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A Novel Method for the Preparation of Amino-Substituted Hypocrellin B

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Abstract—A series of amino-substituted hypocrellins derived from hypocrellin B (HB) were synthesized by a novel mild method, in which the peri-hydroxylated perylenequinone structure of hypocrellin was preserved by the reaction of HB with an amine. The red absorption of the resulting products was significantly enhanced relative to the parent hypocrellins, which will significantly improve its photodynamic therapy effectiveness.

Photodynamic therapy is a medical treatment which employs a combination of light and a photosensitizing agent to bring about a cytotoxic or modifying effect of cancerous or other unwanted tissue. Active oxygen species generated during the irradiation of the dyes with light play a predominant role in the photodynamic activity.

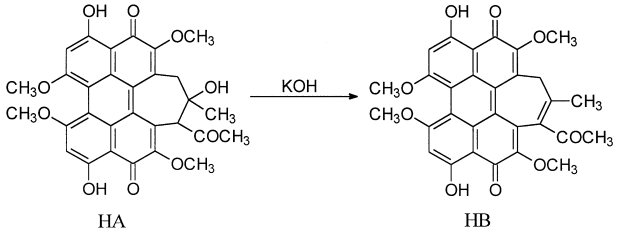
The naturally occurring polycyclic quinones, hypocrellins (including hypocrellin A and hypocrellin B) (Scheme 1), isolated from the fungus *Hypocrella bambuase* (B. et Br) sacc, have gained considerable attention because of their light-induced antitumor and antiviral activity, most notably against the human immunodeficiency virus, HIV.[1,2] In addition, the hypocrellins have several advantages over the presently used photodynamic therapeutic agent, *photofrin II*,[3] including easy preparation and purification, low aggregation tendency and rapid metabolism in vivo.[4,5]

However, hypocrellins exhibit little absorption in the photodynamic window (600–900 nm), which limits their applications in PDT.

A group of hypocrellin derivatives has been synthesized in recent years,[6–10] but with few promising results. However, the amino-substituted hypocrellin derivatives were found to possess higher photodynamic activity than any other derivatives.[11] The original peri-hydroxylated perylenequinone structure of hypocrellin was altered by the amino substitution, which impeded the intramolcular proton transfer process. The possible role of labile protons in the photodynamic activity of the polycyclic quinones was discussed in detail in the literature.[12–15]

To overcome these limitations and to extend the photoresponse of the PDT agents, an electron-donating alkylamino group was introduced into the peri-hydroxylated perylenequinone ring of HB, which made the intramolecular charge transfer (ICT) readily between the hydroxyl group and the carbonyl group in HB. The ICT distinctly red-shifted the absorption spectra of the sensitizers.[16,17]

This paper describes a useful method by which the peri-hydroxylated perylenequinone structure is preserved and the photoresponse of the dye is enhanced remarkably. The solvent pyridine was chosen as the key reaction media. The reaction of HB and an amine occurred



Scheme 1. The structures of HA and HB.

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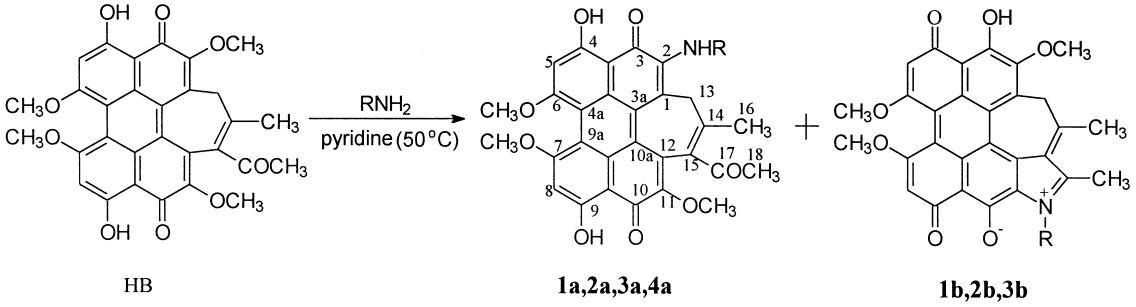

Scheme 2. Pathway for the amino-substituted hypocrellin B.

at 50 °C with good selectivity. The chemical structure of hypocrellin suggests multiple possible reaction sites, such as the aromatic ring (positions 5 and 8), quinonoid carbonyl groups[11] and the attached ring,[18] but the yields in previous research using these reactions were rather low due to polymerization during the reaction at high temperature or in strongly basic media.

The high yield of the amination in our one-step reaction is also noteworthy.

The current one-step method[20] for the amino substitution described in Scheme 2 has a high yield.

Comparison of the ^{1}H NMR spectra from the parent HB, showed the product had only three methoxy groups. The quinonoid carbonyl groups were retained in the amino-substituted HB as evidenced by their IR spectra which were similar to that of HB. Their molecular ion peaks, UV–vis spectra data and the absence of 2-OMe or 11-OMe protons, were used to assign their structures to 2-butylamino demethoxy HB and 11-butylaminodemethoxy HB (Table 1).[21]

In the reaction process and later processing, the reaction solution temperature was always less than 55 °C to prevent significant side reactions.

Using pyridine as the reaction medium provided significantly better results than any other solvent tested. At similar conditions, the amination cannot occur in any other solvent that we find. The reaction mechanism will be studied with further investigations of the interaction between hypocrellin and pyridine to be reported later.

The amino-substituted hypocrellins, which showed a strong absorption band above 600 nm with $\lg \varepsilon > 4$, were tested using spin trapping EPR technique to determine whether they possessed photodynamic activities. The results indicated that they displayed strong EPR signals due to the singlet oxygen and superoxide anion radical,[19] which are essential for a potent photodynamic agent. Moreover, cellular studies showed that compound **1a** had much greater phototoxicity (with a 200- to 400-fold photopotentiation factor) than their parent HB (with a 10-fold photopotentiation factor), with 50% cytotoxicity at concentrations of 0.25–0.5 μM at a light dose of 4 J/cm. Cellular and animal experiments of other compounds are in progress.

Table 1. Main products of the amino-substituted hypocrellin B

R =	n-C_4H_9	n-C_5H_{11}	$PhCH_2$	C_6H_{11}
2-NHR	**1a** (54%)	**2a** (62%)	**3a** (56%)	**4a** (88%)
11-NR	**1b** (25%)	**2b** (19%)	**3b** (24%)	

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20. Experimental. HB (200 mg) was dissolved in freshly distilled pyridine (250 mL) containing alkyl amine (20 mL). The resulting solution was stirred for 10–20 h at 50 °C in the dark. The solvent was removed under reduced pressure. Then chloroform was added and the solution was washed with dilute hydrochloric acid several times until the pH value of the water layer was neutral. Chloroform was evaporated to afford a black solid, which was separated by thin layer chromatography (TLC) on a 1–2% KH_2PO_4 silica gel plate using 4:2:1 petroleum ether/ethyl acetate/95% ethanol as eluent. The products were obtained and identified by ^{1}H NMR, UV–vis, IR, mass spectra and elemental analysis.
21. Data for **1a**: 54%. FAB-MS: *m*/*e* 569. UV–vis [($CHCl_3$) $\lambda_{max,nm}$ (log ε)]: 463 (4.06), 583 (4.09), 621 (4.10). IR (KBr): 3340, 2922, 1706, 1600. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.07 (3H, t, CH_3-22), 1.50 (2H, m, CH_2-21), 1.78 (3H, s, CH_3-16), 1.95 (2H, m, CH_2-20), 2.34 (3H, s, CH_3-18), 2.80 (1H, d, H-13a, J_{AB} = 11.5), 3.70 (2H, m, NCH_2), 3.90 (1H, d, H-13b, J_{AB} = 11.5), 4.05 (6H, s, OCH_3-6,7), 4.09 (3H, s, OCH_3-11), 6.38 [1H, s, H-5(8)], 6.60 [1H, s, H-8(5)], 15.8 [1H, OH-3(4)], 16.1 [1H, OH-9(10)]. Elemental analysis: requires: C, 69.60; H, 5.45; O, 22.50; N, 2.45. Found: C, 69.58; H, 5.57; O, 22.40; N, 2.56. **1b**: 25%. FAB-MS: *m*/*e* 550. UV–vis [($CHCl_3$) $\lambda_{max,nm}$ (log ε)]: 400 (3.60), 470 (3.60), 600 (3.60). IR (KBr): 2923, 1596. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03 (3H, t, CH_3-22), 1.42 (2H, m, CH_2-21), 1.80 (2H, m, CH_2-20), 2.63 (3H, s, CH_3-16), 2.65 (1H, d, H-13a), 2.73 (3H, s, CH_3-18), 3.75 (1H, d, H-13b), 4.05 (6H, s, OCH_3-6,7), 4.15 (3H, s, OCH_3-2), 4.68 (2H, m, NCH_2), 6.65 [1H, s, H-5(8)], 7.05 [1H, s, H-8(5)], 17.12 (1H, OH-3). Elemental analysis: requires: C, 72.00; H, 5.09; O, 20.36; N, 2.55%. Found: C, 72.10; H, 5.13; O, 20.25; N, 2.62%. **2a**: 62%. FAB-MS: *m*/*e* 583. UV–vis [($CHCl_3$) $\lambda_{max,nm}$ (log ε)]: 471 (3.92), 625 (4.14). IR (KBr): 3435, 2940, 1682, 1604. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (3H, t, CH_3-23), 1.27 (2H, m, CH_2-22), 1.42 (2H, m, CH_2-21), 1.62 (2H, m, CH_2-20), 1.78 (3H, s, CH_3-16), 1.90 (2H, m, NCH_2), 2.28 (3H, s, CH_3), 2.63 (1H, d, H-13a), 3.98 (1H, d, H-13b), 4.04 (3H, s, OCH_3-6), 4.06 (3H, s, OCH_3-7), 4.13 (3H, s, OCH_3-11), 6.37 [1H, s, H-5(8)], 6.59 [1H, s, H-8(5)], 16.59 [1H, OH-3(4)], 16.80 [1H, OH-9(10)]. Elemental analysis: requires: C, 70.00; H, 5.65; O, 21.95; N, 2.40%. Found: C, 70.04; H, 5.68; O, 21.92; N, 2.36%. **2b**: 19%. FAB-MS: *m*/*e* 536. UV–vis [($CHCl_3$) $\lambda_{max,nm}$ (log ε)]: 467 (3.86), 618 (4.06). IR (KBr): 2945, 1648, 1600. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12 (3H, t, CH_3-23), 1.25 (2H, m, CH_2-22), 1.38 (2H, m, CH_2-21), 1.58 (1H, d, H-13a), 1.75 (2H, m, CH_2-20), 2.05 (3H, s, CH_3-16), 2.34 (3H, s, CH_3-18), 3.60 (2H, m, NCH_2), 3.86 (1H, d, H-13b), 4.02 (3H, s, OCH_3-6), 4.06 (3H, s, OCH_3-7), 4.18 (3H, s, OCH_3-2), 6.45 [1H, s, H-5(8)], 6.61 [1H, s, H-8(5)], 16.80 [1H, OH-3(4)], 17.00 [1H, OH-9(10)]. **3a**: 56%. FAB-MS: *m*/*e* 603. UV–vis: [($CHCl_3$) $\lambda_{max,nm}$ (log ε)]: 458 (4.15), 579 (4.20), 628 (4.06). IR (KBr): 3380, 2945, 1675, 1604, 1580, 1450. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.68 (3H, s, CH_3-16), 2.25 (3H, s, CH_3-18), 2.63 (1H, d, H-13a), 3.56 (2H, m, NCH_2), 3.96 (1H, d, H-13b), 4.03 (3H, s, OCH_3-6), 4.05 (3H, s, OCH_3-7), 4.20 (3H, s, OCH_3-11), 6.49 [1H, s, H-5(8)], 6.50 [1H, s, H-8(5)], 7.38 (3H, m, Ph-H), 7.54 (2H, m, Ph-H), 15.8 [1H, OH-3(4)], 16.1 [1H, OH-9(10)]. **3b**: 24%. FAB-MS: *m*/*e* 585. UV–vis: [($CHCl_3$) $\lambda_{max,nm}$ (log ε)]: 467 (4.09), 579 (4.14), 625 (3.98). IR (KBr): 3410, 2954, 1643, 1600, 1578, 1445. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.85 (3H, s, CH_3-16), 2.63 (1H, d, H-13a), 2.72 (3H, s, CH_3-18), 4.03 (3H, s, OCH_3-6), 4.04 (3H, s, OCH_3-7), 4.09 (1H, d, H-13b), 4.14 (3H, s, OCH_3-2), 4.65 (2H, m, NCH_2), 6.27 [1H, s, H-5(8)], 6.66 [1H, s, H-8(5)], 7.02 (3H, m. Ph–H), 7.48 (2H, m, Ph–H), 15.92 [1H, OH-3(4)], 16.15 [1H, OH-9(10)]. **4a**: 88%. FAB-MS: *m*/*e* 595. UV–vis: [($CHCl_3$) $\lambda_{max,nm}$ (log ε)]: 474 (4.06), 581 (4.10), 620 (3.86). IR (KBr): 3360, 2935, 1675, 1605. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 [2H, m, (CH_2-22)], 1.51 [4H, m, CH_2-21,23], 1.76 (3H, s, CH_3-16), 1.94 (4H, m, CH_2-20,24), 2.32 (3H, s, CH_3-18), 2.76 (1H, m, NCH), 4.04 (3H, s, OCH_3-6), 4.05 (3H, s, OCH_3-7), 4.17 (3H, s, OCH_3-11), 6.48 [1H, s, H-5(8)], 6.52 [1H, s, H-8(5)], 16.42 [1H, OH-3(4)], 16.77 [1H, OH-9(10)]. Elemental analysis: requires: C, 70.58; H, 5.56; O, 21.51; N, 2.35%. Found: C, 70.66; H, 5.56; O, 21.39; N, 2.38%.