reaction vial for a small scale (ca. 0.2 mmol - 1 mmol), or a general purpose bomb for a large scale (over 2 mmol). In an open flask, the yield of desired product was lowered, impurities were increased and longer reaction times did not improve the reaction yield. The transformation of crude protohypericin into hypericin took place readily upon exposure of an acetone solution of **2a** to bright sunlight.[12] Using our optimized procedure pure (98.7% by HPLC) hypericin (**3a**) was obtained in 45% yield. The conversion of **1a** into **3a** was in fact much greater than 45% (by ^{1}H NMR), but a substantial amount of material was lost during chromatographic purifications. Both, small and large scale procedures gave essentially the same results.

This optimized procedure was also used to prepare previously synthesized hypericin dicarboxylic acid (**3b**) [8b, 10] and hypericin monocarboxylic acid (**3c**).[8b] In contrast to hypericin, **3b** and **3c**

were slightly soluble in water. This increased solubility in water could make **3b** and **3c** more useful as drugs. Emodic acid (**1b**), the starting material for the preparation of **3b**, was produced in 78% yield by acetylation of emodin (**1a**) followed by chromium trioxide oxidation and subsequent hydrolysis of the resulting acetate.[13] Pure hypericin dicarboxylic acid (**3b**) was obtained in 42% yield. Emodic acid was also coupled with emodin, using optimized conditions, followed by exposure to bright sunlight to produce the expected mixture of three compounds **3a**, **3b** and **3c**. The mixture was separated by a combination of silica gel chromatography and gel filtration on Sephadex LH-20. The major product of the mixed coupling was found to be consistent in all properties (^{1}H NMR, UV and IR spectra) with the expected unsymmetrical hypericin monocarboxylic acid (**3c**). Pure **3c** (99.7% by HPLC) was separated from the mixture of three products in 20% yield.

In conclusion, we report here a modified, convenient and efficient procedure for the preparation of high purity hypericin, and related compounds.

EXPERIMENTAL SECTION

Emodin was purchased from Aldrich Chemical Co. Capped heavy wall reaction vials were bought from Pierce Chemical Co. and ACE Glass, Inc. A general purpose bomb was purchased from Parr Instrument Co. Melting points were taken on a Kofler hot stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker WM-300 spectrometer equipped with an Aspect 2000 or a Varian XL-400 spectrometer. IR spectra were recorded on a Nicolet 520 FT spectrophotometer. UV-VIS spectra were recorded on a Perkin Elmer Lambda 19 spectrophotometer. Mass spectra were measured on a VG 70-SE, 2 sector, forward geometry instrument. HPLC analyses were performed on a Hewlett-Packard HP 1090 system using a C_{18} reversed-phase, 5 µm, 250 x 4 mm column, mobile phase: MeOH / H_2O, program: A=MeOH, B=H_2O, start with A:B = 50:50, after 5 min. A=100%, flow rate = 1 mL/min. TLC chromatography was performed with EM Science precoated silica gel 60 F-254 plates. Spots were detected with shortwave UV light. Column chromatography was carried out on ICN Industries, Inc. 60-200 µm silica gel. Gel filtration was performed on Sigma Chemical Co. 25-100 µ lipophilic Sephadex LH-20.

Hypericin (1,3,4,6,8,13-hexahydroxy-10,11-dimethylphenanthro(1,10,9,8-*opqra*)perylene-7,14-dione, **3a**).- A solution of emodin (**1a**, 1.351 g, 5.0 mmol) and hydroquinone (1.101 g, 10.0 mmol) in 0.8 N KOH (25 mL) was placed in a general purpose bomb (Parr Instrument Co.) and flushed with nitrogen. The reaction mixture was then heated in a sealed high pressure reactor, under nitrogen, at 155° for 6 days. The reactor was cooled to room temperature and the reaction mixture was acidified to pH 1 with 0.1 N HCl; the resulting precipitate was removed by filtration, then washed with dil. HCl followed by water, dried and finally taken up in acetone (500 mL). The insoluble material was filtered off and washed with acetone (ca. 1.5 L) until the washings were colorless. The combined violet-colored acetone solution was concentrated under reduced pressure and the concentrate (ca. 1 L) was exposed to bright sunlight for 20 min. The reaction was monitored by TLC. The resulting cherry-red solution was evaporated to dryness, under reduced pressure. The residue was purified by silica gel column chromatography using as eluent a mixture of CH_2Cl_2/CH_3COCH_3/CH_3OH in ratios varying from 90:8:2 (by volume) to 75:15:10. Repeated chromatography of the enriched material on silica gel,

elution with a mixture of $CHCl_3/CH_3OH$ (95:5 to 90:10) followed by a gel filtration on Sephadex LH-20 using methanol as eluent gave pure (98.7% by HPLC) **3a** in 45% yield (0.567 g) as a black-violet solid. mp. >300°; TLC (silica gel, $CH_2Cl_2/CH_3COCH_3/CH_3OH$ = 75:15:10) R_f 0.45; ^1H NMR (acetone-d_6 + 2 drops of D_2O): δ 7.37 (s, 2H, CH-9,12), 6.66 (s, 2H, CH-2, 5), 2.77 (s, 6H, CH_3); UV-VIS spectra in methanol and IR spectra in KBr pellet were identical to those previously described, respectively.[14a,b] HRMS calcd for $C_{30}H_{17}O_8$ $(M+1)^+$ 505.0923, found 505.0924.

Hypericin Dicarboxylic Acid (1,3,4,6,8,13-hexahydroxy-10,11-dicarboxyphenanthro(1,10,9,8-*opqra*)perylene-7,14-dione, **3b**).- Compound **3b** was prepared in analogy to **3a** from **1b**. In the purification procedure, the solvent system used as eluent in silica gel column chromatography was $CHCl_3/CH_3OH$ in ratios varying from 99:1 (by volume) to 60:40. Pure **3b** was obtained in 42% yield after gel filtration on Sephadex LH-20 using methanol as eluent. mp. >300°; TLC (silica gel, $CHCl_3/CH_3OH$ = 1:1) R_f 0.12; ^1H NMR (DMSO-d_6 + $CDCl_3$ = 7:3): δ 7.90 (s, 2H, CH-9,12), 6.66 (s, 2H, CH-2,5); UV-VIS (CH_3OH) λ [nm] (ε): 588 (22200), 545 (10500), 507 (3600), 473 (6100), 387 (4500), 329 (14800), 286 (16800), 251 (21700), 227 (35600), 213 (42500); IR (KBr) ν_{max} [cm^{-1}]: 3427, 2957, 2924, 2853, 1616, 1576, 1559, 1506, 1497, 1457, 1385, 1247, 1178, 1107, 840, 693 , 657.

Hypericin Monocarboxylic Acid (1,3,4,6,8,13-hexahydroxy-10-carboxy-11-methylphenanthro(1,10,9,8-*opqra*)perylene-7,14-dione, **3c**).- Compound **3c** was prepared in analogy to **3a** from **1a** and **1b**. The crude material - a mixture of three products **3a-c**, was chromatographed on silica gel column using as eluent a mixture of $CHCl_3/CH_3OH$ in ratios varying from 99:1 (by volume) to 60:40. All collected fractions were analyzed by TLC. The nonhomogeneous fractions, containing **3c**, were rechromatographed on silica gel column. Subsequent gel filtration on Sephadex LH-20 of all combined fractions containing hypericin monocarboxylic acid, elution with methanol, gave **3c** in 20% yield. mp. >300°; TLC (silica gel, $CHCl_3/CH_3OH$ = 3:2) R_f 0.40; ^1H NMR (CD_3OD): δ 7.91 (s, 1H, CH-9), 7.30 (s, 1H, CH-12), 6.77 (s, 1H, CH-2 or CH-5), 6.74 (s, 1H, CH-2 or CH-5), 3.03 (s, 3H, CH_3); UV- VIS (CH_3OH) λ [nm] (ε): 588 (24600), 546 (12200), 509 (4300), 470 (7300), 388 (5700), 328 (16500), 287 (19400), 251 (21700), 228 (33300), 211 (42100); IR (KBr) ν_{max} [cm^{-1}]: 3423, 2955, 2923, 2853,1617, 1576, 1559, 1506, 1497, 1464, 1419, 1385, 1289, 1251, 1187, 1111, 1073, 943, 846, 802, 754, 701, 667, 624.

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