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First Synthesis of Methylated Hypocrellin and Its Fluorescent Excited State: A Cautionary Tale

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Received October 10, 2002

Abstract: Methylated hypocrellins were obtained and characterized by satisfactory ^{1}HNMR, UV−vis, IR, and mass data, and their absorption and fluorescence emission spectra were studied. A previous report of methylated hypocrellin (*J. Phys. Chem. A* **1999**, *103*, 7949) appears to be in error.

Photodynamic therapy (PDT) is a medical treatment that employs a combination of light and a photosensitizing agent to bring about a cytotoxic or modifying effect of cancerous or other unwanted tissue. PDT exhibits high selectivity of light-orientation and low damage to normal tissues, suggesting its high drug safety.

Investigations of the intramolecular H-atom transfer process in perylenequinone pigments have suggested that the intramolecular H-atom transfer process might play a significant role in photodynamic therapy.[1−6] Petrich and co-workers reported the synthesis of methylated hypocrellin A (HA, Figure 1) and studies of its absorption spectrum, emission spectrum, and fluorescence[7] and described such intramolecular H-atom transfers.

We prepared the monomethylated (**3**) and dimethylated hypocrellin B (**4**) using the reaction conditions as shown in Figure 1. The structures of **3** and **4** were identified by satisfactory data of IR, ^{1}H NMR, UV−vis, and MS. By comparing the ^{1}H NMR spectral data of **3** and **4** with hypocrellin B, we found that five methoxy groups existed in **3** and six in **4**. Quinonoid carbonyl groups were presented, as the IR spectrum is similar to that of HB, except that the band of the hydroxyl group above 3000 cm^{-1} is absent for **4**. On the basis of the molecular ion peaks (M + 1, 543.1654 and 557.1809 for **3** and **4**, respectively), the structures of **3** and **4** are assigned as 3-methoxy-substituted hypocrellin B and 3,-10-dimethoxy-substituted hypocrellin B, respectively. The methylated HA prepared by Petrich was apparently incompletely identified, with only ^{1}HNMR spectra reported. We repeated the reported synthesis of the methylated hypocrellin[7] and did not obtain the methylated derivatives.

Hypocrellins have three absorption bands at 450, 535, and 584 nm in the region of the wavelength between 400 and 800 nm. The shorter wavelength absorption band (at 450 nm) was assigned to the $\pi-\pi^*$ transition, while the longer wavelength bands (at 535 and 584 nm) were considered to be related to the intramolecular H-atom transfer process between the *peri*-hydroxyl group and the quinonoid carbonyl group. In methylated hypocrellin molecules, the H-atoms of the *peri*-hydroxyl group were substituted with methyl groups and the intramolecular H-atom transfer process should be inhibited so that the 535- and 584-nm bands no longer appear. The absorption spectra of **3** and **4** were observed as shown in Figure 2. When the H-atom of the *peri*-hydroxyl group was substituted with a methyl group, the 450-nm absorption band blue-shifted to some degree and the 540- and 580-nm bands disappeared simultaneously, which further confirmed that the contributions of the longer wavelength bands are derived from the intramolecular H-atom transfer process. Nevertheless, a previous paper[7] by Petrich indicated that the absorption spectrum of the methylated hypocrellin was very similar to that of HB and still had absorption bands at 535 and 584 nm, which conflicted with the assignment of longer wavelength bands.

The fluorescence excitation and emission spectra of HB were observed as shown in Figure 3. Figure 3 shows that both the longer and shorter wavelength lights contribute to the fluorescence emission.

Moreover, Figure 3 also implies that the longer wavelength light contributes to the fluorescence emission more effectively than does shorter wavelength light. This suggests that the fluorescent excited state might be the intramolecular H-atom transfer state.

The fluorescence emission spectra of HB **3** and **4** are shown in Figure 4. The results show that when HB was replaced with **3**, the fluorescence quantum yield decreased by 70% and the emission band red-shifted distinctly. It is supposed that the methylation of HB heightened the vibration energy of the ground state and upon irradiation the excited state of compound **3** transited to the higher vibration energy level of the ground state, not directly to the 0 vibration energy level, and then was quenched by internal conversion. The fluorescence quantum yield of **4** decreased by almost 100% compared with that of HB.

The distinct fluorescent quenching indicated that the excited intramolecular H-atom transfer process might play a significant role in the hypocrellin fluorescence emission. It confirms Petrich's previous report that a protonated carbonyl group is needed for the hypericin-like fluorescence emission.[8] It is the intramolecular

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10.1021/jo026554x CCC: $25.00
Published on Web 02/01/2003

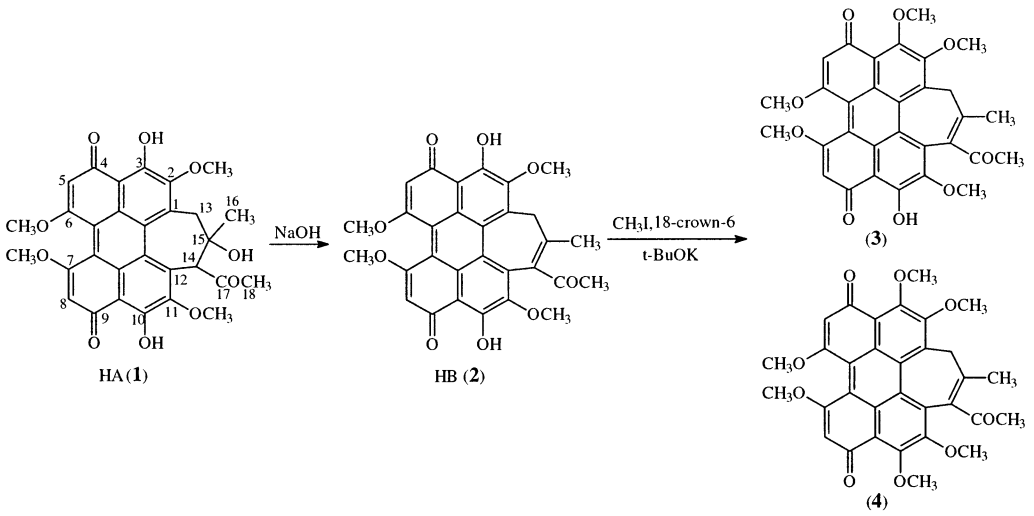

FIGURE 1. Pathway for the synthesis of methylated HB **3** and **4**.

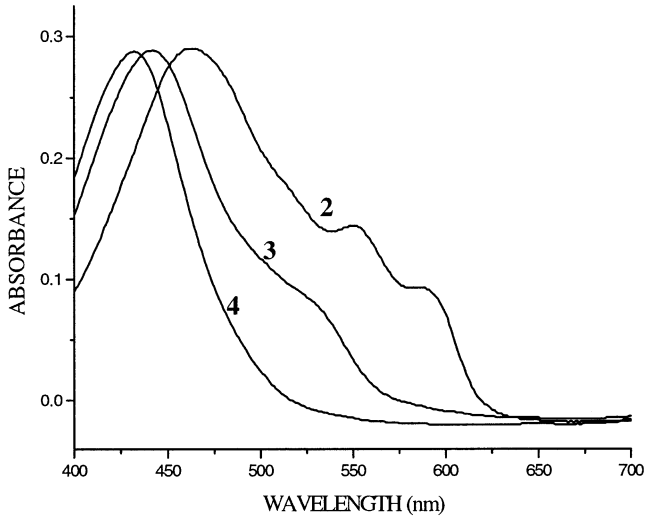

FIGURE 2. Absorption spectra of compounds **2**−**4** in chloroform.

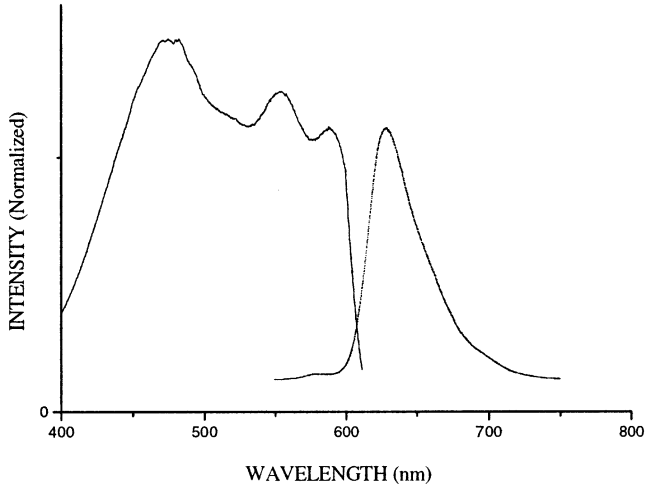

FIGURE 3. Fluorescence excitation (solid line) and emission spectra (dash line) of compound **2** (normalized) in chloroform.

H-atom transfer process from hydroxyl group to carbonyl group that produces the protonated carbonyl group.

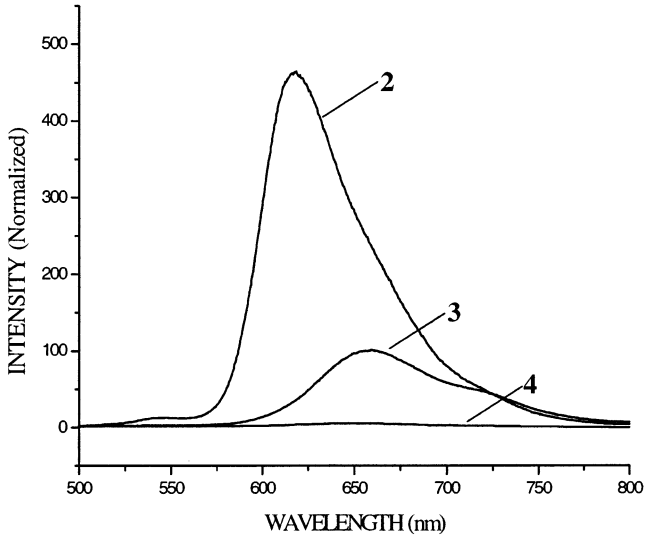

FIGURE 4. Fluorescence emission spectra of compounds **2**−**4** excited at 440 nm in chloroform.

The absorption spectrum and emission spectrum indicated that the fluorescent state of hypocrellin was derived from the intramolecular H-atom transfer process. According to our experimental results, we inferred that the intramolecular H-atom transfer process was impeded in methylated hypocrellin, and thus, the hypericin-like fluorescence emission is distinctly quenched.

Experimental Section

Synthesis of Compounds 3 and 4. Hypocrellin B (**2**) (100 mg) was dissolved in freshly distilled DMF (100 mL), and potassium *tert*-butoxide (100 mg) was added dropwise over 30 min. The resulting solution was stirred for 1 h below 5 °C in the dark. Then the mixture was transferred to a freezer, and the filtrate was filtered and dried under vacuum to afford KHB. KHB was dissolved in CH_3I (20 mL), and 18-crown-6 (200 mg) was added. The solution was refluxed for 48 h. The mixture was cooled, and chloroform and hydrochloride acid were added. The aqueous phase was extracted with chloroform several times, and the organic phase was washed with water and brine. Desiccant $MgSO_4$ was added and contained for 24 h. $MgSO_4$ was removed, and chloroform was evaporated to afford an orange solid that

was separated and purified by column chromatography on a 1−2% KH_2PO_4 silica gel column using petroleum ether/ethyl acetate (4:1 v/v) as eluent. The products **3** and **4** were obtained in yields of 27% and 56%, and they were identified by satisfactory ^{1}H NMR, UV−vis, IR, and mass spectral data.

Data for Compounds 2−4. Compound **2**: purity (HPLC analysis): 99.2%; UV−vis [($CHCl_3$), λ_{max}, nm (log ϵ)] 466 (4.16), 548 (3.70), 580 (3.52); IR [(KBr), ν_{max}, cm^{-1}] 3430, 2928, 1691, 1604, 1530; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm) 1.84 (3H, s, 16-CH_3), 2.37 (3H, s, 18-CH_3), 3.22−3.86 (2H, d, 13-CH_2), 4.04−4.15 (12H, 4s, 2,3,6,7-OCH_3), 6.42 (1H, s, 5(8)-H), 6.44 (1H, s, 5(8)-H), 15.9 (1H, 3-OH), 16.1 (1H, 10-OH); *m*/*z* (FAB-MS) 529.51 (M + 1). Compound **3**: purity (HPLC analysis) 98.2%; UV−vis [($CHCl_3$), λ_{max}, nm (log ϵ)] 442 (4.16); IR [(KBr), ν_{max}, cm^{-1}] 3450, 2928, 1688, 1614, 1530; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm) 1.79 (3H, s, 16-CH_3), 2.40 (3H, s, 18-CH_3), 3.00−3.07 (2H, d, 13-CH_2), 3.90−4.15 (15H, 5s, 2,3,6,7,11-OCH_3), 6.16 (1H, s, 5(8)-H), 6.30 (1H, s, 8(5)-H), 15.8 (1H, 10-OH); *m*/*z* (FAB-MS) 543.1654 (M + 1, $C_{31}H_{27}O_9$ requires 543.1649), 543 (44), 529 (4), 511 (6), 395 (4), 154 (70), 136 (72), 107 (50), 89 (79), 77 (100). Compound **4**: purity (HPLC analysis) 97.8%; UV−vis [($CHCl_3$), λ_{max}, nm (log ϵ)] 432 (4.22); IR [(KBr), ν_{max}, cm^{-1}] 2937, 1691, 1629, 1554; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm) 1.75 (3H, s, 16-CH_3), 2.38 (3H, s, 18-CH_3), 2.90−2.96 (2H, d, 13-CH_2), 3.90−4.10 (18H, 6s, 2,3,6,7,10,11-OCH_3), 6.13 (2H, s, 5,8-H); *m*/*z* (FAB-MS) 557.1809 (M + 1, $C_{32}H_{29}O_9$ requires 557.1806), 557 (22), 543 (6), 154 (36), 136 (41), 105 (44), 91 (75), 77 (100).

Acknowledgment. This research is supported by the National Natural Science Foundation of China (No: 20172058).

Supporting Information Available: IR, MS, and ^{1}H NMR spectra for compounds **3** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO026554X