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Structure and Properties of Hematein Derivatives

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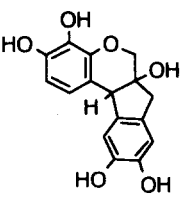
ABSTRACT

The oxidation of hematoxylin to hematein under various conditions was investigated, and the formation and absorption spectra of the hematein metal complexes studied. Conformational structures of hematoxylin and hematein were calculated by MOPAC PM3. The possibilities of their structural isomers, heat of formation, and their three-dimensional structures were evaluated and the most stable conformers were thus elucidated. Copyright © 1996 Elsevier Science Ltd

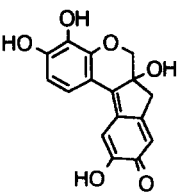
Keywords: Hematein, hematoxylin, metal complex, MOPAC, conformational analysis, PPP MO.

INTRODUCTION

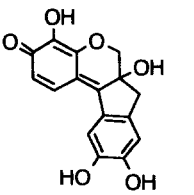
Hematein metal complexes are important stains for use in histology and cytology, as well as dyes for human hair. Hematein as a dye is well established, i.e. C. I. Natural Black 1, and has been widely used for many years. The chemistry and applications of hematoxylin (**1**) and hematein (**2**), extracted



Hematoxylin (**1**)



Hematein (**2**)



Hematein isomer (**3**)

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from logwood, have been investigated by numerous authors. A comprehensive review has been given by Puchtler *et al.*[1]

However, it has not yet been possible to formulate a uniform and completely satisfying description of the various chemical processes which take place during staining and dyeing, and the structures of the hematein metal complexes have not yet been unambiguously elucidated. One of the reasons for these difficulties is that hematoxylin is a polyhydroxy aromatic compound and that the oxidation to hematein can result in many possible conformations including isomeric structures.

The isomeric structure (**3**) of hematein (**2**) has been reported by Roubani-Kalantzopoulou *et al.*[2] Structural identifications of hematoxylin and hematein have been recently conducted and determined by Zimmermann *et al.*,[3] by means of ^{1}H- and ^{13}C-NMR, mass and other spectral data, together with elemental analyses.

In this paper, we attempt to clarify the conformational structures of hematoxylin and hematein by means of MOPAC PM3. The possible structural isomers, heat of formation, and three-dimensional structures can be evaluated by computer simulation, and the most stable conformers were elucidated from these evaluations.

Hematein exists as a hydrate and strongly interacts with water or alcohols, which makes the structural identification of hematein derivatives somewhat ambiguous. We evaluate here the interactions between hematein and protic solvents.

EXPERIMENTAL

General

Commercial hematein and hematoxylin were used after purification. Visible spectra were recorded on a Hitachi 220A spectrophotometer and ^{1}H-NMR spectra on a JEOL JNM-GX 270 (270 MHz) spectrometer using D_6-DMSO as solvent and TMS as internal reference. CHN analyses were carried out with a Yanaco CHN Corder MT-3. Column chromatography was conducted on cellulose (Toyo Roshi, 100–200 mesh) using water: ethanol = 4:1 (v/v) as the eluent. PPP MO and MOPAC PM3 calculations were conducted by using commercialized software.

Purification of hematein

Hematein (1 g) was dissolved in EtOH (300 ml), heated at 50°C with stirring for 3 h, and immediately filtered. The filtrate was evaporated and the residue

was dried *in vacuo*. The residue was dissolved in a mixture of water and EtOH (v/v = 4:1) and isolated by column chromatography on cellulose. The structure of hematein was determined by ^{1}H-NMR.

Oxidation of hematoxylin to hematein

Air oxidation of hematoxylin to hematein was carried out by blowing air through an aqueous and an aqueous alkali solution of hematoxylin, respectively, with stirring for 1 week; the degree of oxidation was evaluated spectrophotometrically. The oxidation of hematoxylin by iron(III) salt proceeded smoothly and the hematein iron complex was thus obtained. When ethylenediaminetetraacetic acid (EDTA) as a competitive ligand was added, EDTA preferentially formed the EDTA iron complex, and consequently the oxidation of hematoxylin to hematein with iron(III) salt could be controlled by adding EDTA to an aqueous solution of hematoxylin.

Hematein metal complexes and their spectra

An aqueous solution and an aqueous ammonia solution of hematein were freshly prepared, respectively, and their UV spectra determined. Hematein metal complexes were prepared by mixing aqueous solutions of hematein (2 mol) and metal salts (1 mol), filtering the solution and washing the precipitate with water. Metal salts used were copper(II) sulphate, iron(II) chloride, and iron(III) chloride. CHN analyses of the metal complexes were determined.

RESULTS AND DISCUSSION

Oxidation of hematoxylin to hematein

The oxidation of hematoxylin to hematein by air and metal salts is shown in Fig. 1. The air oxidation of hematoxylin to hematein proceeded very slowly in water, but it was very fast in an aqueous alkali solution, accompanied by slow degradation of hematein. The oxidation of hematoxylin by iron(II) salt was unsuccessful because iron(II) salt has no oxidation ability. The oxidation of hematoxylin by iron(III) salt proceeded rapidly and formed the black-colored iron(III) complex. When an aqueous EDTA solution was added to the resulting iron(III) complex solution, the precipitate disappeared, leaving hematein. From the results, it was found that oxidation of hematoxylin to hematein could be controlled by the iron(III)/EDTA system.

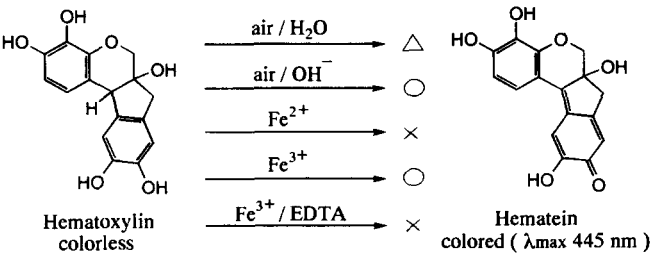


Fig. 1. Oxidation of hematoxylin to hematein under various conditions.

Substituent effects on the absorption spectra of hematein by PPP MO method

The oxidation of hematoxylin (**1**) gives the red-colored hematein (**2**). Hematoxylin is colorless because it has the central sp^3 carbon between the two phenylene rings and π-conjugation through a whole molecule is not attained. The π-electron density changes accompanying the first excitation of **2** were calculated by the PPP MO method, and the results are shown in Fig. 2, which indicates the pertinent electron density changes. (White circles in Fig. 2 show increase of π-electron density and black circles show decrease of π-electron density.) From these results, it is concluded that hematein has a

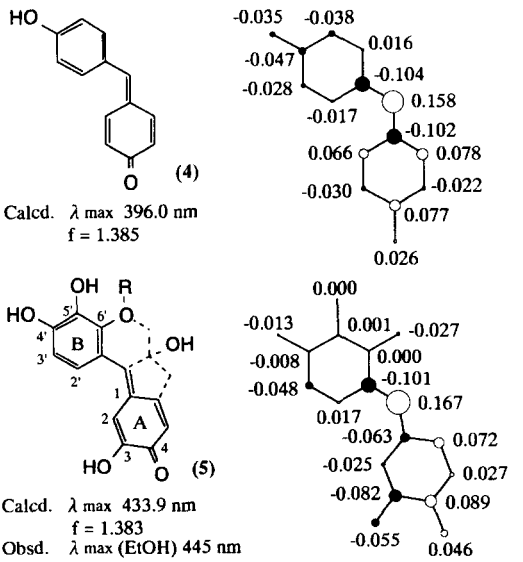


Fig. 2. π-Electron density changes accompanying the first excitation of indophenol **4** and hematein framework **5**.

TABLE 1
Observed and Calculated Absorption Spectra of Dyes **1–3**

Compound	Observed[a] (nm)	Calculated (nm)	(f)
1	293	—	—
2	445	434	(1.38)
Anion[b]	560	512	(0.95)
3	—	496	(0.38)
Anion[b]	—	617	(0.34)

[a]Measured in EtOH.
[b]Deprotonated oxide form.

chromophoric system similar to that of indophenol, as shown in structure **4**. Increase of electron density was observed mainly at the carbonyl group and at the 6-position, and decreases were mainly observed at the 3-, 3′- and 4′-positions. These positions are therefore important when introducing substituents which might cause large spectral changes of the hematein derivatives.

It was found that hematein has an intramolecular charge-transfer (CT) chromophoric system in which the hydroxybenzene moiety acts as a donor and the benzoquinone moiety acts as an acceptor. Therefore, the dissociation of the phenolic hydroxyl groups to oxide ions increase the donor nature of **2**, thus causing a bathochromic shift of λ_{max}; the π-electron system of **2** is shown in structure **5**. From the π-electron density changes of **5**, it is proposed that the dissociation of the hydroxyl group at the 3-position in ring A induces a bathochromic shift of λ_{max}, and that of the hydroxyl group at the 4′-position also has a similar effect, whereas the effect of the hydroxyl group at the 5′-position had little effect on λ_{max}, because no electron density change was observed at the 5′-position.

The results of the PPP MO calculation well reproduced the experimental results, as shown in Table 1.

Substituent effects on the absorption spectra of the phenolic hydroxyl groups of hematein framework **5** were evaluated by the PPP MO method. The correlation between the valence state ionization potential (IP) of the substituents and λ_{max} or oscillator strength (f) of **2** is shown in Figs 3 and 4. A decrease of IP (an increase of the electron donating property), of substituents X in ring B produces a small bathochromic shift (434–441 nm) of λ_{max} as well as a small increase (1.384–1.442) in the f value, as shown in Fig. 3. On the other hand, a decrease of the IP of substituent Y in ring A produces a large bathochromic shift (434–456 nm) of λ_{max} and a decrease (1.38–1.13) in the f value, as shown in Fig. 4.

From these results, it is concluded that the substituent Y has a large effect on the absorption spectra, producing a bathochromic shift of hematein but

that substituents X had little effect. The hydroxyl group at the 3-position can form a six-membered hydrogen bonded system with the neighboring carbonyl group, and the push–pull effect of the substituents produces a large bathochromic shift. Therefore the absorption spectra of hematein can be controlled mainly in the following manners: namely, a bathochromic shift of λ_{max} can be attained by introducing a strong donor substituent at Y, and the color value (increase of ϵ_{max}), can be obtained by introducing strong donor substituents at X-positions.

^{1}H-NMR spectra of hematoxylin and hematein were determined in D_6-DMSO, and the results are summarized in Fig. 5. These results are in accord with those reported by Zimmermann *et al.*[3] The phenolic protons of hematoxylin showed broad peaks at around 8.2–8.6 ppm but disappeared by addition of a drop of D_2O. The four aromatic protons, the two sets of germinal coupled methylene protons, a methine proton at 3.85 ppm, and the hydroxyl proton at 5.27 ppm were also observed, and identified as shown in Fig. 5.

The *ortho*-coupling of benzenoid protons at 6.55 and 7.34 ppm (J = 8.54 Hz) confirmed the indicated quinoid structure of hematein; disappearance of a methine proton at 3.85 ppm also indicated oxidation of hematoxylin to

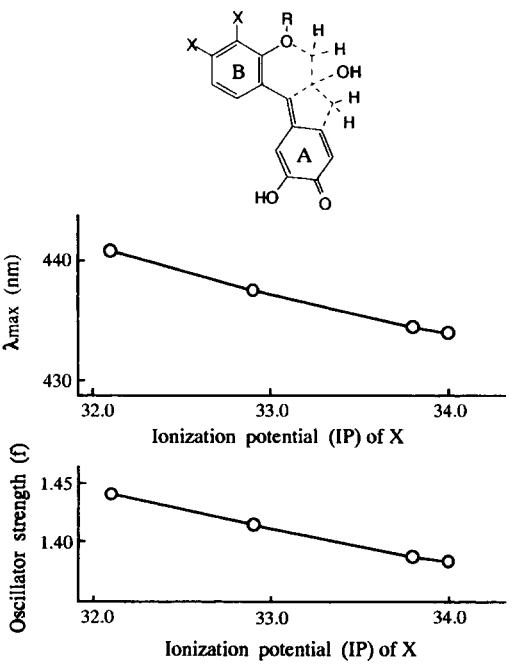


Fig. 3. Effect of substituent X on the absorption spectra of hematein.

hematein. Hematein exists as a hydrate and the hydrate protons were observed at around 3.5–4.5 ppm of very broad peaks which were shifted to lower field of 5.7 ppm when a drop of trifluoroacetic acid was added. From these results, it was concluded that hydrated water strongly interacted with hematein molecules.

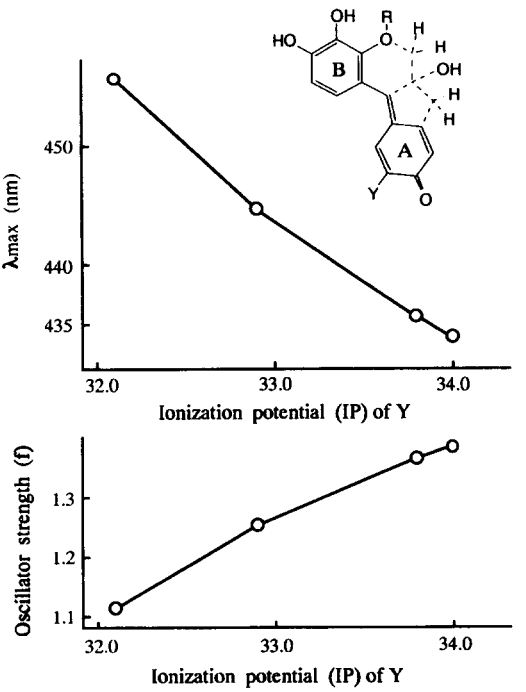


Fig. 4. Effect of substituent Y on the absorption spectra of hematein.

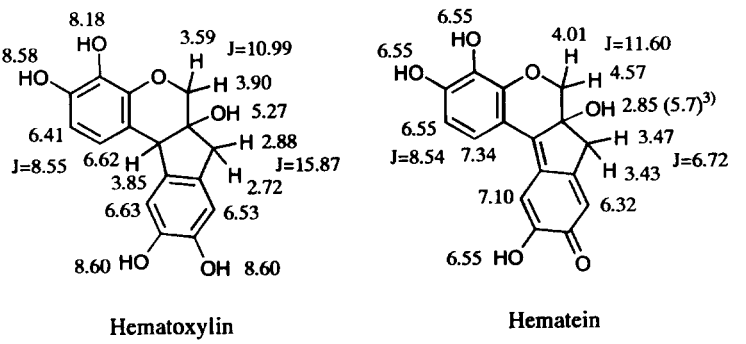
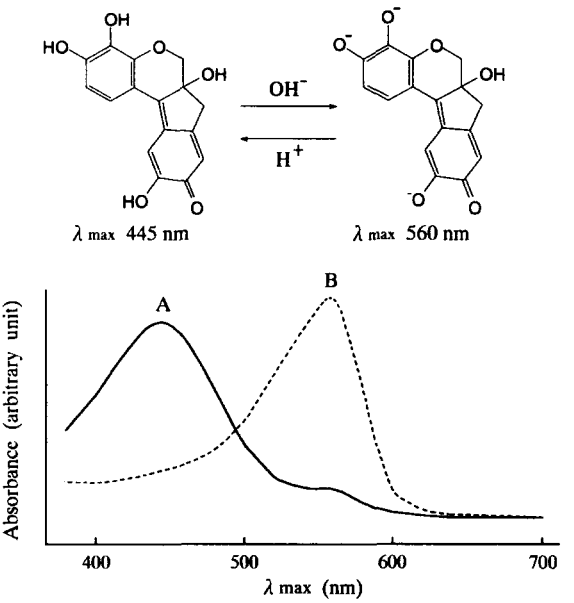


Fig. 5. ^{1}H-NMR spectra (270 MHz) of hematoxylin and hematein.



A : Hematein in aq. EtOH, B : Hematein in aq. EtOH + NH_4OH

Fig. 6. Acid–base equilibrium of hematein in solution. (The shoulder around 560 nm of hematein arises from partial deprotonation in the solvent.)

Absorption spectra of hematein and its metal complexes

The λ_{max} values of hematein and its metal complexes were determined. Hematein absorbs at 445 nm (yellow) in water but absorbs at 560 nm (purplish red) in aqueous ammonia, depending on the deprotonation of hydroxyl groups. Hematein was unstable in alkali solution and was gradually discolored. The acid–base equilibrium of hematein in solution is shown in Fig. 6.

TABLE 2
Elemental Analysis of Hematein Metal Complexes

Compound	Molecular formula	C (%)		H (%)	
		Calculated	Found	Calculated	Found
Hematein–Fe(III) 1:1 complex H_2O	$C_{16}H_{11}O_6Fe$ H_2O	51.51	51.80	3.51	4.04
Hematein–Cu(II) 1:1 complex 1.5 H_2O	$C_{16}H_{11}O_6Cu$ $1.5H_2O$	49.30	49.24	3.36	3.93

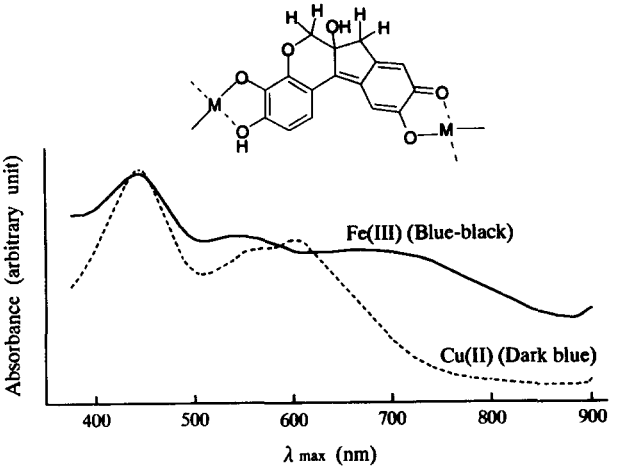


Fig. 7. Absorption spectra of hematein metal complexes (hematein/metal = 1/1) in DMSO.

The results of CHN analyses of hematein metal complexes are shown in Table 2. From these results, it was found that hematein formed a 1:1 metal complex, and that the hematein–Fe complex has 1 molecule of H_2O and that the hematein–Cu complex has 1.5 molecules of H_2O. The metal complexes showed a broad absorption spectrum in DMSO. The absorption spectra Fe(III) and Cu(II) complexes of hematein in DMSO are shown in Fig. 7.

A large bathochromic shift of λ_{max} on metal complexation was generally observed on formation of the chelate complex. One typical example is in the case of the indonaphthol chromophore, in which a large bathochromic shift of 200 nm, an increase of the ϵ value ($\approx$7 times), and broadening of the spectrum were observed on formation of the 2:1 metal complex.[4]

Conformational analyses of hematoxylin and hematein by MOPAC PM3

The most stable conformations and the heats of formations of hematoxylin (*trans*-form) **6**, hematoxylin (*cis*-form) **7**, hematein **8**, and of the hematein isomer **9** were optimized using the MOPAC PM3, and the results are shown in Fig. 8. The optimized conformations of the three-dimensional structures, as drawn by 'the balls and sticks model' are shown in Fig. 8. The structures of **6A**, **7A**, **8A**, and **9A** are the front views of π-plane of ring A in each structure, and those of **6B**, **7B**, **8B**, and **9B** are the right side-views of the π-plane. The H_f values are the calculated heat of formation for each structure.

In hematoxylin, it is considered that there are two isomeric forms, namely, the *trans*-form **6** and the *cis*-form **7**, with respect to the hydroxyl group and

the hydrogen in the central part of hematoxylin. The heat of formation of the *cis*-form 7 (−208.1 kcal/mol) was lower, by about 10 kcal/mol, than that of the *trans*-form 6 (−198.4 kcal/mol), and indicates that **7** is more stable than **6**.

On the other hand, the heat of formation of **8** (−174.7 kcal/mol) was a little lower than that of **9** (−171.7 kcal/mol). The coplanarity of **8** was found to be much better than that of **9** from observations of the three-dimensional molecular structures in Fig. 8. The absorption maxima of **8** and **9** were calculated by the PPP MO method, and the values were 434 and 496 nm, respectively. The λ_{max} of hematein observed by a visible/UV spectrophotometer was 445 nm in ethanol (445 nm[5] in MeOH) and these values were well reproduced by the structure of **8**, but not **9**.

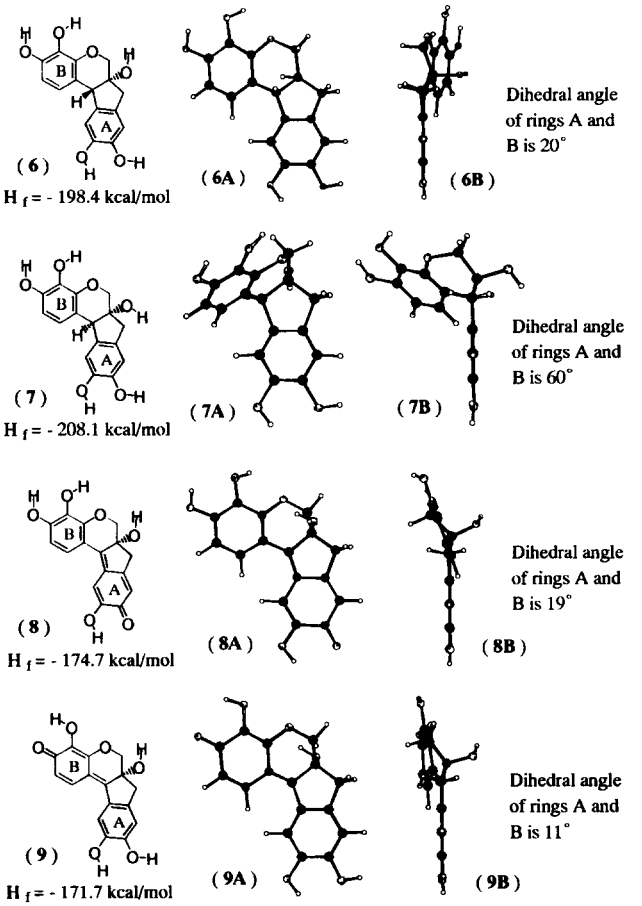


Fig. 8. The most stable conformation of hematoxylin *trans*-form (**6**), hematoxylin *cis*-form (**7**), hematein (**8**), and isomer of hematein (**9**) optimized by MOPAC-PM3.

CONCLUSION

Application of the hematoxylin/EDTA/Fe(III) system is of value for a black hair dye, which is initially colorless in application, but gradually develops a black color on hair after air oxidation. Various colors (on hair) can be obtained by mixing the hematein metal complexes.

Substituent effects on the absorption spectra of hematein are well reproduced by the PPP MO method. It was found that hematein has an indophenol type intramolecular charge-transfer (CT) chromophoric system, as apparent from the π-electron density changes accompanying the first excitation.

The structures of hematoxylin (**1**) and hematein (**2**) were confirmed by ^{1}H-NMR. The most stable conformations of hematoxylin, hematein and its isomer were optimized by MOPAC PM3 were shown to be **6**, **7**, **8** and **9**, respectively. From these results, it was concluded that hematein has a more planar structure than hematoxylin because of the π-conjugation throughout the whole molecule, and the conventional structure of **2** is thus supported.

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REFERENCES

1. Puchtler, H.,, Meloan, S. N. & Waltrop, F. S., *Histochemistry*, **85** (1986) 353.
2. Roubani-Kalantzopoulou, F. & Katsanos, N. A., *Z. Phys. Chem. (Munich)*, **1492** (1986) 165.
3. Bettinger, C. & Zimmermann, H. W., *Histochemistry*, **95** (1991) 279.
4. Kubo, Y., Sasaki, K. & Yoshida, K., *Chemistry Lett.* (1987) 1563.
5. Marshall, P. N. & Horobin, R. W., *Stain Technol.*, **493** (1974) 137.