Preparation and Crystal Structure of Acetyl Hemerocallin and Structural Revision of Hemerocallin

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Abstract: **Objective** To revise the structure of hemerocallin, an active and toxic compound isolated from the roots of *Hemerocallis fulva*, which was first reported in 1974 based on the chemical and spectral data. **Methods** The structure of acetyl hemerocallin was determined through the acetyl derivative preparing and X-ray diffraction. **Results** The title compound acetyl hemerocallin (**1**) has been prepared and structurally characterized by X-ray single-crystal diffraction method. X-ray analysis revealed that the two naphthalene rings in compound **1** were significantly twisted with an average dihedral angle of 44.1°. The two acetate groups in each naphthalene ring adopted anti-parallel conformation. In addition, the π-π stacking interactions were found in the crystal structure, the molecules were linked into a supramolecular network, and the crystal structure was stabilized. **Conclusion** The structure of hemerocallin is identified as 2,2′-bi(1,8-dihydroxy-6-methyl-7-acetylnaphthalene) (**3**).

Keywords: acetyl hemerocallin; binaphthalene; crystal structure; hemerocallin; *Hemerocallis fulva*
DOI: 10.3969/j.issn.1674-6348.2013.02.014

Introduction

Hemerocallin (structure **1**, Fig. 1), a dark-red crystal powder, is a major active compound isolated from the roots of *Hemerocallis fulva* L., an herbaceous perennial plant of Liliaceae native to Asia from the Caucasus east through the Himalaya to China, Japan, Korea, and southeastern Russia. The root of *H. fulva* has been used as a herbal medicine for the treatment of mumps, jaundice, cystitis, hematuria, urination, agalactosis, irregular menstruation, nose bleeding, and bloody stool with the function of cleaning the body-heat and diuresis, cooling blood and hemostasis (Jiangsu New Medical College, 1986). The external use is for the treatment of mastitis. Hemerocallin showed significant anti- schistosomiasis activity. But the strong toxicity has limited its clinical use (Xia, 2005). Earlier structural study established its structure as a symmetrical 2,2′-binaphthyl compound with eight substituents (structure **2**, Fig. 1) mainly based on the chemical evidence and some spectral data, especially the inaccurate IR spectrum (Huang *et al*, 1974). But, the other possibility with different symmetrical substitution (structure **3**, Fig. 1) remains unresolved for years. Recently, Yang *et al* (2003; 2008a; 2008b) investigated the chemical constituents in the roots of *H. fulva* collected from Lingchuan (Guangxi, China). Total 31 compounds including steroids, triterpenes, and phenolic acids of small molecule had been identified from chloroform and butanol soluble fractions of ethanol extract, but there was no hemerocallin and related compounds found. The possibility may be that the extractive solvent is 95% ethanol instead of chloroform, but it is also possible that the experimental materials are from different sources. In order to confirm the structure with more evidences, especially the substitution style, the acetyl derivative of hemerocallin was prepared. Single crystal successfully achieved for acetyl hemerocallin made the crystal structure possible. Herein, we described the preparation of acetyl hemerocallin, the crystallographic study of acetyl hemerocallin, and the structure revision of hemerocallin as structure **3**.

Materials and methods

Isolation of hemerocallin

The roots (1 kg) of *Hemerocallis fulva*, collected at

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Received: July 26, 2012; Revised: September 18, 2012; Accepted: October 19, 2012

Gaoli Mountain (Jiangsu, China) and authenticated by Prof. CHEN Chang, National Institute of Parasitic Diseases, Chinese Center for Diseases Control and Prevention, were ground to crude powder and extracted in Soxlet extractor with chloroform. The chloroform extraction was concentrated to small volume and the precipitate was formed after natural cooling to room temperature. The filtrated precipitate, showing anti-schistosomiasis activity while the mother liquid did not, was separated over aluminum oxide column chromatography eluted with acetone containing 5% sodium hydroxide. The elute containing yellow-band was concentrated and neutralized with 5% hydrochloric acid, and hemerocallin (yellow crystal powder) was obtained. The yield is 0.3%—0.4%, showing activity for antischistosomiasis and host toxicity.

Preparation of acetyl hemerocallin

Hemerocallin (1 g) was added to acetic anhydride (7 mL) containing 7 mL of anhydrous pyridine and stood for overnight at room temperature. Ethyl ether (15 mL) and water (25 mL) were added to the reactant during stirring. Yellowish crystallized powder obtained was washed with acetone and then re-crystallized in acetone. Colorless platelet crystallized acetyl hemerocallin (0.95 g) was obtained.

Preparation of single crystal

Acetyl hemerocallin (10 mg) was dissolved with methanol (1 mL) in a 5-mL vial. The cap of the vial was pricked to let the solvent evaporate slowly. After 2 weeks, the colorless thick platelet single crystal was formed for crystallographic study. Because of the cracking property of the crystal once left the solvent, the gel film was used before X-ray crystallographic determination.

Crystal data and structure determination

A colorless single crystal with dimensions of 0.18 mm × 0.14 mm × 0.11 mm was selected for X-ray diffraction analysis. The unit cell determination and data collection were performed with a Mo *Kα* radiation (λ = 0.710 73 Å) on a Rigaku Saturn 724 CCD diffractometer. A total of 15 615 reflections were collected by using an ω-2θ scan mode at (298 ± 2) K in the range of 2.57°—25.00°. The 5388 reflections were independent with R_{int} = 0.0367 and 4123 observed reflections with [$I > 2\sigma(I)$] were used in the structure determination and refinements. The calculations were performed with Shelel-97 program and expanded by using the Fourier technique (Sheldrick, 1997). The structure was refined by full-matrix least-squares method on F^2 with anisotropic thermal parameters for all non-hydrogen atoms. All hydrogen atoms were placed in the calculated positions and constrained to ride on their parent atoms. Semi-empirical absorption correction was applied by using CrystalClear program. The final full-matrix least-squares refinement gave R = 0.0688, wR = 0.1847 ($w = 1/[\sigma^2(F_o^2) + (0.1167P)^2 + 0.4285P]$, where $P = (F_o^2 + 2F_c^2)/3$), S = 1.017, $(\Delta\rho)_{max}$ = 0.253, $(\Delta\rho)_{min}$ = −0.189 e/Å^3.

Structural revision of hemerocallin

Based on the crystal structure of acetyl hemerocallin, the structure of hemerocallin should be revised as structure **3** in Fig 1. The structure is identical with stypandrol, a toxic binaphthalenetetrol isolated from Australian plant *Stypandra imbricate* R. Br. of the same family with *H. fulva* (Colegate *et al*, 1985)

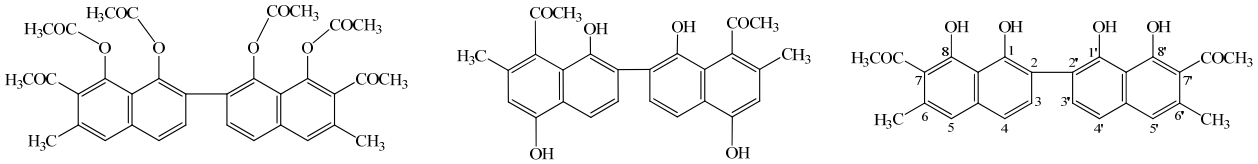

Fig. 1 Structures of hemerocallin and related compounds

Results

Preparation of acetyl hemerocallin

Hemerocallin was first isolated from the roots of *H. fulva* as a dark-red crystal powder. Many solvent systems failed to achieve single crystal for crystallographic study. So, its acetyl derivative has been prepared in order to get ideal crystal (Fig. 2). Yellowish powdered acetylated hemerocallin was successfully cultured in methanol to get desirable colorless single crystal for X-ray crystallographic study.

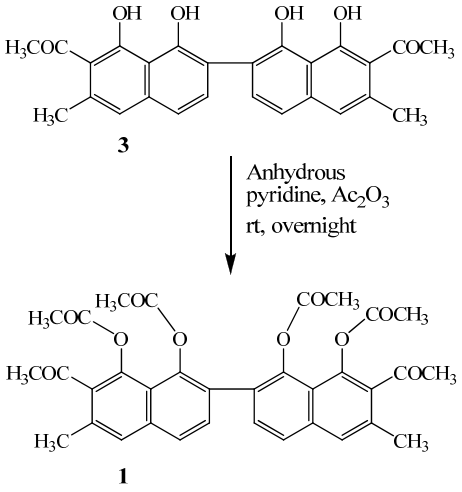

Fig. 2 Preparation of acetyl derivative of hemerocallin

Table 1 Crystallographic data and structure refinement for compound 1

Complex	Unit	Compound **1**
empirical formula		$C_{17}H_{15}O_5$
formula weight		299.29
temperature	K	293(2)
crystal system		Triclinic
space group		P-1
a	Å	10.178(2)
b	Å	11.672(2)
c	Å	14.350(3)
α	(°)	80.99(3)
β	(°)	86.53(3)
γ	(°)	65.76(3)
volume	$Å^3$	1535.2(5)
Z		4
ρ	$g·cm^{-3}$	1.295
μ	mm^{-1}	0.096
F		628
crystal sizes	mm	0.18 × 0.14 × 0.11
R	int	0.0367
data/restraints/parameters		5388 / 0 / 405
goodness-of-fit on F^2		1.017
final R^a indices [$I > 2\sigma(I)$]		$R1 = 0.0688$
		$wR_2 = 0.1847$
R indices (all data)		$R_1 = 0.0914$
		$wR_2 = 0.2043$
larfest diff. peak and hole	$(e·Å^{-3})$	0.23 and −0.189

Crystal structure description

The title compound is colorless block-shaped crystal and stable in air at room temperature. Crystallographic parameters and structural refinement data were summarized in Table 1.

Selected bond lengths and bond angles were listed in Tables 2 and 3, respectively. The molecular structure of the title compound and packing diagram were shown in Figs. 3 and 4, respectively. The single-crystal structural determination reveals that acetyl hemerocallin crystallizes in triclinic system with Pī space group. The asymmetric unit of this compound contains two naphthalene rings, and each naphthalene ring carries one methyl group, one acetyl group, and two acetate groups. The naphthalene rings C(1)-C(10) and C(1′)-C(10′) are each essentially planar, with mean deviations of 0.0492 and 0.0468 Å, respectively. The two naphthalene rings are significantly twisted and the dihedral angle between them is 44.1°. The aromatic C-C bond distances are between 1.353 and 1.430 Å, and the aromatic C-C-C bond angle is ranging from 116.3° to 123.3°, almost within the normal ranges. But

Table 2 Selected bond length (Å) of acetyl hemerocallin

Bonds	Dist.	Bonds	Dist.	Bonds	Dist.
O(1)-C(12)	1.176 (4)	C(2)-C(3)	1.426 (4)	C(1′)-C(6′)	1.420 (4)
O(2)-C(14)	1.357 (4)	C(2)-C(11)	1.510 (4)	C(2′)-C(3′)	1.423 (4)
O(2)-C(4)	1.406 (3)	C(3)-C(4)	1.365 (4)	C(2′)-C(11′)	1.515 (4)
O(3)-C(14)	1.201 (4)	C(3)-C(12)	1.505 (4)	C(3′)-C(4′)	1.366 (4)
O(4)-C(16)	1.359 (3)	C(4)-C(5)	1.421 (4)	C(3′)-C(12′)	1.511 (4)
O(4)-C(10)	1.417 (3)	C(5)-C(10)	1.411 (4)	C(4′)-C(5′)	1.429 (4)
O(5)-C(16)	1.193 (4)	C(5)-C(6)	1.430 (4)	C(5′)-C(10′)	1.418 (4)
C(13′)-C(12′)	1.480 (5)	C(6)-C(7)	1.408 (4)	C(5′)-C(6′)	1.427 (4)
O(2′)-C(14′)	1.375 (4)	C(7)-C(8)	1.361 (4)	C(6′)-C(7′)	1.406 (4)
O(2′)-C(4′)	1.405 (3)	C(8)-C(9)	1.420 (4)	C(7′)-C(8′)	1.350 (4)
O(3′)-C(14′)	1.186 (4)	C(9)-C(10)	1.367 (4)	C(8′)-C(9′)	1.413 (4)
O(4′)-C(16′)	1.370 (3)	C(9)-C(9′)	1.489 (4)	C(9′)-C(10′)	1.374 (4)
O(4′)-C(10′)	1.403 (3)	C(12)-C(13)	1.480 (5)	C(12′)-O(1′)	1.204 (4)
O(5′)-C(16′)	1.181 (3)	C(14)-C(15)	1.493 (5)	C(14′)-C(15′)	1.485 (5)
C(1)-C(2)	1.356 (4)	C(16)-C(17)	1.485 (4)	C(16′)-C(17′)	1.482 (4)
C(1)-C(6)	1.415 (4)	C(1′)-C(2′)	1.353 (4)		

Table 3 Selected bond angles (°) of acetyl hemerocallin

Bonds	Angle	Bonds	Angle	Bonds	Angle
C(14)-O(2)-C(4)	117.6 (2)	C(8)-C(9)-C(9′)	120.6 (2)	C(10′)-C(5′)-C(4′)	126.4 (2)
C(16)-O(4)-C(10)	115.4 (2)	C(9)-C(10)-C(5)	123.3 (2)	C(6′)-C(5′)-C(4′)	116.3 (2)
C(14′)-O(2′)-C(4′)	117.0 (2)	C(9)-C(10)-O(4)	117.7 (2)	C(7′)-C(6′)-C(1′)	121.4 (2)
C(16′)-O(4′)-C(10′)	115.8 (2)	C(5)-C(10)-O(4)	119.0 (2)	C(7′)-C(6′)-C(5′)	119.4 (2)
C(2)-C(1)-C(6)	123.2 (3)	O(1)-C(12)-C(13)	122.2 (4)	C(1′)-C(6′)-C(5′)	119.2 (3)
C(1)-C(2)-C(3)	118.1 (3)	O(1)-C(12)-C(3)	119.9 (3)	C(8′)-C(7′)-C(6′)	121.1 (2)
C(1)-C(2)-C(11)	121.9 (3)	C(13)-C(12)-C(3)	117.8 (3)	C(7′)-C(8′)-C(9′)	121.3 (3)
C(3)-C(2)-C(11)	120.0 (3)	O(3)-C(14)-O(2)	122.6 (3)	C(10′)-C(9′)-C(8′)	118.2 (2)
C(4)-C(3)-C(2)	120.0 (3)	O(3)-C(14)-C(15)	126.3 (4)	C(10′)-C(9′)-C(9)	121.6 (2)
C(4)-C(3)-C(12)	119.0 (3)	O(2)-C(14)-C(15)	111.1 (3)	C(8′)-C(9′)-C(9)	120.2 (2)
C(2)-C(3)-C(12)	121.0 (3)	O(5)-C(16)-O(4)	122.4 (3)	C(9′)-C(10′)-O(4′)	118.5 (2)
C(3)-C(4)-O(2)	116.7 (2)	O(5)-C(16)-C(17)	125.8 (3)	C(9′)-C(10′)-C(5′)	122.4 (2)
C(3)-C(4)-C(5)	123.0 (3)	O(4)-C(16)-C(17)	111.8 (3)	O(4′)-C(10′)-C(5′)	119.1 (2)
O(2)-C(4)-C(5)	120.3 (2)	C(2′)-C(1′)-C(6′)	123.1 (2)	O(1′)-C(12′)-C(13′)	122.9 (3)
C(10)-C(5)-C(4)	126.4 (2)	C(1′)-C(2′)-C(3′)	118.0 (2)	O(1′)-C(12′)-C(3′)	119.9 (3)
C(10)-C(5)-C(6)	117.1 (2)	C(1′)-C(2′)-C(11′)	122.2 (3)	C(13′)-C(12′)-C(3′)	117.2 (3)
C(4)-C(5)-C(6)	116.5 (2)	C(3′)-C(2′)-C(11′)	119.7 (3)	O(3′)-C(14′)-O(2′)	122.7 (3)
C(7)-C(6)-C(1)	122.0 (3)	C(4′)-C(3′)-C(2′)	120.7 (3)	O(3′)-C(14′)-C(15′)	126.5 (3)
C(7)-C(6)-C(5)	119.0 (2)	C(4′)-C(3′)-C(12′)	120.2 (2)	O(2′)-C(14′)-C(15′)	110.8 (3)
C(1)-C(6)-C(5)	119.0 (3)	C(2′)-C(3′)-C(12′)	119.1 (2)	O(5′)-C(16′)-O(4′)	122.4 (3)
C(8)-C(7)-C(6)	121.4 (3)	C(3′)-C(4′)-O(2′)	117.2 (2)	O(5′)-C(16′)-C(17′)	126.3 (3)
C(7)-C(8)-C(9)	120.8 (3)	C(3′)-C(4′)-C(5′)	122.4 (2)	O(4′)-C(16′)-C(17′)	111.4 (3)
C(10)-C(9)-C(8)	117.9 (2)	O(2′)-C(4′)-C(5′)	120.4 (2)	C(10′)-C(5′)-C(6′)	117.2 (2)
C(10)-C(9)-C(9′)	121.4 (2)				

compared with the normal aromatic bond values, the shorter C1-C2 (1.356 Å), C1′-C2′ (1.353 Å) and longer C4-C5 (1.421 Å), C5-C6 (1.430 Å), C4′-C5′ (1.429 Å), C5′-C6′ (1.427 Å) may be owing to the hyper-conjugation effect (Zhou and Duan, 1995). Compared with the structure of hemerocallin, in acetyl hemerocallin, four acetyl groups are introduced to the four hydroxyl groups of hemerocallin. In addition, the two acetate groups in each naphthalene ring adopt anti-parallel conformation. The bond lengths of O1-C12, O5-C16, O3-C14, O1′-C12′, O5′-C16′, O3′-C14′ range from 1.176(4) to 1.204(4) Å, belonging to the typical C=O double bond.

It should be noted that there is intermolecular π-π stacking interactions between the neighboring naphthalene rings, which are parallel to each other. The centroid-to-centroid, plane-to-plane, and displacement distances are 4.335, 3.514, and 2.424 Å, respectively, showing the existence of intermolecular π-π interaction. In the solid state, the π-π stacking interactions mentioned above could link the molecules into a supramolecular network and stabilize the crystal structure.

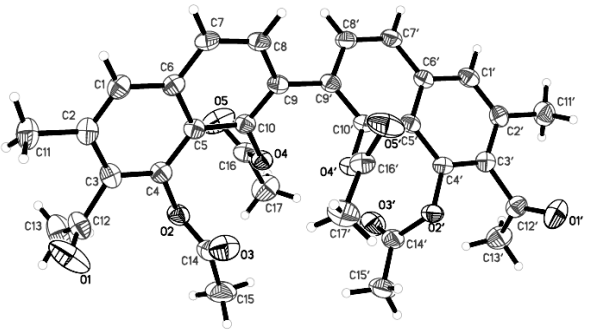

Fig. 3 Crystal structure of acetyl hemerocallin with atomic labeling scheme at 30% probability displacement ellipsoids

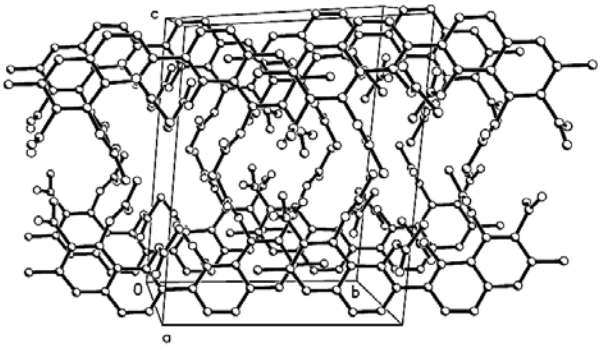

Fig. 4 Crystal packing of acetyl hemerocallin

Discussion

Hemerocallin from the roots of *H. fulva* was first discovered as an active constituent against schistosomiasis. Until 1974, the structure was established based on the limited IR, low-res NMR, and chemical degradation data. But, the uniqueness of the substitutes on the skeleton was not unambiguous since then. One of the original contributors, Prof. XIE Jing-xi has never given up the effort to clarify the structure by solid evidence for years. Recently, we successfully prepared its acetyl derivative and achieved the single crystal. The crystallographic study revealed the detail arrangement of eight substitutes on the skeleton. The crystal structure of acetyl hemerocallin corrected the wrong sequence of substitutions and indirectly established the correct structure of hemerocallin.

The flower buds of *H. fulva* has been traditionally used as delicious and nutritional vegetable for thousands of years in fresh or dry form, while the roots are used as herbal medicine. But the serious poisoning cases had been reported either for sheep or human. The attention must be seriously paid although the mechanism has not been clarified. The structural clarification of hemerocallin as a toxic component will help researchers deeply understand the poisoning mechanism and find the way for the prevention and better use of the herbal medicine.

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