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Biotransformation of Bioactive (–)-Mellein by a Marine Isolate of Bacterium *Stappia* sp.

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The biological transformation of the bioactive dihydroisocoumarin, (–)-mellein, isolated from the marine-derived fungus *Cladosporium* sp., was studied. The preparative-scale culture of (–)-mellein with a marine isolate of a bacterium *Stappia* sp. resulted in the isolation of its oxidized metabolite, (3*R*,4*S*)-4-hydroxymellein. The stereostructure of the metabolite obtained was assigned on the basis of detailed physicochemical data analyses.

Keywords: Biotransformation, dihydroisocoumarin, (–)-mellein, (3*R*,4*S*)-4-hydroxymellein, *Stappia* sp.

The aim of our program is to explore the biological transformation of bioactive metabolites produced by microorganisms isolated from marine habitats. As part of this program, we identified two species of marine-derived *Streptomyces* (MFAac18 and 67) and one *Penicillium* species (MFAac49) that regioselectively biotransform terreusinone into the unsymmetrical alcohol derivative terreusinol [8]; 6-*n*-pentyl-α-pyrone into two oxidized metabolites, 6-*n*-(4-oxopentyl)-α-pyrone and 6-*n*-[(*S*)-1-hydroxypentyl]-α-pyrone [7]; cyclonerodiol into three metabolites, 10(*Z*)-, 10(*E*)-cyclonerotriol, and cyclonerodiol mannopyranoside [9]; and geraniol into its 7-oxidized metabolite, 1,7-dihydroxy-3,7-dimethyl-(*E*)-oct-2-ene [6]. In our continuing studies on applications of biological transformation [6], we screened 50 cultures for their ability to biotransform (–)-mellein (**1**) [1], which was isolated from the marine algicolous fungus *Cladosporium* sp. [13]. The marine-derived bacterium *Stappia* sp. was selected as the biotransforming target strain.

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Isolation of the Marine-Derived Bacterium *Stappia* sp.

The *Stappia* sp., isolated from the surface of the edible marine green alga *Enteromorph compressa* (Korean name: NapJag PaRae), had light gray, downy, and soft white vegetative mycelia, and was identified on the basis of morphology and 16S rRNA analysis (SolGent Co., Ltd., Daejeon, Korea), identity of 99%. The *Stappia* sp., designated as BAac008, is deposited at Pukyong National University, South Korea.

Biotransformatin of (–)-Mellein (1)

A two-stage culture protocol [12] was used to obtain metabolites of **1** on a preparative scale. The SWS medium contained soytone (0.1%), soluble starch (1.0%), and seawater (100%), and was autoclaved at 121°C for 15 min. The preparative culture (stage 1) was incubated in 1 l of sterile medium in a 3-l culture flask on a rotary shaker (130 rpm) at 29°C for 1 week. A 10% inoculum derived from the 1-week-old preparative culture was used to initiate the stage II culture, which was incubated for a further 24 h under the same conditions before addition of 21 mg of **1** in 0.75 ml of *N,N*-dimethyl formamide (DMF). Incubation was continued for two weeks in the same manner as described above. The substrate control consisted of sterile medium and substrate incubated under the same conditions, but without microorganisms. The culture control consisted of the microorganism grown under the same condition, but without substrate. After three weeks, each control was harvested and analyzed by TLC. The culture was filtered through cheesecloth, and the filtrate was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered through sintered glass, and vacuum-concentrated to yield a crude extract (80 mg) that was subjected to Si gel flash column chromatography using *n*-hexane:EtOAc (1:2) as eluent to give a fraction containing compound **2**, which

Table 1. NMR spectral data for (3*R*,4*S*)-4-hydroxymellein (2)[a].

(3*R*,4*S*)-4-hydroxymellein (**2**)			
Carbon No.	δ_C, mult	δ_H (*J* in Hz)	HMBC (H to C)
1	168.1, qC		
3	79.8, CH	4.54, m	1, 4, 10, 11
4	67.3, CH	4.55, m	3, 9, 10, 11
5	116.1, CH	6.93, d (7.5)	7, 9
6	136.5, CH	7.59, dd (7.5, 8.5)	8, 10
7	116.4, CH	7.05, d (8.5)	5, 9
8	160.4, qC		
9	106.8, qC		
10	143.6, qC		
11	17.6, CH_3	1.39, d (4.9)	3, 4
4-OH		6.11, d (5.9)	3, 4, 10
8-OH		10.88, s	7, 8, 9

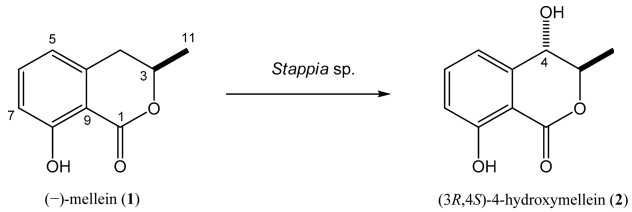
[a]Recorded in DMSO-d_6 at 400 MHz (^{1}H) and 100 MHz (^{13}C).

was further purified by HPLC (Appolo-ODS, 10×250 mm, 1 ml/min) utilizing a 30 min gradient program of 50% to 100% MeOH in H_2O to furnish (3*R*,4*S*)-4-hydroxymellein (**2**) (15.0 mg) [1, 3].

(3*R*,4*S*)-4-Hydroxymellein (**2**): colorless solid; TLC ($CHCl_3$: EtOAc 5:1), R_f 0.65, color reaction with $FeCl_3$ (pink); $[\alpha]_D$ -28 (*c* 0.5, MeOH) and -28 ($CHCl_3$) [(3*R*,4*S*): -29 (MeOH) and (3*R*, 4*R*): -31 (MeOH) and -41 ($CHCl_3$)] [3, 4]; m.p.: 130~132°C [(3*R*, 4*S*): 131~132°C; (3*R*, 4*R*): 123~124°C] [3, 4]; UV (MeOH) λ_{max} (log ε) 245 (3.8), 313 (3.6) nm; IR (neat) ν_{max} 3,305, 1,683 cm^{-1}; ^{1}H and ^{13}C NMR (DMSO-d_6), see Table 1, and ^{1}H ($CDCl_3$, 400 MHz) δ 4.61 (1H, m, H-3), 4.62 (1H, m, H-4), 7.03 (1H, d, *J*=7.7 Hz, H-5), 7.54 (1H, dd, *J*=8.4, 7.7 Hz, H-6), 7.00 (1H, d, *J*=8.4 Hz, H-7), 1.52 (3H, d, *J*=5.9 Hz, H_3-11), 10.99 (1H, s, 8-OH); LR–EI–MS *m/z* 194 $[M]^+$ (57), 176 $[M-H_2O]^+$ (2), 150 $[M-CO_2]^+$ (91), 147 (4), 122 (85), 121 (100), 93 (26), 65 (38).

Structural Determination of (3*R*,4*S*)-4-Hydroxymellein (2)

(−)-Mellein (**1**) is a dihydroisocoumarin derivative widely distributed in fungi [1, 5], and it is noteworthy that some of the mellein derivatives are produced in substantial amounts, facilitating their use for chemotaxonomic purposes [11]. (−)-Mellein (**1**) was also isolated from the fungus *Aspergillus ochraceus* as a potent inhibitor of the hepatitis C virus (HCV) NS3 protease [2]. The biofunctional role of NS3 protease makes this protein an attractive target as an antiviral therapy [10]. In this context, we are interested in the chemical and biological aspects of the derivatives of **1** and the microbial synthesis of mellein derivatives. As part of a program to find antiviral compounds produced by the biological transformation of the bioactive metabolites produced by fungi isolated from marine habitats, the biotransformation of **1** was performed by a marine-derived bacterium, *Stappia* sp., using a two-stage culture protocol [12]. After culture of *Stappia* sp. with **1** (20 mg) for 72 h, the culture broth was harvested, filtered, and extracted with EtOAc to yield the crude extract. The extract was purified by repeated Si gel flash chromatography (*n*-hexane in ethyl acetate) to yield the oxidized metabolite (**2**). The IR absorption spectrum of **2** showed bands characteristic of hydroxyl (3,305 cm^{-1}) and conjugated lactone (1,683 cm^{-1}) functionalities. The general features of the UV, IR, and NMR spectra of **2** closely resembled those of the substrate, (−)-mellein (**1**), except that the NMR signal at C-4 indicated a change from the sp^3-methylene [δ2.96 (2H, m, H_2-4), 33.4 (CH_2, C-4)] of **1** to an sp^3-methine [δ4.55 (1H, m, H-4), 67.3 (CH, C-4)] bearing an aliphatic secondary alcohol [δ6.11 (1H, d, *J*=5.9 Hz, 4-OH)] in **2** [5]. Detailed analysis of the ^{1}H and ^{13}C NMR spectra of **2**, including the results of DEPT, HMQC, and HMBC experiments, suggested that metabolite **2** is the 4-hydroxyl derivative of **1**. The key HMBC correlations from H-3 to C-4, C-10, and C-11, from 4-OH to C-3, C-4, and C-10, and from H_3-11 to C-3 and C-4 were critical in establishing the location of the 4-hydroxyl group, as shown in Fig. 1 (Table 1). On the basis of these data, the structure of the metabolite is proposed to be 4-hydroxymellein (**2**) [1, 3]. To obtain additional stereochemically relevant information, NOE data were recorded in different solvents and with different delay values, but no further useful cross-peaks between the chiral centers at C-3 and C-4 were observed because of the overlapping signals of H-3 and H-4. Therefore, the stereochemistry of **2** is based on optical rotation, which established that **2** has a 3*R* and 4*S* configuration from a comparison of its optical rotation ($[\alpha]_D^{20}$ -28 (MeOH) and -28 ($CHCl_3$)) with those of (3*R*,4*S*)-4-hydroxymellein ($[\alpha]_D^{20}$ -29) [3] and (3*R*,4*R*)-4-hydroxymellein ($[\alpha]_D^{20}$ -31 (MeOH) and -41 ($CHCl_3$)) [3, 4], which indicated that both compounds share the same configuration at the asymmetric center. This conclusion was further supported by a comparison of the melting point, ^{1}H NMR data, and TLC of **2** with those of (3*R*,4*S*)-4-hydroxymellein and (3*R*,4*R*)-4-hydroxymellein [3, 4]. The melting point (130~132°C), chemical shifts of ^{1}H NMR ($CDCl_3$), and TLC ($CHCl_3$: EtOAc 5:1, R_f 0.65, pink with $FeCl_3$) of **2** corresponded well to those of (3*R*,4*S*)-4-hydroxymellein (131~132°C) [3]. Based on the evidence described above, the stereostructure



(−)-mellein (**1**) (3*R*,4*S*)-4-hydroxymellein (**2**)

Fig. 1. Biotransformation of (−)-mellein (**1**) by the marine-derived bacterium *Stappia* sp.

of the metabolite was deemed to be (3*R*,4*S*)-4-hydroxymellein (Fig. 1) [1, 3]. To the best of our knowledge, compound **2** is the first example of a compound derived from the biological transformation of **1**. Compounds **1** and **2** exhibited mild radical scavenging activity against 1,1-diphenyl-2-picryl-hydrazyl (DPPH), with IC_{50} values of 34 and 54 μM, respectively. Compound **2** also showed mild antibacterial activity against methicillin-resistant and multidrug-resistant *Staphylococcus aureus*, with a minimum inhibitory concentration (MIC) of 62.5 μg/ml for both, whereas **1** was inactive. We are now intensively examining compounds **1** and **2** for antiviral activity, and the results will be reported in due course.

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