Microbial Transformation of Terreusinone, an Ultraviolet-A (UV-A) Protecting Dipyrroloquinone, by *Streptomyces* sp.

XiFeng Li,[a] Sang Mi Lee,[a] Hong Dae Choi,[b] Jung Sook Kang,[c] and Byeng Wha Son*,[a]

[a] *Department of Chemistry, Pukyong National University; Busan 608–737, Korea:* [b] *Department of Chemistry, Dongeui University; Busan 614–714, Korea: and* [c] *College of Dentistry, Pusan National University; Busan 602–739, Korea.*
Received September 5, 2003; accepted September 24, 2003

Biotransformation study was conducted on the marine dipyrroloquinone, terreusinone (1) isolated from the marine-derived fungus *Aspergillus terreus*. Preparative-scale fermentation of terreusinone with *Streptomyces* sp. has resulted in the isolation of a new oxidized metabolite, terreusinol (2). The structure was elucidated as 2-[(1*R*)-1-hydroxyisobutyl]-6-[(1*R*)-1,2-dihydroxyisobutyl]-1*H*,5*H*-pyrrolo[2,3-*b*]indole-4,8-dione (2) on the basis of physicochemical evidence. Terreusinol (2) showed an ultraviolet-A (UV-A) (320—390 nm) protecting activity with ED_{50} values of 150 μM, which is more active than oxybenzone (ED_{50}, 350 μM) currently being used as sunscreen.

Key words biotransformation; terreusinone; terreusinol; dipyrroloquinone; *Aspergillus terreus*; ultraviolet-A (UV-A) protecting agent

Selectivity is an essential requirement in the synthetic organic chemistry. The regioselectivity of enzymes even on complex or symmetric molecules without the need of protecting groups is the fundmental strength of biocatalysis. The application of biocatalysis to marine natural products has been shown to be a powerful tool for the generation of new, active, and less toxic derivatives for lead optimization and for establishing the stereostructure and structure–activity relationships (SAR).[1—4)]

Terreusinone (**1**), a chiral and symmetrical dimer, is an ultraviolet-A (UV-A) protecting dipyrroloquinone isolated from the marine-derived fungus *Aspergillus terreus*.[5)]

In an attempt to clarify the orientation of symmetrical dimer **1** by regioselective oxidation, compound **1** was subjected to a microbial transformation studies. The present study describes the isolation and identification of this polar biotransformed metabolite (**2**).

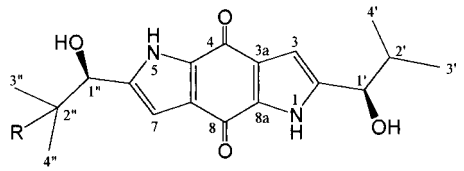
The marine actinomycete (MFAac18) was isolated from a sample of sea plant *Zostera marina* collected at Bijin Island, Gyeongnam, using YPG (yeast, peptone, and glucose) agar. The fermentation of the bacterium, isolate MFAac18, was carried out in the presence of a substance, terreusinone (**1**) by a two-stage fermentation protocol.[6,7)] The incubation was harvested after five weeks, and the broth was extracted with EtOAc. Chromatography of the extract (75 mg) gave the metabolite, terreusinol (**2**, 5 mg).

Terreusinol (**2**) was isolated as a red solid. A molecular formula of $C_{18}H_{22}N_2O_5$, which gave nine degrees of unsaturation, was established by high resolution electron impact mass spectrum (HR-EI-MS) and ^{13}C-NMR methods.

The general features of its UV, IR, and NMR spectra (Table 1) closely resembled those of **1**, except that the ^{1}H- and ^{13}C-NMR signals were doubled due to the presence of two slightly different side chain units from the regioselective oxidation on a direction of the two side chains.

In the ^{1}H- and ^{13}C-NMR spectra of **2**, additional signals of two new methyls [δ_H 1.04, 1.06 (each 3H, s, H_3-3″, 4″); δ_C 26.2, 25.3 (C-3″, 4″)] and one new quaternary carbon having a hydroxyl group [δ_H 4.38 (1H, s, 2″-OH); δ_C 71.7 (C-2″)] appeared at the lower field, indicating that the hydroxyl group is attached to C-2″.

Detailed analyses of the ^{1}H- and ^{13}C-NMR spectra of **2**, including the results from distortionless enhancement by polarization transfer (DEPT), ^{1}H-detected heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC) experiments, revealed the diagnostic signals for two different isobutyl-pyrroloketone



terreusinone (**1**) : R = H
terreusinol (**2**) : R = OH

Table 1. NMR Spectral Data for Terreusinol (**2**)[a,b)]

Position	δ_H (mult., *J*)	δ_C (mult)	HMBC (H to C)
1	12.21 (s)		2, 3, 3a, 8, 8a
2		143.4 (s)	
3	6.28 (s)	104.4 (d)	1′, 2, 3a, 4, 8a
3a		125.9 (s)	
4		173.9 (s)	
4a		130.9 (s)	
5	11.91 (s)		4, 4a, 6, 7, 7a
6		141.4 (s)	
7	6.31 (s)	105.5 (d)	1″, 4a, 6, 7a, 8
7a		125.7 (s)	
8		174.0 (s)	
8a		131.3 (s)	
1′	4.23 (dd, 6.0, 5.0)	71.5 (d)	2, 2′, 3, 3′, 4′
1′-OH	5.18 (d, 5.0)		1′, 2′
2′	1.90 (qqd, 6.8, 6.6, 6.0)	33.8 (d)	1′, 2, 3′, 4′
3′	0.86 (d, 6.6)[c)]	18.1 (q)[d)]	1′, 2′, 4′
4′	0.76 (d, 6.8)[c)]	18.7 (q)[d)]	1′, 2′, 3′
1″	4.31 (d, 5.3)	73.6 (d)	2″, 3″, 4″, 6, 7
1″-OH	5.34 (d, 5.3)		1″, 2″
2″		71.7 (s)	
2″-OH	4.38 (s)		1″, 2″, 3″, 4″
3″	1.06 (s)	26.2 (q)	1″, 2″, 4″
4″	1.04 (s)	25.3 (q)	1″, 2″, 3″

a) Recorded in DMSO-d_6 at 400 MHz (^{1}H) and 100 MHz (^{13}C). *b*) Assignments aided by DEPT, HMQC and HMBC. *c*, *d*) Interchangeable in each column.

* To whom correspondence should be addressed. e-mail: sonbw@pknu.ac.kr

halves. One half of the molecule was assigned to be identical to **1** by comparison of NMR data. The other half was assigned to be 1,2-dihydroxyisobutyl pyrroloketone unit by comparison of the NMR data derived from **1**.

On the basis of the evidence presented above, the structure of terreusinol was determined to be 2-[(1*R*)-1-hydroxyisobutyl]-6-[(1*R*)-1,2-dihydroxyisobutyl]-1*H*,5*H*-pyrrolo[2,3-*b*]indole-4,8-dione (**2**).

The orientation of **1** was presumed to be a pyrrolo[2,3-*b*]indole-4,8-dione by comparing the NMR data with those of pyrrolo[3,2-*b*]indole-4,8-dione and pyrrolo[2,3-*b*]indole-4,8-dione and through quantum chemistry calculation.[5,8]

This orientation (pyrrolo[2,3-*b*]indole-4,8-dione) of dipyrroloquinone unit in **2** was further supported by the key HMBC correlations from H-3 [δ_H 6.28 (1H, s)] to C-4 (δ_C 173.9) and from H-7 [δ_H 6.31 (1H, s)] to C-8 (δ_C 174.0).

Terreusinol (**2**) displayed an UV-A protecting activity with ED_{50} value of 150 μM. This activity was more potent than oxybenzone (ED_{50}, 350 μM) currently being used as sunscreen.

The effects of an UV-A, the major type of UV radiation in sunlight, are well understood. The UV-A-absorbing compounds have been described for terrestrial and marine cyanobacteria.[9] Such compounds may have potential industrial application as sunscreen cosmetics.

Experimental

General Melting point was determined on a Electrothermal model IA 9100 micro-melting point apparatus and was uncorrected. Optical rotation was determined on a Perkin Elmer model 341 polarimeter. IR spectrum was recorded on a Bruker FT-IR model IFS-88 spectrometer. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) spectra were obtained on a JEOL JNM-ECP 400 NMR spectrometer, using TMS or solvent peaks as reference standard. MS spectra were obtained on a JEOL JMS-700 spectrometer. UV/visible spectra were measured on a Hitachi U-2001 UV/Vis spectrometer. CD spectra were taken on a JASCO J-715 spectropolarimeter.

Isolation of the Bacterium *Streptomyces* Species The actinomycete species, isolate MFAac18, was isolated using YPG agar medium, composed of 0.5% yeast extract, 0.5% peptone, 1.0% glucose, 1.6% agar, 40% deionized water, and 60% seawater, containing penicillin and streptomycin (each 250 μg/ml), from the sea plant *Zostera marina* collected at the Bijin Island, Tongnyeong, Gyeongnam, Korea in 2000. The culture showed ash gray vegetative mycelia and demonstrated fatty acid composition (Korean Culture Center of Microorganisms, Seoul, Korea) and growth characteristics typical of actinomycete belong to the *Streptomyces* and related genera.

Biotransformation of 1 A two-stage fermentation protocol[6,7] was used for preparative scale formation of the metabolite of terreusinone (**1**). The SWS medium contained soytone (0.1%), soluble starch (1.0%), and seawater (100%), and it was autoclaved at 121 °C for 15 min. Preparative incubation was conducted in 1 l of sterile medium held in 3 l culture flask that was incubated at 29 °C for 1 week (static). A 10% inoculum derived from one week old stage I culture was used to initiate stage II culture, which was incubated for 24 h more before receiving 20 mg of **1** in 0.75 ml of dimethyl formamide (DMF), and incubation was continued at 29 °C for five weeks (static). Substrate control consisted of sterile medium and substrate incubated under the same conditions but without microorganism. Also, culture control was composed of fermentation blanks in which the microorganism was grown under identical condition but without the addition of substrate. After five weeks of incubation, 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 pooled, dried over anhydrous Na_2SO_4, filtered through sintered glass, and vacuum-concentrated to yield a viscous brown extract (75 mg).

Isolation of Metabolite The brown extract (75 mg) was subjected to silica gel flash column chromatography using in sequence *n*-hexane–EtOAc (100 : 0—0 : 100) to give two fractions A and B, which contained substrate (**1**) and metabolite (**2**), respectively. Fractions A and B were separately purified by reversed-phase YMC ODS-A gel flash column chromatography using MeOH, followed by HPLC (ODS-A, MeOH) to furnish the substrate (**1**) (10 mg) and the metabolite (**2**) (5 mg), respectively.

Terreusinol (**2**): A red solid; $[\alpha]_D^{20}$ +33.8° (c=0.6, MeOH); IR (KBr) ν_{max} 3403, 3200, 1626, 1467, 1162, 1056, 990, 837, 744 cm^{-1}; UV (MeOH) λ_{max} nm (log ε) 356 (3.87), 285 (4.10), 247 (4.53); low resolution (LR)-EI-MS m/z 346 $[M]^+$ (2), 328 $[M-H_2O]^+$ (9); 310 $[M-2H_2O]^+$ (7); 288 $[M-(CH_3)_2CO]^+$ (45); 270 $[288-H_2O]^+$ (100); 59 (94); HR-EI-MS m/z 346.1529 (Calcd for $C_{18}H_{22}N_2O_5$, 346.1518). See Table 1 for NMR spectral data.

Acknowledgments Mass spectral data were kindly provided by the Korea Basic Science Institute. This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (02-PJ2-PG3-21604-0003).

References and Notes

1) El Sayed K. A., Yousaf M., Hamann M. T., Avery M. A., Kelly M., Wipf P., *J. Nat. Prod.*, **65**, 1547—1553 (2002), and references cited therein.
2) Fraga B. M., Hernandez M. G., Gonzalez P., Chamy M. C., Garbarino J. A., *Phytochemistry*, **53**, 395—399 (2000).
3) Hosny M., Johnson H. A., Ueltschy A. K., Rosazza J. P. N., *J. Nat. Prod.*, **65**, 1266—1269 (2002).
4) Garcia-Granados A., Gutierrez M. C., Parra A., Rivas F., *J. Nat. Prod.*, **65**, 1011—1015 (2002).
5) Lee S. M., Lee X. F., Jiang H., Cheng J. G., Seong S., Choi H. D., Son B. W., *Tetrahedron Lett.*, **44**, 7707—7710 (2003).
6) Orabi K. Y., Clark A. M., Hufford C. D., *J. Nat. Prod.*, **63**, 396—398 (2000).
7) Smith R. V., Rosazza J. P., *J. Pharm. Sci.*, **64**, 1737—1759 (1975).
8) Qiao G. G., Meutermans W., Wong M. W., Traubel M., Wentrup C., *J. Am. Chem. Soc.*, **118**, 3852—3861 (1996).
9) Matsunaga T., Burgess J. G., Yamada N., Komatsu K., Yoshida S., Wachi Y., *Appl. Microbiol. Biotechnol.*, **39**, 250—253 (1993).