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Crystal Structure of 3S-hydroxy-7 Melleine

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A new compound, 3S-hydroxy-7 melleine was isolated from the endophytic fungus *Xylariasp* No.2508 from the mangrove tree on the South China Sea coast. It was the first time that this kind of compound was isolated from marine fungus. The structure was elucidated by NMR data, infrared spectrum (IR) and mass spectrometry (MS). In addition, its structure was determined by the single-crystal X-ray diffraction analysis. It crystallized in monoclinic, space group $P2_1$ with a=10.8884(19) Å, b=7.2284(13) Å, c=13.398(2) Å, β=104.217(3)°, $C_{10}H_{10}O_4{\cdot}H_2O$, M_r=212.20, V=1022.2(3) Å^3, Z=4, D_c=1.379 mg/m^3, $F(000)$=448, μ=0.112 mm^{-1}, the final R=0.0498, wR=0.101 for 2407 observed reflections ($I>2\sigma(I)$). The molecular backbone of the compound includes a benzopyran ring. By comparing with the melting point and the optical rotation of the known 3R-hydroxy-7 melleine in literature, the absolute configuration of the compound was determined as 3S. It didn't exhibit antibacterial activity against Gram-positive bacterium *Staphylococcus aureus* at 200 μg/disk in the preliminary test.

Key words: 3S-hydroxy-7 melleine, Isolation, Crystal structure

I. INTRODUCTION

The mangrove fungus strain No.2508 collected from the seeds of an angiosperm tree and identified as *Xylaria* species (*Ascomycota*), has already been found to produce rich secondary metabolites, which consists of a series of new ketal compounds and novel allenic compounds [1-3]. In order to find more new compounds, we continued the study of the metabolites from this mangrove fungus, which resulted in the isolation and structure elucidation of a new compound 3S-hydroxy-7 melleine. This kind of compound has been isolated from the plant [4] and terrestrial fungus [5] before and was synthesized as racemate [6]. While it was isolated from the marine fungus and identified as the hydroxy-7 melleine with 3S configuration for the first time.

II. EXPERIMENTS

A. Instrument

The melting point was determined on X-4 micro-melting point apparatus and was uncorrected. The mass spectra were measured with VG-ZABHS mass spectrometer, and the IR spectra were obtained with Bruker EQUINOX 55. The UV spectra were measured with SHIMADZU UV-3150 UV-Vis-NIR spectrophotometer, and the optical rotation were achieved with SCHMIDT+HAENSCH POLARTRONIC HH W5 polarimeter. The NMR spectra were performed on Varian Inova 300NB NMR spectrometer using TMS as an internal standard. The NMR experiments include ^{1}H NMR, ^{13}C NMR and DEPT. And the X-ray data were generated on Bruker Smart 1000 CCD system diffractometer.

B. Extraction and isolation

A strain of the fungus *Xylariasp.*(No.2508) was isolated from the seeds of an angiosperm tree in Mai Po, Hong Kong, and was stored in the Department of Applied Chemistry, at Sun Yat-sen University, Guangzhou, China. The starter cultures (from Professor E. B. G. Jones and Dr. L. L. P. Vrijmoed) were maintained on cornmeal seawater agar. The plugs of agar supporting mycelial growth were cut and transferred aseptically to a 500 mL Erlenmeyer flask containing 250 mL of liquid medium (glucose 10 g/L, peptone 2 g/L, yeast extract 1 g/L, NaCl 30 g/L). The flask was incubated at 30 °C on a rotary shaker for 5-7 days. The mycelium was aseptically transferred to a 300 L fermenter containing 170 L of GYT medium, incubated at 30 °C for 80 h. The cultures (170 L) were filtered through cheesecloth. The filtrate was extracted five times by shaking with an equal volume of EtOAc. The combined extracts were concentrated to give a brown oil, and chromatographed on silica gel using a gradient elution from petroleum to ethyl acetate to obtain compound 3-hydroxy-7 melleine

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(48.5 mg) from the 10%-15% ethyl acetate/petroleum ether fraction.

The compound crystallized as colorless crystals (MeOH-$CHCl_3$). mp: 81-82 °C. $[\alpha]_D^{20}$ 93°±2° (c=0.5, $CHCl_3$). IR(KBr): ν_{max} 3346(br), 3134, 3095, 2982, 2936, 1672, 1588, 1505, 1455, 1383, 1274, 1128, 877 cm^{-1}; UV (CH_3OH, c 0.03): λ_{max} 334(logε=0.85), 257(logε=1.12), 224(logε=1.56) nm; ^1H NMR(acetone-d_6, 300 MHz): δ11.09 (1H,s,8-OH), 8.05 (1H,s,7-OH), 7.04 (1H,d,J=7.2 Hz,H-6), 6.66 (1H,d,J=7.2 Hz,H-5), 4.77 (1H,ddq,J=6.3 Hz,H-3), 2.95 (2H,d,J=6.3 Hz,H-4), 1.48 (3H,d,J=6.3 Hz,H-11); ^{13}C NMR (Acetone-d_6, 75 MHz): δ 173.0 (C-1,C=O), 79.9 (C-3,CH), 36.4 (C-4,CH_2), 111.2 (C-10,C), 120.3 (C-5,CH), 124.1 (C-6,CH), 147.2 (C-7,C), 152.8 (C-8,C), 132.6(C-9,C), 23.0 (C-11,CH_3); FABMS: m/z 195(M+1)$^+$, 177, 165, 136.

C. Crystallographic data collection and structure

The crystals of the title compound suitable for single crystal X-ray diffraction analysis were obtained by recrystallization from methanol. A colorless plate of the title compound, approximately 0.47×0.33×0.23 mm, was mounded on a glass fiber. The determination of the unit cell and the data collection were on a Bruker SMART 1000 CCD system diffractometer with graphite-monochromatic Mo-Kα radiation (λ=0.71073 Å). At 293(2) K, a total of 6182 reflections (R_{int}=0.0180) were collected in the range of 1.93°≤θ≤27.10°, of which 2407 observed reflections with $I\geq 2\sigma(I)$ were used in the succeeding structure determination and refinements. All empirical absorption corrections were applied by using SADABS program [7]. The crystal structure was solved using the direct method, which yielded the positions of all non-hydrogen atoms. These were refined isotropically first, and then refined anisotropically. All the hydrogen atoms of the title compound were placed at the calculated positions with the fixed isotropic thermal parameters and included in the structure factor calculations in the final stage of the full-matrix least-squares refinement. The hydrogen atoms of water molecules were located from the difference Fourier map and refined isotropically, the O−H distances of the water molecules were refined with a DFIX restraint of 0.86 Å. All calculations were performed using the SHELXTL system of computer programs [8]. The final refinement converged at R=0.0498, wR=0.101. The largest peak and the deepest hole on the final difference Fourier map were 0.136 and −0.140 e/Å^3, respectively.

D. Antibacterial assay

The preliminary screening of the antibacterial activity was assessed against *Staphylococcus aureus* by disk diffusion method [9]. The solutions of two kinds of concentrations (100 and 200 μg/disk) of the title compound were dissolved in dimethyl sulfoxide (DMSO) and were added to the Petri dishes (containing beef extract, sea salt, peptone and agar).

III. RESULTS AND DISCUSSION

The FABMS spectrum analysis indicated the compound has the molecular formula $C_{10}H_{10}O_4$. The number of the hydrogen atom and the carbon atoms observed in the ^1H and ^{13}C NMR spectra were in agreement with this molecular formula. Its IR spectrum showed the presence of one ester carbonyl group with band at 1672 cm^{-1} and the hydroxyl absorption at 3346 cm^{-1}, respectively. In the ^{13}C NMR spectrum and the DEPT spectrum, one carbonyl carbon signal (173.0), six phenyl carbon signals (152.8, 147.2, 132.6, 124.1, 120.3, 111.2), one methine bearing oxygen signal (79.9), one methylene signal (36.4) and one methyl group (23.0) were observed. The ^1H NMR signals of the two coupling protons at δ 6.66 (d,J=7.2 Hz) and 7.04 (d,J=7.2 Hz) indicated a pair of adjacent protons on the benzene ring, and the other three coupling protons at δ 4.77 (ddq,J=6.3 Hz), 2.95 (d,J=6.3 Hz) and 1.48 (d,J=6.3 Hz) suggested that both the methyl and the methylene groups were attached to the methine group. Two downfield signals at 11.09 and 8.05 indicated the two phenol hydroxyls, while the occurrence of an innermolecular hydrogen bonding between 8-OH and 1-C=O, caused a downfield shift of the signal of the former. The structure of the hydroxy-7 melleine was finally confirmed by X-ray diffraction analysis. But its absolute stereochemistry was not defined in the X-ray structure. However, the m.p. and $[\alpha]_D^{20}$ of 3R-hydroxy-7 melleine in literature was 100-101 °F (47.1-47.5 °C) and $-97° \pm 3°$ [5], respectively. While those of the title compound were 81-82 °C and $93° \pm 2°$, respectively. Therefore, its absolute configuration was able to elucidate as 3S. This is the first report of 3S-hydroxy-7 melleine and its isolation from marine fungus.

The molecular formula and relative molecular weight of the crystal with one H_2O molecule were $C_{10}H_{10}O_4 \cdot H_2O$ and 212.20, respectively. The crystal system was monoclinic, and the space group of the crystal was $P2_1$. The unit cell dimensions were shown as following: a= 10.8884(19) Å, α=90°, b=7.2284(13) Å, β=104.217(3)°, c=13.398(2) Å, γ=90°; V=1022.2(3) Å^3, Z=4, D_c=1.379 mg/m^3, F(000)=448, μ(Mo Kα)=0.112 mm^{-1}. The final value of R was 0.0498, wR=0.101[$I>2\sigma(I)$]. The detailed crystallographic data were summarized in Table I. Further crystallographic data can be reached from the IUCr electronic archives using http://www.ccdc.cam.ac.uk (Reference: CCDC 294735). The selected bond lengths and angles for title compound were listed in Table II. An ORTEP drawing of the title compound showing the molecular conformation and atom-labeling scheme was

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TABLE I Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters (10^3Å^2)

Atom	x	y	z	U(eq)	Atom	x	y	z	U(eq)
O(1)	465(2)	−1015(3)	7333(2)	69(1)	C(6)	2671(2)	2816(5)	5213(2)	57(1)
O(2)	−428(2)	1681(3)	7362(1)	65(1)	C(7)	2905(2)	1040(4)	5592(2)	49(1)
O(3)	3831(2)	−62(3)	5399(2)	66(1)	C(8)	2144(2)	297(4)	6194(2)	45(1)
O(4)	2384(2)	−1468(3)	6521(1)	60(1)	C(9)	1173(2)	1370(4)	6412(2)	44(1)
O(5)	4464(2)	4933(3)	7590(2)	71(1)	C(10)	−1626(3)	4427(7)	7406(3)	89(1)
O(6)	5540(2)	2353(3)	7744(1)	63(1)	C(11)	4634(2)	3381(4)	7968(2)	51(1)
O(7)	1350(2)	4205(3)	9776(1)	56(1)	C(12)	5973(2)	667(4)	8340(2)	55(1)
O(8)	2786(2)	5557(3)	8626(2)	61(1)	C(13)	4872(2)	−427(4)	8501(2)	52(1)
O(9)	5202(2)	1378(4)	4267(2)	91(1)	C(14)	3978(2)	764(4)	8916(2)	45(1)
O(10)	193(2)	7676(4)	9251(2)	79(1)	C(15)	3212(2)	81(4)	9506(2)	55(1)
C(1)	400(2)	606(5)	7057(2)	52(1)	C(16)	2341(2)	1221(4)	9806(2)	53(1)
C(2)	−392(3)	3696(5)	7228(2)	65(1)	C(17)	2205(2)	3037(4)	9512(2)	45(1)
C(3)	−202(2)	4149(5)	6191(2)	63(1)	C(18)	2966(2)	3772(4)	8912(2)	43(1)
C(4)	935(2)	3157(4)	6000(2)	52(1)	C(19)	3861(2)	2629(4)	8628(2)	42(1)
C(5)	1693(2)	3855(5)	5410(2)	60(1)	C(20)	6760(3)	−374(6)	7735(2)	74(1)

U (eq) is defined as one third of the trace of the orthogonalyzed U_{ij} tensor.

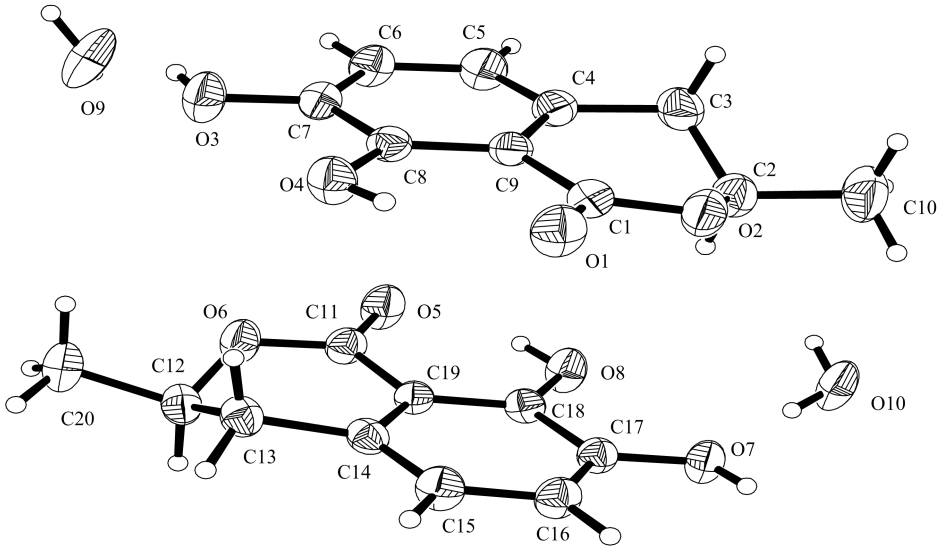

FIG. 1 Molecular structure of title compound at 30% ellipsoid probability.

depicted in Fig.1. The molecular backbone of the title compound was one benzopyran as shown in Fig.2. The C−C bond distances in the benzene ring were from 1.372(4) to 1.403(4) Å and the C−C−C angle were from 119.6°(2) to 121.2°(3), which are normal.

There existed intermolecular hydrogen-bonding O(3)−H(3)···O(9) between the title compound and the one H_2O molecule. The bond lengths of O(3)···O(9) and H(3)···O(9) were 2.593(3) and 1.78 Å, respectively, and the bond angle of O(3)−H(3)···O(9) was 170.5° [10]. The bond lengths and angles for the title compound concerning hydrogen-bonding were shown in Table III, and the important structural feature concerning hydrogen-bonding could be found in its

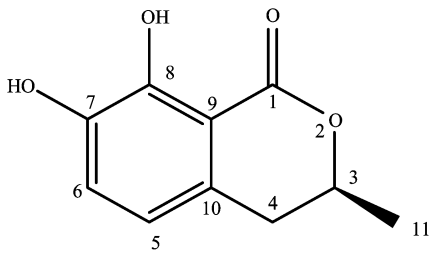

FIG. 2 The structure of 3S-hydroxy-7 melleine.

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TABLE II Bond lengths (Å) and angles (°)

Bond	Lengths/Å	Bond	Lengths/Å	Bond	Lengths/Å
O(1)−C(1)	1.225(4)	C(3)−H(3A)	0.9700	C(13)−C(12)	1.495(4)
O(2)−C(1)	1.329(3)	C(3)−H(3B)	0.9700	C(13)−H(13A)	0.9700
O(2)−C(2)	1.469(4)	C(4)−C(5)	1.372(4)	C(13)−H(13B)	0.9700
O(3)−C(7)	1.358(3)	C(4)−C(3)	1.505(4)	C(14)−C(15)	1.374(3)
O(3)−H(3C)	0.8200	C(5)−H(5A)	0.9300	C(14)−C(13)	1.505(3)
O(4)−H(4)	0.8200	C(6)−C(5)	1.380(4)	C(15)−H(15)	0.9300
O(5)−C(11)	1.226(3)	C(6)−C(7)	1.381(4)	C(16)−C(15)	1.387(4)
O(6)−C(11)	1.327(3)	C(6)−H(6A)	0.9300	C(16)−H(16)	0.9300
O(6)−C(12)	1.470(3)	C(8)−O(4)	1.354(3)	C(17)−O(7)	1.366(3)
O(7)−H(7)	0.8200	C(8)−C(7)	1.398(3)	C(17)−C(16)	1.368(4)
O(8)−C(18)	1.346(3)	C(8)−C(9)	1.399(3)	C(17)−C(18)	1.394(3)
O(8)−H(8)	0.8200	C(9)−C(4)	1.403(4)	C(19)−C(14)	1.399(4)
O(9)−H(9B)	0.8632	C(9)−C(1)	1.455(4)	C(19)−C(18)	1.400(3)
O(9)−H(9A)	0.8082	C(10)−C(2)	1.516(4)	C(19)−C(11)	1.466(3)
O(10)−H(10D)	0.8379	C(10)−H(10A)	0.9600	C(20)−C(12)	1.516(4)
O(10)−H(10E)	0.8067	C(10)−H(10B)	0.9600	C(20)−H(20A)	0.9600
C(2)−H(2)	0.9800	C(10)−H(10C)	0.9600	C(20)−H(20B)	0.9600
C(3)−C(2)	1.490(4)	C(12)−H(12)	0.9800	C(20)−H(20C)	0.9600
Bond	Angle/(°)	Bond	Angle/(°)	Bond	Angle/(°)
O(4)−C(8)−C(7)	117.0(2)	H(3A)−C(3)−H(3B)	108.0	O(5)−C(11)−C(19)	122.5(3)
O(4)−C(8)−C(9)	123.4(2)	C(14)−C(19)−C(18)	121.1(2)	O(6)−C(11)−C(19)	119.8(2)
C(7)−C(8)−C(9)	119.6(2)	C(14)−C(19)−C(11)	119.8(2)	H(9B)−O(9)−H(9A)	106.3
C(7)−O(3)−H(3C)	109.5	C(18)−C(19)−C(11)	119.1(2)	C(12)−C(20)−H(20A)	109.5
C(1)−O(2)−C(2)	120.1(2)	C(11)−O(6)−C(12)	119.9(2)	C(12)−C(20)−H(20B)	109.5
C(8)−O(4)−H(4)	109.5	C(15)−C(14)−C(19)	118.5(2)	H(20A)−C(20)−H(20B)	109.5
C(8)−C(9)−C(4)	120.3(2)	C(15)−C(14)−C(13)	123.1(3)	C(12)−C(20)−H(20C)	109.5
C(8)−C(9)−C(1)	119.4(2)	C(19)−C(14)−C(13)	118.2(2)	H(20A)−C(20)−H(20C)	109.5
C(4)−C(9)−C(1)	120.3(2)	O(7)−C(17)−C(16)	123.5(2)	H(20B)−C(20)−H(20C)	109.5
C(5)−C(6)−C(7)	121.2(3)	O(7)−C(17)−C(18)	116.9(2)	C(2)−C(10)−H(10A)	109.5
C(5)−C(6)−H(6A)	119.4	C(16)−C(17)−C(18)	119.6(2)	C(2)−C(10)−H(10B)	109.5
C(7)−C(6)−H(6A)	119.4	C(18)−O(8)−H(8)	109.5	H(10A)−C(10)−H(10B)	109.5
O(1)−C(1)−O(2)	117.4(3)	C(17)−C(16)−C(15)	121.4(2)	C(2)−C(10)−H(10C)	109.5
O(1)−C(1)−C(9)	122.9(3)	C(17)−C(16)−H(16)	119.3	H(10A)−C(10)−H(10C)	109.5
O(2)−C(1)−C(9)	119.7(3)	C(15)−C(16)−H(16)	119.3	H(10B)−C(10)−H(10C)	109.5
C(5)−C(4)−C(9)	119.1(3)	C(12)−C(13)−C(14)	111.4(2)	O(6)−C(12)−C(13)	110.82(19)
C(5)−C(4)−C(3)	124.0(3)	C(12)−C(13)−H(13A)	109.3	O(6)−C(12)−C(20)	105.6(2)
C(9)−C(4)−C(3)	116.9(3)	C(14)−C(13)−H(13A)	109.3	C(13)−C(12)−C(20)	113.2(3)
C(4)−C(5)−C(6)	120.8(3)	C(12)−C(13)−H(13B)	109.3	O(6)−C(12)−H(12)	109.0
C(4)−C(5)−H(5A)	119.6	C(14)−C(13)−H(13B)	109.3	C(13)−C(12)−H(12)	109.0
C(6)−C(5)−H(5A)	119.6	H(13A)−C(13)−H(13B)	108.0	C(20)−C(12)−H(12)	109.0
O(3)−C(7)−C(6)	123.7(3)	C(17)−O(7)−H(7)	109.5	O(2)−C(2)−C(3)	110.2(2)
O(3)−C(7)−C(8)	117.2(3)	O(8)−C(18)−C(17)	117.6(2)	O(2)−C(2)−C(10)	105.9(3)
C(6)−C(7)−C(8)	119.1(3)	O(8)−C(18)−C(19)	123.4(2)	C(3)−C(2)−C(10)	113.7(3)
C(2)−C(3)−C(4)	111.2(2)	C(17)−C(18)−C(19)	118.9(2)	O(2)−C(2)−H(2)	109.0
C(2)−C(3)−H(3A)	109.4	C(14)−C(15)−C(16)	120.5(3)	C(3)−C(2)−H(2)	109.0
C(4)−C(3)−H(3A)	109.4	C(14)−C(15)−H(15)	119.7	C(10)−C(2)−H(2)	109.0
C(2)−C(3)−H(3B)	109.4	C(16)−C(15)−H(15)	119.7	H(10D)−O(10)−H(10E)	111.7
C(4)−C(3)−H(3B)	109.4	O(5)−C(11)−O(6)	117.6(3)		

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TABLE III Hydrogen-bonding

D−H···A	$d_{(H\cdots A)}$/Å	$d_{(D-H)}$/Å	$d_{(D\cdots A)}$/Å	∠DHA/(°)
O(3)−H(3C)···O(9)	1.78	0.82	2.593(3)	170.5
O(4)−H(4)···O(1)	1.89	0.82	2.602(3)	144.2
O(8)−H(8)···O(5)	1.88	0.82	2.592(3)	145.1
O(10)−H(10D)···O(7)	2.00	0.84	2.818(3)	164.0
O(7)−H(7)···O(10)	1.80	0.82	2.611(2)	170.1
O(9)−H(9B)···O(3)	1.93	0.86	2.775(4)	164.9
O(9)−H(9A)···O(5)	2.00	0.81	2.803(3)	174.2
O(10)−H(10E)···O(1)	2.02	0.81	2.822(3)	174.4

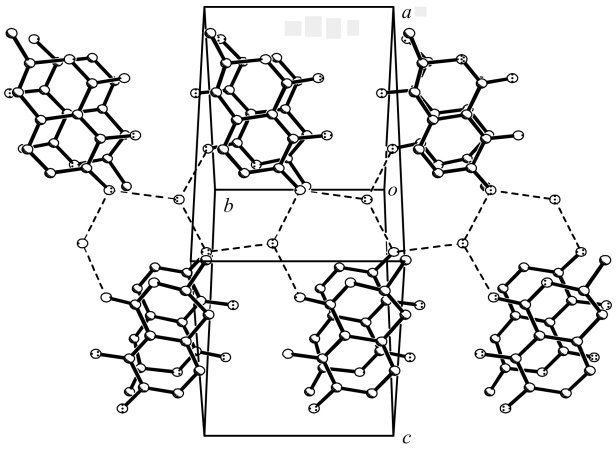

FIG. 3 Packing diagram of the title compound, showing the intermolecular hydrogen-bonding network along the *b* axis.

molecular packing (Fig.3).

The title compound was tested against the Gram-positive bacterium *Staphylococcus aureus*. The disk diffusion method used in the preliminary screening showed that it is inactive at the amount of 200 μg/disk.

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