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Synthesis and ESR Investigation of Hypocrellin Glycoside

Yu-Ying He, Jing-Yi An and Li-Jin Jiang*

Institute of Photographic Chemistry, Academia Sinica, Beijing 100101, People's Republic of China

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Abstract: A glycoside derived from hypocrellin B was designed and synthesized using an improved Konigs-Knorr reaction. The water-solubility and red absorption of the resulting product was enhanced significantly over hypocrellin B. Electron spin resonance (ESR) measurements indicated that this glycoside remained photodynamically active in terms of Type I and Type II mechanisms. © 1998 Elsevier Science Ltd. All rights reserved.

Hypocrellins, including hypocrellin A (HA) and hypocrellin B (HB, **1**), are new photodynamic agents isolated from the fungus of *Hypocrella bambuase* (B.et Br) sacc. grown abundantly in Yunnan province of China[1]. These lipid-soluble agents exhibit strong light absorption in the phototherapeutic window (600-900nm), high photosensitizing efficiency and low degree of delayed skin photosensitivity[2], and have been used in the clinical treatment of some skin diseases[1]. However, hypocrellins are insoluble in water and their red absorption is not strong enough for photodynamic therapy (PDT). In addition, it has been reported that some carbohydrate substituted porphyrins exhibit enhanced selectivity to cancer cells[3] and are easily built into model membranes due to their amphiphilic character[4]. Until recently, however, no effort has been devoted to the synthesis of carbohydrate substituted hypocrellin. We herein describe the synthesis and some preliminary investigations of this new amphiphilic hypocrellin derivative that exhibits favorable PDT properties.

Previous studies on hydroxylperylenequinones suggested that the peri-hydroxyl groups at 3 and 10 positions played an essential role in their PDT processes[5]. Also direct glycosylation rarely occurred under

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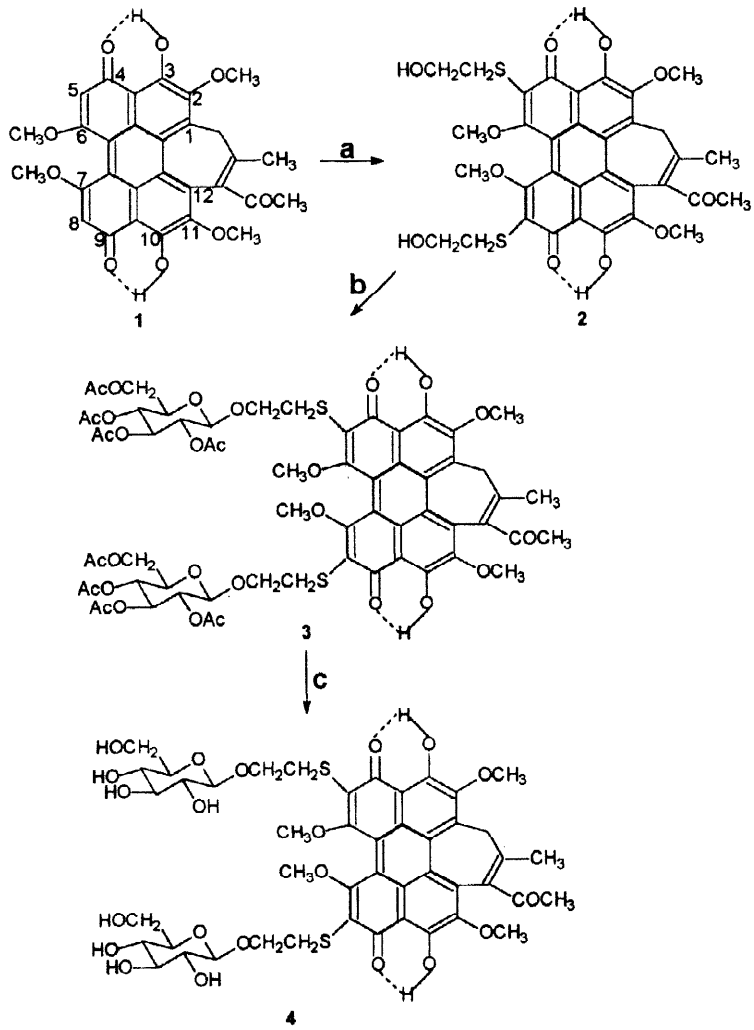
Königs-Knörr conditions on monitoring by ^{1}H-NMR spectroscopy and TLC analysis, with either hypocrellin A or hypocrellin B.

We have successfully synthesized 5,8-dimercaptoethanol-substituted hypocrellin B (**2**) photochemically (**scheme 1**). Irradiation of the aerated ethanol-buffer (1:3 by volume, pH 11.0) of HB (0.1mM) and mercaptoethanol (10mM) with light of wavelength longer than 470 nm afforded **2** with quantum

Scheme 1



a. $HSCH_2CH_2OH$, $h\nu$(>470nm), C_2H_5OH-buffer (1:3 by volume pH11.0); **b.** α-D-acetobromoglucose, Ag_2O, I_2, molecular sieves, benzene/chloroform (8:1); **c.** CH_3ONa/CH_3OH.

yield of approximately 0.02. Compound **2** was isolated in about 95% yield after chromatographic work up. As compared with a thermal method, the photochemical method has several advantages were attained such as higher yield, higher reaction rate and no byproducts.

Coupling of diol **2** with acetobromo-α-D-glucose yielded β-D-hypocrellin glycoside precursor **3** via the optimized Königs-Knorr reaction[6], in which the ratio of I_2/Ag_2O/ acetobromoglucose/alcohol was 1:2:2:1 with the addition of powdered 4 Å molecular sieves (MS) activated at 500 °C for 2 hours. Dichloromethane and chloroform proved to be unsuitable for the glycosylation and the reaction yield of glycoside precursor **3** decreased significantly. Benzene was suitable with regard to reaction rate, but its solubility for **2** was insufficient. Therefore we used an 8:1 mixture of absolute benzene and chloroform.

Deacetylation of **3** was complete within 2 hours using 0.05N CH_3ONa in absolute methanol. Hypocrellin glycoside (**4**) was obtained with nearly quantitative yield, followed by extraction with chloroform, evaporation under reduced pressure, purification by TLC ($CHCl_3$:CH_3OH 5:1) and recrystallization from methanol. All products were proved to be pure and stereochemically uniform by elemental analysis, ^{1}H-NMR, IR, MS and TLC. The yield of **4** was about 65% based on the amount of hypocrellin B used.

Partition coefficients of **1**, **2**, **3** and **4** between n-octanol and phosphate buffer (pH 7.4) were determined at 20 °C, which are 46.4, 20.6, 38.5, and 1.3, respectively. Also the molar extinction coefficient (ε) of **4** at 600 nm in chloroform has increased up to 10260 from 2329 for that of **1**. As expected, both the water solubility and red absorption of **4** have been enhanced greatly over **1**.

Compound **2** and **4** were tested by ESR spectroscopy to determine whether they still possessed photodynamic properties. The experimental results indicated that irradiation of the deoxygenated DMSO solution of **2** or **4** generated a strong ESR signal ascribed to the semiquinone anion radical based on a series of experiments, and thus the superoxide anion radical in DMSO and hydroxyl radical in water in the presence of oxygen, but with enhanced efficiencies compared with HB. The singlet oxygen-generating quantum yields of both **2** and **4** were determined to be 0.19 by 9,10-diphenylanthracene bleaching method, and lower than that of HB (0.76). It can be inferred that the Type I mechanism is predominant for **4** as well as for **2**, the changes from the predominant Type II mechanism observed for HB to the predominant Type I mechanism for **2** and **4** resulted from thiylation on the 5 and 8 positions. Glycosylation of **2** had little influence on the photodynamic action derived from the perylenequinone chromophore. Work is under way in our laboratory to study the photodynamic action of **4** *in vitro* and *in vivo* in detail.

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7 Data for **2:** FAB-MS: m/e 680. Uv-Vis ($CHCl_3$): λ (logε) = 514 (4.28), 580 (4.04) nm. IR (KBr): 3439, 1710, 1605 cm^{-1}. ^{1}H-NMR (300MHz,$CDCl_3$) δ: 16.00 (s,1H), 15.96 (s,1H), 4.11 (s,3H), 4.09 (s,3H), 3.96 (d,1H,J=12Hz), 3.90 (s,3H), 3.85 (s,3H), 3.75 (m,4H), 3.25 (m,4H), 3.20 (d,1H,J=12Hz), 2.36 (s,3H) and 1.86 (s,3H). **3.** FAB-MS: m/e 1340. Uv-Vis ($CHCl_3$): λ (logε) = 516 (4.28), 582 (4.04) nm. IR (KBr): 3439, 1744, 1595, 1229, 1030, 895, 800 cm^{-1}. ^{1}H-NMR (300MHz,$CDCl_3$) δ: 15.96 (s,1H), 15.92 (s,1H), 5.78 (d,1H,J=4Hz), 5.56 (d,1H,J=4Hz), 5.38 (t,1H,J=10Hz), 5.20 (t,1H,J=10Hz), 5.00 (t,1H,J=10Hz), 4.92 (t,1H,J=10Hz), 4.78 (dd,1H,J=4Hz), 4.60 (dd,1H,J=4Hz), 4.20 (m,6H), 4.10 (s,3H), 4.05 (s,3H), 4.00 (d,1H,J=12Hz), 3.97 (s,3H), 3.83 (s,3H), 3.78 (m,4H), 3.28 (m,4H), 3.20 (d,1H,J=12H), 2.37 (s,3H), 2.12 (s,6H), 2.08 (s,12H), 2.02 (s,6H) and 1.96 (s,3H). **4.** FAB-MS: m/e 1004. Uv-Vis ($CHCl_3$): λ (logε) = 510 (4.28), 586 (4.04) nm. IR (KBr): 3416, 1695, 1590, 1259, 1100, 1070, 1018, 900, 800 cm^{-1}. ^{1}H-NMR (300MHz,$CDCl_3$) δ: 15.96 (s,1H), 15.93 (s,1H), 4.45-5.40 (br,10H), 4.16 (s,3H), 4.06 (s,3H), 4.02 (s,3H), 4.00 (s,3H), 3.60-3.96 (m,15H), 3.12-3.18 (m,7H), 2.38 (s,3H) and 1.96 (s,3H) . (Due to the insufficient solubility of glucal moiety in $CDCl_3$, no well resolved NMR spectra could be obtained for it.) Anal. calc. for $C_{46}H_{52}O_{21}S_2$ (1004.24): C,54.97; H,5.22; S,6.37. Found: C,54.76; H,5.01; S,6.42.