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New Long-wavelength Perylenequinones. The Reaction Between Hypocrellin B and Mercapto Compounds

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ABSTRACT

Certain new mercapto-substituted hypocrellin B (HB) derivatives have been synthesized and characterized. The possible mechanism for this reaction, and the properties of the resulting compound, are discussed. Interaction of HB with mercapto compounds in dimethyl sulfoxide containing ammonium hydroxide proceeded very quickly, and the mono- or di-substituted HB derivatives were formed. Their significantly enhanced red absorptivities and strong $^1O_2$-generating functions may qualify them as promising PDT agents. In addition, the semiquinone radical anion of HB (HB^-.) was one of the reaction intermediates detected by ESR and Uv–vis spectrum. Moreover, the effect of free radical quenchers on this reaction have also been investigated. On the basis of the experimental evidence, the mechanism of free radical addition and nucleophilic addition are proposed.
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Keywords: hypocrellin B, mercapto compounds, free radical and nucleophilic addition, reaction mechanism, ESR.

INTRODUCTION

Hypocrellin A and B (HA and HB) have recently been isolated from natural fungus sacs of *Hypocrella bambusae* in China [1]. These lipid-soluble 4,9-dihydroxy-3,10-perylenequinone derivatives [2], employed in pioneering photodynamic therapy (PDT) applications of perylenequinonoid pigments

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(PQPS), exibit several advantages over the presently used hematoporphyrin derivatives (HPD), i.e. ready preparation and easy purification relative to HPD, small aggregation tendency (which decreases the HPD efficiency of HPD), strong red light absorptivity and significantly reduced normal tissue photosensitivity because of their fast metabolism *in vivo* [3, 8]. As a result, hypocrellins have been successfully employed in the clinical PDT treatment of a number of skin diseases, such as white lesions of vulva, keloid, vitiligo, psoriasis, tinea capitis and lichen amyloidosis [4–7] without observing the prolonged normal tissue photosensitivity that occurs with HPD [8]. Recently, the chemistry, photochemistry and photophysics of hypocrellins have been studied extensively and have been reviewed by Z. J. Diwu [9, 10]. It has also been shown that hypocrellins are efficient singlet oxygen generators, and demonstrate some advantages over the classic $^1O_2$ sensitizers such as porphyrins, rose bengal, methylene blue, etc.), including high molor extinction coefficients, wide Uv–vis absorption, high quantum yields of singlet oxygen generation, high stability, good solubility and small solvent and concentration effects [11, 12].

The latest studies indicate that the target of the photodynamic action of the hypocrellins is the cell membrane; this photodynamic action caused the evident reduction in the quantity of mercapto groups in membrane proteins [13, 14]. Until recently relatively few efforts have been devoted to the study of the mechanism of this photodynamic action. These facts encouraged us to study the reaction of HB with mercapto compounds.

In this study, certain new mercapto-substituted HB derivatives have been successfully synthesized in high yield and characterized. The reaction indicated that HB could easily react with mercapto compounds to form mono- or di-substituted HB derivatives, and hence reduce the total quantity of mercapto groups in reaction solutions. It seems reasonable to assume that the reduction of the mercapto groups in membrane proteins may be closely related to the photodynamic action of HB. The study of the interactions of HB with mercapto compounds may be helpful for the understanding of the mechanism of photodynamic damage of cell membrane by HB, and furthermore elucidate the mechanism of the phototherapeutic actions on some skin diseases or cancers by HB.

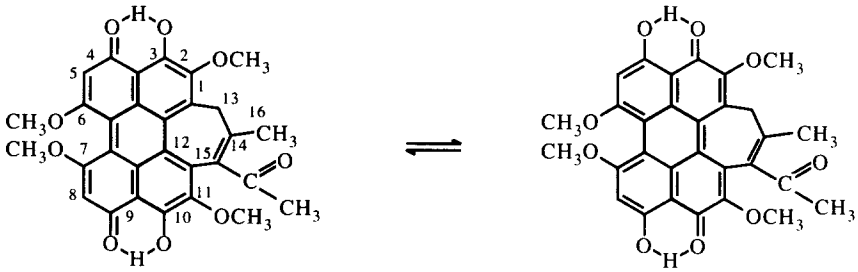
In addition, the semiquinone radical anion of HB ($HB^{-\cdot}$) was one of the reaction intermediates detected by ESR and Uv–vis; moreover, the effect of free radical quenchers to the reaction has also been studied. Nevertheless, existing PQPS do not display sufficiently strong absorptivity at wavelengths longer than 600 nm and this limits their PDT application at present. As described in this report, we have significantly enhanced their red absorptivities by means of mercapto substitution with the preservation of their $^1O_2$-generating function which is required for PDT.

RESULTS AND DISCUSSION

Reaction of HB with mercapoto compounds and characterization of products

According to the chemical structure of HB (Fig. 1), there are two kinds of positions expected to be reactive towards mercapto compounds, i.e. the aromatic ring (positions 5 and 8), and the side ring. As in the case of other PQPS, HB existed in an equilibrium of two tautomers (structures A and B in Fig. 1) at ambient temperature. These tautomers have been identified and studied by NMR, ESR, and circular dichorism methods [10]. It has been shown by the reaction of HB with either aldehydes or ketones that the several α-active hydrogens on the side ring of HB are relatively deactivated because of the steric hindrance in the ring. As expected, the aromatic ring (positions 5 and 8) of HB is more reactive than the α-active hydrogens on the side ring of HB. Several HB derivatives were synthesized as shown in Scheme 1.

On the basis of the spectra data and specific chemical tests, compound 1 was identified to be the 5-OH or 8-OH substituted HB; its molecular ion peak (m/z) appeared at 544 in the mass spectrum indicating that compound 1 contained one more atom of oxygen than HB. In the ^{1}H NMR spectra, there should be two aromatic protons for HB (^{1}H at positions 5 or 8) but in fact, for compound 1, only one aromatic proton was observed; meanwhile, a new broad signal at 6.82 ppm, which disappeared after addition of D_2O to the sample solution, was detected. These results imply the presence of an additional hydroxyl group substituted at the 5- or 8-position of the HB molecule in compound 1. Moreover, two phenolic hydroxy protons at 14.31 and 15.85 ppm, respectively, were also detected in the ^{1}H NMR spectrum of compound 1. The signal of 14.31 ppm which was at a higher field than that of the HB molecule (which is approx. 16.00 ppm) may result from the weakening of the hydrogen bonding ability between the quinonoid carbonyl group



(A) (B)

Fig. 1. Structure of HB.

Substrates
R:

A:$-CH_2COOH$
B:$-(CH_2)_7CH_3$
C:$-(CH_2)_{11}CH_3$

Compounds

A,B,C:(1)a:R_1=OH R_2=H
(1)b:R_1=H R_2=OH
A: (2)R_1=$-SCH_2COOH$(-H)
R_2=H(SCH_2COOH)
(3)R_1=R_2=$-SCH_2COOH$
B: (4)R_1=$-S(CH_2)_7CH_3$(H)
R_2=H($-S(CH_2)_7CH_3$)
(5)R_1=R_2=$-S(CH_2)_7CH_3$)
C: (6)R_1=$-S(CH_2)_{11}CH_3$(H)
R_2=H($-S(CH_2)_{11}CH_3$)
(7)R_1=R_2=$-S(CH_2)_{11}CH_3$)

Scheme 1.

and the *para*-hydroxyl groups (at positions 3, 4 or 9, 10) by its neighbouring additional hydroxyl substitutent (positions 5 or 8). Nevertheless, in the IR spectrum of compound 1, two bands at 1614 and 1592 cm^{-1} of the quinonoid carbonyl groups were observed and the band at 1592 cm^{-1} in the lower absorption frequency may result from the interaction of the quinonoid carbonyl group with its neighbouring additional hydroxyl substituent group (position 5 or 8). In addition, it was noted that in the ^1H NMR spectrum of compound 1 every peak was composed of two closely related peaks (intensity ratios of the two peaks were all about 2:1). So we assumed that compound 1 was a mixture of the two isomers 1A and 1B derived from the additional hydroxyl group substituted at a different position in the HB molecule (position 5 or 8) which could not be separated even by thin layer chromatography (TLC) or high pressure liquid chromatography (HPLC).

The *m/z* of compound 2 was 618. Only one aromatic proton was observed in the ^1H NMR spectrum indicating also that either position 5- or 8-hydrogen in the HB molecule was substituted the characteristic absorption of a carboxylic carbonyl group was also observed in the IR spectrum of compound. Thus, we assigned compound 2 to be a 5- or 8-mono-mercapto acid substituted HB derivative. It is noteworthy that compound 2 was also a

mixture of two isomers, *viz* of 5- or 8-substituted derivatives. Unfortunately, these could not be distinguished in the ^1H NMR spectrum, and could not be separated by TLC and HPLC. The observation of the carboxylic carbonyl group at 1721 and 1717 cm^{-1} in the IR spectrum may be evidence of compound 2 being a mixture of two isomers.

On the basis of the spectra data, compound 3 was assigned to be the di-substituted HB derivative: no aromatic proton at 6–7 ppm in the ^1H NMR spectrum of compound 3 was observed; the *m/z* was at 708 in the mass spectrum. The presence of two carboxyl carbonyl groups in compound 3 is evidenced by the strong 1725 and 1717 cm^{-1} IR absorption bands, respectively. Additionally the Uv–vis absorption bands of compound 3 shifted towards 518 nm (in $CHCl_3$). Subsequently, the chemical structure of compounds 4–7 were confirmed by IR, Uv–vis, MS and ^1H NMR spectrum (Scheme 1).

In the course of isolation and characterization of the reaction products between HB and cysteamine, not only compound 8 but also compound 9 were obtained (see Scheme 2).

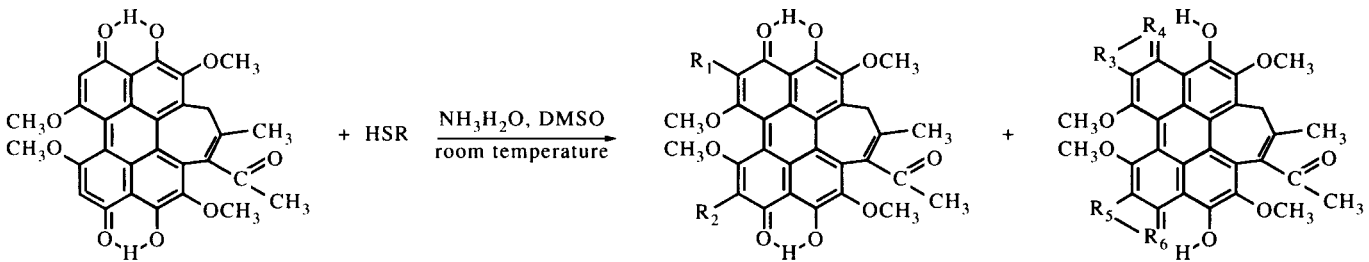
The spectrtal data (MS, IR, Uv–vis and ^1H NMR) of compound 9 were very similar to those of compound 8. The only difference was that in the ^1H NMR spectrum of compound 9, no signal at 4.40 ppm, which had been assigned to be the two active protons of HN_2 in the -SR substituent group of compound 8, was observed. The *m/z* at 585 in the MS spectrum of compound 9 was 18 less than the calculated value 601 for the mono-$SCH_2CH_2NH_2$ substituted HB (compound 8), which indicated that elimination of H_2O between NH_2 in the -SR substituent group and the quinonoid carbonyl group in the HB molecule probably occurred. As early as 1952, Burton *et al.* [15] reported that the interaction between a simple quinone and cysteamine could yield a mono-substituted quinone, which could then, further, lose a water molecule between the amino group in cysteamine and the quinonoid carbonyl group to form a cycloproduct [15] (see Scheme 3).

A similar reaction could occur between 2-methyl-1,4-naphoquinone and cysteamine [16] (see Scheme 4).

In addition, the base could accelerate the elimination of a water molecule. The reaction of HB with cysteamine was very similar to the reactions mentioned above the initially formed mono-SR substituted compound 8 then subsequently could lose a water molecule to produce the cyclic compound 9 in basic media.

Discussion of reaction mechanism

Previous research indicated that the possible mechanism for the reaction between mercapto compounds (RS) and quinonoid compounds was a



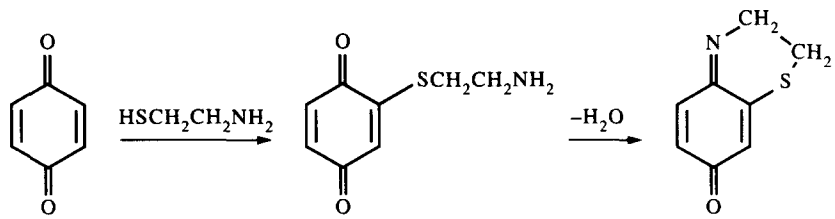
Substrate

R:D–$SCH_2CH_2NH_2$

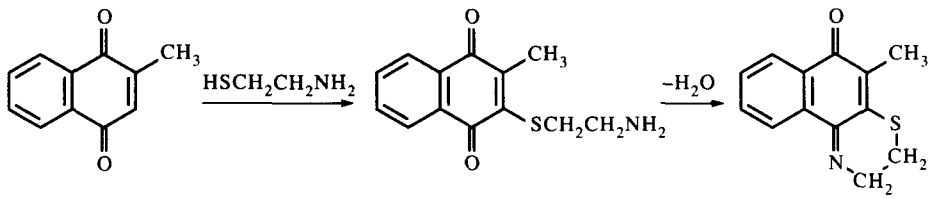
Compounds

D: (8)R_1=–$SCH_2CH_2NH_2$(H)
R_2=H(–$SCH_2CH_2NH_2$)
(9)R_3=–SCH_2CH_2–R_4=N(R_5=H R_6=O=)
or R_3=H R_4=O=(R_5=–SCH_2CH_2–R_6=N)

Scheme 2.



Scheme 3.



Scheme 4.

nucleophilic addition reaction. Concurrently, it has been reported that the reaction plausibly proceeded via an oxido–reduction reaction, the semiquinone radical anion being observed in the reaction system. We have reported that the maximal absorption of the semiquinone radical anion of HB ($HB^{-}\cdot$) was at 618 nm. Therefore, we detected the change in the Uv–vis absorption of the reaction solution after HB reacted with mercaptoacetic acid for 5 min (Fig. 2). As a result a new strong absorption at 618 nm was observed under an argon atmosphere, the colour of the sample changing from red to green. It can be seen that the intensity of the 466 nm absorpiton band of HB decreases and a new band of the green intermediate with λ_{max} 618 nm appears, which is coincident with the absorption maxima of the semiquinone radical anion of HB ($HB^{-}\cdot$) previously identified at 618 nm [17]. However, no absorption at 618 nm was observed under an air atmosphere. In order to confirm the existence of $HB^{-}\cdot$ the following procedure was carried out. In a deoxygenated DMSO solution containing HB (1×10^{-3}M) and 28% ammonium hydroxide, a weak ESR signal was generated within seconds, but after addition of the mercaptoacetic acid, the intensity of the ESR signal increased rapidly (Fig. 3). When the sample was exposed to the air, the ESR signal disappeared completely, which was in good agreement with the increase and disappearance of the absorption at 618 nm. The hyperfine structure of the ESR spectrum was shown in Fig. 4 in which 24 lines can be observed. It corresponded well with that reported for $HB^{-}\cdot$ with $g = 2.0030$ [17,18]. The probable coupling constants for the ESR spectrum of $HB^{-}\cdot$ were reported to be $a^{H(-OH)} = 1.66$ G; $a^{2H(aro-)} = 0.46$; $a^{6H(-OCH_3)} = 0.225$ G;

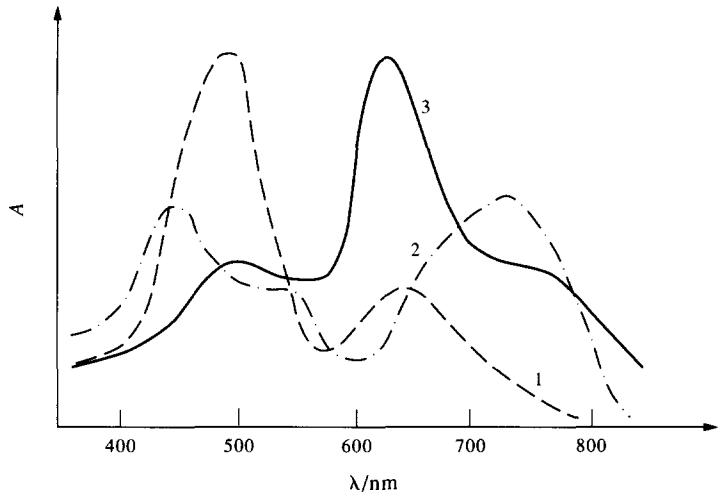


Fig. 2. Uv–vis absorption of HB when HB has reacted with mercaptoactic acid for 20 min. Solvent: DMSO, HB(M = 1×10^{-5} mol^{-1}), $HSCH_2COOH$(M = 2×10^{-4} mol^{-1}), PH = 9–10; (1) in the absence of $HSCH_2COOH$; (2) air-saturated solutions; (3) argon-saturated solution.

$a^{3H(16\text{-}CH_3)} = 0.81$ G and $a^{2H(13\text{-}H)} = 0.85$ G [17]. Simulation performed using these parameters produced a spectrum (Fig. 4) which matched the spectrum observed experimentally. Subsequently, quenching experiments were carried out using 2,6-di-*tert*-butylphenol and oxygen as free radical quenches, and the results are listed in Table 1.

On the basis of the above ESR and absorption measurements the semiquinone radical anion of HB($HB^{-}\cdot$) may be one of the reaction intermediates, i.e. a radical mechanism is probably involved. We have previously reported that the semiquinone radical anion of HB($HB^{-}\cdot$) could be easily quenched by oxygen and produced the superoxide anion O_2^{-}[17, 18] ($HB^{-} + O_2 \rightarrow HB + O_2^{-}$). So oxygen can here act as a strong radical quencher and therefore the reaction proceeds more quickly under an argon atmosphere than under an air atmosphere. However, from Table 1 it can be seen that free radical quenchers (oxygen and 2,6-di-*tert*-butylphenol) do not completely hinder the reaction. Accordingly, we suggest that nucleophilic addition plays a certain role in this reaction, with the exception of free radical addition.

The reaction did not proceed in neutral and acidic solutions because in such media no RS- exists, and this was the only active species to proceed via nucleophilic addition and electron transfer to the HB molecule, In addition, Table 1 shows that compound 1 was only obtained when the reaction system contained oxygen. We could explain the formation of compound 1 as follows: it has been reported that OH can react with quinones to form hydroxyl substituted quinone [19]. When our experiments were carried out in the presence of oxygen, one of reaction the intermediates, i.e. the semiquinone

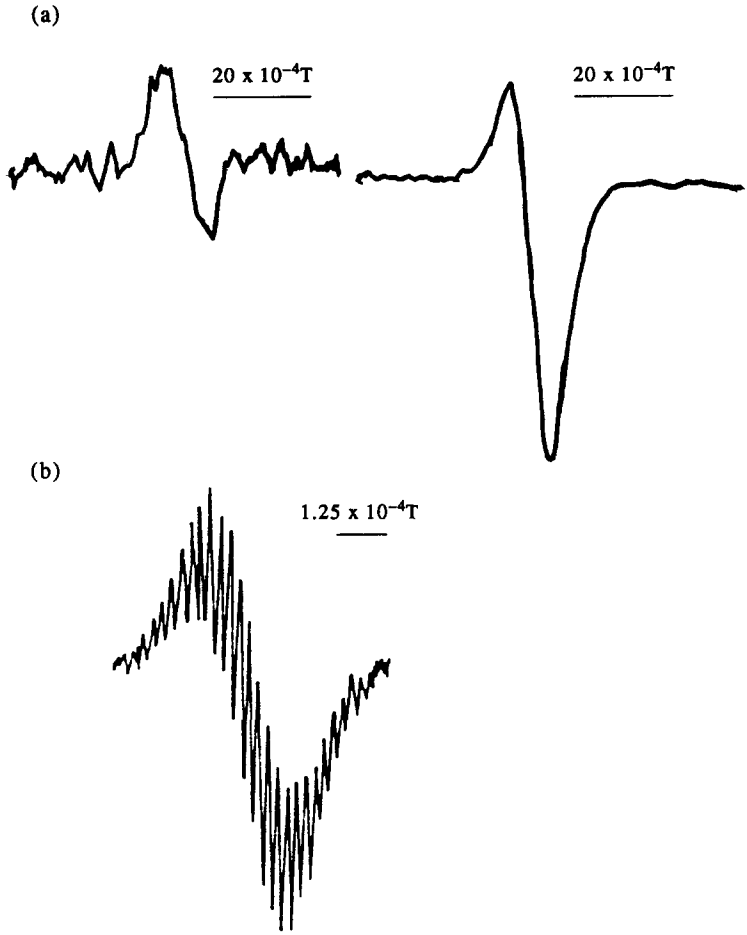


Fig. 3. ESR spectrum obtained on reaction of HB (2×10^{-3} mol^{-1}) with $HSCH_2COOH$ in DMSO (pH = 9–10); (a) in the absence of $HSCH_2COOH$; (b) addition of $HSCH_2COOH$(4×10^{-2} mol^{-1}; (c) hyperfine structure of (b). Spectrometer settings: microwave power: (a,b) 10 mW, (c) 1 mW. Modulation amplitude; (a,b) 1G, (c) 0.16 G. Receiver gain: (a) 2×10^4, (b) 4×10^3, (c) 2×10^4.

radical anion of HB($HB^{-}\cdot$) could be quenched by oxygen and produce $O_2^{-}\cdot$ which can generate ·OH in a water containing system (water was present in our system). Therefore the ·OH generated in our system can react with HB to produce compound 1.

As expected, compounds 2–9 possess the general properties of HB. Significantly, they can produce singlet oxygen on visible light illumination. Interestingly, compound 9 can also produce singlet oxygen on illumination and it also possesses very strong red absorption, longer than 600 nm, which is useful for PDT of tumors, since light scattering in tissue is inversely and exponentially

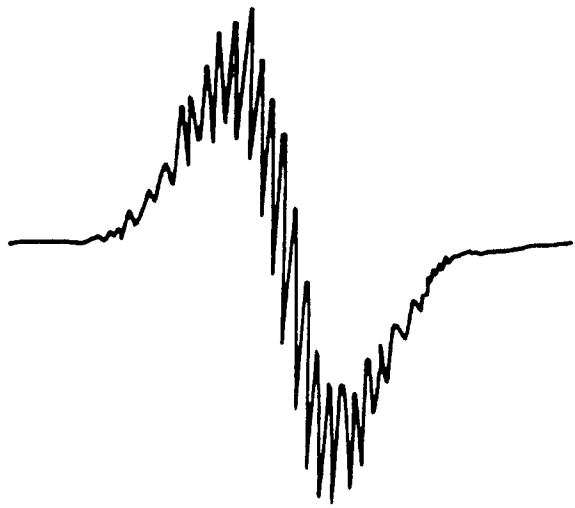


Fig. 4. (a) Computer simulated ESR spectrum with $a\frac{H}{OH}$ = 1.66G(1H), $a\frac{H}{aro}$ = 0.46G(2H), $a\frac{H}{OCH_3}$, = 0.225G(6H), $a\frac{H}{CH_3}$ = 0;81G(3H), $a\frac{H}{CH_2}$ = 0.85G(2H).

proportional to wavelength. These two characteristics might qualify compounds 2–9 as promising photosensitizers for the photodynamic therapy of human tumors. Such properties are under investigation and will be reported in due course.

EXPERIMENTAL SECTION

Chemicals

HA was crystallized twice from acetone. HB was obtained by dehydration of HA. Mercaptoacetic acid (analytical grade) was purchased from Beijing chemical plant, China. Cysteamine was purchased from Fluka AG. Water

TABLE 1
Reaction Rate and Products Distribution of HB With Mercaptoacetic Acid Under Different Conditions[a]

Reaction condition	*Consumption percentage of HB*[b]	*Products distribution* 1	2	3
Without O_2 and quenchers	80%	–	+	+
With O_2, without quenchers[c]	60%	+	+	+
Without O_2, with quenchers[c]	50%	–	+	+

[a]HB(M = 1.9×10^{-3} mol^{-1}), HSR (M = 3.8×10^{-2} mol^{-1}), pH = 9–10; solvent, DMSO.
[b]Consumption percentage of HB was the ratio of reacted HB with ross HB after HB reacted with mercaptoacetic acid for 20 min.
[c]Quenchers is 2,6-di-tert-butylphenol(M = 2×10^{-1} mol^{-1}).

was freshly distilled and the organic solvents were all of analytical grade. The required high-purity solvents were prepared by further purification of the commercial products, and no impurities were detected by absorption and/or fluoresence spectroscopy. The solutions were purged with argon, air, or oxygen according to the experimental requirements. The reaction products were separated by thin layer chromatography (TLC) on silica gel GF254 (Qingdao Marine Chemical Factory) containing 1% citric acid. The silia gel plates were activated at 105–110 for 60 min.

Instrumentation

Uv–vis absorption spectra were recorded with either an HP diode-array spectrophotometer model 8451A or a Hitachi-340. IR spectra were measured with a Perkin Elmer 557 grating spectrophotometer ^{1}H NMR spectra were run on either a Varian XL-400 or XL-200 spectrometer in deuterated chloroform with TMS as internal standard. Mass spectra were obtained using a ZAB-HS (Faster Atom Bombardment Mass Spectrometer). Electron spin resonance (ESR) spectra were recorded using a Varian E-1700B spectrometer operating at room temperature.

General procedure for the reaction of HB with mercapto compounds

HB (40 mg) was dissolved in DMSO (40 ml), 28% ammonium hydroxide was added to adjust the pH 9–10, and the mercapto compounds (2 ml) were then added in the dark under argon. The resulting solution was stirred in the dark for 3–6 h depending on the individual mercapto compounds used. The mixture was poured into ice-water, neutralized with 10% hydrochloric acid, and extracted with chloroform 3–4×. The chloroform layer was washed with water 3×. Chloroform was evaporated under reduced pressure to give a violet–red solid. The solid was purified by TLC on a 1% citric acid–silica gel plate using 4:2:1 (v/v/v) petroleum ether–ethyl acetate–ethanol or 2:1 (v/v) ether–ethyl acetate, according to experimental requirements. Compounds 2–9 were thus obtained.

Reaction of HB with mercaptoacetic acid

When carried out according to the above procedure, this reaction afforded three major products (petroleum ether–ethyl acetate–ethanol 4:2:1 v/v/v) was used as developing agent).

Compound 1 (22% yield): IR: 3334, 1689, 1614 and 1592 cm^{-1}; ^{1}H NMR (1a): 15.85(s,1H, exchanged with D_2O,4(9)-OH), 14.31 (*s*,1H, exchanged with D_2O,9(4)-OH), 6.82 (s,1H, exchanged with D_2O,5(8)-OH). 6.38 (s,1H,8(5)-

H, 2.37 (s,3H,18-CH_3), 1.85 (s,3H,16-CH_3), 4.22 (s,3H,6-OCH_3), 4.12 (s,3H,2-OCH_3), 4.07 (s,3H,7-OCH_3), 4.07 (s,3H,11-OCH_3), 3.92 (d,1H,13-H_2, J = 12 Hz) and 3.26 ppm (d,1H,13-H_2, J = 12 Hz); ^{1}H NMR (1b): 15.33 (*s*,1H, exchanged with D_2O,4(9)-OH), 14.33 (s,1H, exchanged with D_2O,9(4)-OH), 6.82 (s,1H, exchanged with D_2O,5(8)-OH). 6.36 (s,1H,8(5)-H, 2.38 (*s*,3H,18-CH_3), 1.82 (s,3H,16-CH_3), 4.23 (s,3H,6-OCH_3), 4.14 (s,3H,2-OCH_3), 4.04 (s,3H,7-OCH_3), 4.07 (s,3H,11-OCH_3), 3.84 (d,1H,13-H_2, J = 12 Hz) and 3.23 ppm (d,1H,13-H_2, J = 12 Hz); MS(*m*/*z*)(FAB): 5.44(M^+).

Compound 2 (32% yield): IR: 3100-2500, 3431, 1721, 1717, 1690 and 1600 cm^{-1}; ^{1}H NMR: 15.90 (s,1H, exchanged with D_2O,4(9)-OH), 15.77 (s,1H, exchanged with D_2O,9(4)-OH), 5.93 (s,1H,5(8)-H), 2.37 (s,3H,18-CH_3), 1.93 (s,3H,16-CH_3), 3.75 (s,3H,6-OCH_3), 4.16 (s,3H,2-OCH_3), 3.73 (s,3H,7-OCH_3), 4.13 (s,3H,11-OCH_3), 3.98 (d,1H,13-H_2, J = 12 Hz), 3.46 (d,1H,13-H_2, J = 12 Hz), 3.84 (s,2H,-SCH_2COOH) and 8.62 ppm (s,1H exchanged with D_2O,-SCH_2COOH). MS(*m*/*z*)(FAB): 618(M^+).

Compound 3 (36% yield): IR: 3100-2500, 3414, 1725, 1717, 1691 and 1598 cm^{-1}. ^{1}H NMR: 15.76 (s,1H, exchanged with D_2O,4(9)-OH), 15.74 (s,1H, exchanged with D_2O, 9(4)-OH), 2.36 (s,3H,18-CH_3), 1.95 (s,3H,16-CH_3), 3.86 (s,3H,6-OCH_3), 4.16 (s,3H,2-OCH_3), 3.82 (s,3H,7-OCH_3), 4.08 (s,3H,11-OCH_3), 3.96 (d,1H,13-H_2, J = 12 Hz), 3.23 (d,1H,13-H_2, J = 12 Hz), 3.83 (4H,-SCH_2COOH) and 8.68 ppm (2H, exchanged with D_2O,-SCH_2COOH); MS(*m*/*z*)(FAB): 708(M^+).

Reaction of HB with 1-octanethiol

Similarly this reaction afforded three main products (compounds 1,4,5) petroleum ether–ethyl acetate (2:1 v/v) was used as developing agent).

Compound 4 (26% yield): IR: 3368, 1690 and 1600 cm^{-1}; ^{1}H NMR: 15.97 (*s*,1H, exchanged with D_2O,4(9)-OH), 15.95 (s,1H, exchanged with D_2O,9(4)-OH), 6.43 (s,1H,5(8)-H), 2.37 (s,3H,18-CH_3), 1.98 (s,3H,16-OCH_3), 4.03 (s,3H,6-OCH_3), 4.13 (s3H,2-OCH_3), 3.86 (s,3H,7-OCH_3, 4.09 (s,3H,11-OCH_3), 4.05 (d,1H,13-H_2, J = 11.9 Hz), 3.41 (d,1H,13-H_2, J = 11.9 Hz), 3.49-3.82 (m, 4H,-SCH_2CH_2), 1.60 (m,10H,-CH2–) and 1.19 ppm (t,3H,-$SRCH_3$); MS(*m*/*z*)(FAB): 672(M^+).

Compound 5 (32% yield): IR: 3370, 1694 and 1590 cm^{-1}; ^{1}H NMR: 15.96 (s,1H, exchanged with D_2)O,4(9)-OH), 15.93 (s,1H, exchanged with D_2O,9(4)-OH), 2.37 (s,3H,18-CH_3), 1.95 (s,3H,16-CH_3), 3.93 (s,3H,6-OCH_3), 4.15 (s,3H,2-OCH_3, 3.93 (s,3H,7-OCH_3), 4.08 (s,3H,11-OCH_3, 4.05 (d,1H,13-H_2, J = 11.8 Hz), 3.25 (d,1H,13-H_2, J = 11.8 Hz), 3.09-3.51 (m,8H,-SCH_2CH_2), 1.44–1.66 (m,20H,-CH_2-, 1.12 (t,3H,-$SRCH_3$); MS (*m*/*z*)(FAB): 816(M^+).

Reaction of HB with dodecyl mercaptan

Similarly the reaction afforded three main products (compounds 1,6,7) petroleum ether–ethylacetate (2:1 v/v) was used as developing agent).

Compound 6 (26% yield): IR: 3350, 1692 and 1601 cm^{-1}; ^1H NMR: 15.97 (s,1H, exchanged with D_2O,4(9)-OH), 15.94 (s,1H, exchanged with D_2O,9(4)-OH), 6.42 (s,1H,5(8)-H) 2.38 (s,3H,18-CH_3), 1.97 (s,3H,16-CH_3), 4.02 (s,3H,6-OCH_3), 4.14 (s,3H,2-OCH_3), 4.07 (s,3H,7-OCH_3), 4.10 (s,3H,11-OCH_3), 4.04 (d,1H,13-H_2, J = 11.8 Hz), 3.23 (d,1H,13-H_2, J = 11.8 Hz), 3.61–3.81 (m,4H,-SCH_2CH_2), 1.61 (m,18H,-CH_2-), 1.22 (t,3H,-$SRCH_3$); MS(*m/z*)(FAB): 728(M^+).

Compound 7 (38% yield): IR: 3348, 1694 and 1601 cm^{-1}; ^1H NMR: 15.98 (s,1H, exchanged with D_2O,4(9)-OH), 15.95 (s,1H, exchanged with D_2O,9(4)-OH), 2.38 (s,3H, 18-CH_3), 1.96 (s,3H,16-CH_3, 3.88 (s,3H,6-OCH_3), 4.15 (s,3H,2-OCH_3), 3.92 (s,3H,7-OCH_3), 4.08 (s,3H,11-OCH_3), 4.04 (d,1H,13-H_2, J = 11.8 Hz), 3.25 (d,1H,13-H_2, J = 11.8 Hz), 3.09–3.45 (m,8H,-SCH_2CH_2), 1.30–1.68 (m,36H,-CH_2-), 1.21 (t,6H,-$SRCH_3$: MS (*m/z*) (FAB): 928(M^+).

Reaction of HB with cysteamine

This reaction afforded three main products (compounds 1,8,9) (chloroform–ethylacetate (2:1 v/v) was used as developing agent).

Compound 8 (28.6% yield): IR: 3404, 1683 and 1604 cm^{-1}; ^1H NMR: 13.09 (s,1H, exchanged with D_2O,4(9)-OH), 13.05 (s,1H, exchanged with D_2O,9(4)-OH), 6.56 (s,1H,5(8)-H), 3.56 (s,3H,6-OCH_3), 4.08 (s,3H,2-OCH_3), 4.07 (s,3H,7-OCH_3), 4.06 (s,3H,11-OCH_3), 3.92 (d,1H,13-H_2, J = 12 Hz), 3.31 (d,1H,13-H_2, J = 12 Hz), 2.37 (s,3H,18-CH_3), 1.95 (s,3H,16-CH_3), 4.02 (m,2H,-NCH_2CH_2S-), 3.05 (m,2H,-NCH_2CH_2S-) and 4.20 ppm (2H, exchanged with D_2O,-NH_2-R); MS(*m/z*)(FAB): 603(M^+).

Compound 9 (34.8% yield): IR: 3404, 1689 and 1604 cm^{-1}; ^1H NMR: 16.76 (s,1H, exchanged with D_2O,4(9)-OH), 13.38 (s,1H, exchanged with D_2O,9(4)-OH), 6.58 (s,1H,5(8)-H), 3.49 (s,3H,6-OCH_3), 4.14 (s,3H,2-OCH_3), 4.05 (s,3H,7-OCH_3), 4.09 (s,3H,11-OCH_3), 3.92 (d,1H,13-H_2, J = 10 Hz), 3.32 (d, 1H, 13-H_2, J = 10 Hz), 2.35 (s,3H,18-CH_3), 1.83 (s,3H,16-CH_3), 3.56 (m,2H,-NCH_2CH_2S-) and 3.05 ppm (m,2H,-NCH_2CH_2S-); MS(*m/z*) (FAB):585(M^+).

λ_{max}/ ε_{max} values of the products reported here as follows:

	$\lambda_{max}(nm)$	$\varepsilon_{max}(CHCl_3)$
Compound 1	488	3.98×10^3
Compound 2	492,580 (shoulder)	7.94×10^3, 5.01×10^3

Compound 3	516	7.95×10^3
Compound 4	500,586 (shoulder)	8.53×10^3, 6.02×10^3
Compound 5	520,635 (shoulder)	8.88×10^3, 5.65×10^3
Compound 6	508,600 (shoulder)	9.43×10^3, 6.53×10^3
Compound 7	535,655 (shoulder)	9.50×10^3, 6.45×10^3
Compound 8	515	8.23×10^3
Compound 9	532,596,652	2.72×10^4, 8.54×10^3, 1.34×10^4

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