



Tetrahedron Letters 44 (2003) 7707-7710

TETRAHEDRON LETTERS

Terreusinone, a novel UV-A protecting dipyrroloquinone from the marine algicolous fungus *Aspergillus terreus*

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Received 6 August 2003; accepted 28 August 2003

Abstract—A novel chiral dipyrrolobenzoquinone derivative, terreusinone (1), has been isolated as a potent UV-A protectant from the marine algicolous fungus *Aspergillus terreus*. The structure and absolute stereochemistry of the new compound was established by spectral interpretation, Horeau's method and quantum chemistry calculations as 2,6-bis[(1*R*)-1-hydroxyisobuty]]-1*H*,5*H*-pyrrolo[2,3-*b*]indole-4,8-dione (1). Compound 1 exhibited a UV-A absorbing activity with ED_{50} value of 70 µg/mL. © 2003 Elsevier Ltd. All rights reserved.

Marine microorganisms have proven to be a rich source of structurally novel and biologically active natural products required for developing fine chemical agents.^{1,2}

In the course of our search for UV-A absorbing constituents produced by fungi isolated from marine habitats,³ moderate UV-A absorbing activity was detected in the crude extract of the algicolous fungus *Aspergillus terreus*. Here, we report on the structural elucidation of the dipyrroloquinone, terreusinone (1), isolated as the target compound of activity from the marine algicolous fungus *Aspergillus terreus*.

The fungal strain (culture # MFA 460) was isolated from the surface of the marine red alga *Halymenia acuminata* collected in the Bijin Island, Gyeongnam Province in 2002. The fungus, identified to be an *Aspergillus terreus* by fatty acid methyl ester analysis,⁴ was cultured (10 L) in a seawater-based medium.⁵ The resulting broth and mycelium were extracted separately to afford crude extracts of 1.0 g and 8.0 g, respectively. The broth extract (EtOAc) revealed a moderate UV-A absorbing activity and was separated by assay-guided fractionation using repeated silica gel flash chromatography (*n*-hexane in ethyl acetate) and HPLC (ODS-A, MeOH–H₂O=5:1) to yield a new dipyrroloquinone, terreusinone (1, 18 mg).



terreusinone (1)

Terreusinone (1)⁶ was isolated as a yellowish solid which analyzed for $C_{18}H_{22}N_2O_4$ (9 unsaturations) by HREIMS. The IR absorption spectrum of 1 showed bands characteristic of a hydroxyl (3435 cm⁻¹), benzoquinone (1626 cm⁻¹) and pyrrole (3216, 1471 cm⁻¹) functionalities. Two proton signals [δ 12.19 (1H, s, H-1), 5.18 (1H, d, J=5.0 Hz, 1'-OH)] were exchanged by D₂O, suggesting that 1 has an aromatic amine and a hydroxyl group. Although a molecular composition was analyzed to $C_{18}H_{22}N_2O_4$ by HREIMS, both the ¹H and ¹³C NMR spectra of 1 showed signals corresponding to only 11 protons and 9 carbons for one half of the

Keywords: Aspergillus terreus; marine algicolous fungus; terreusinone; dipyrroloquinone; UV-A protection; quantum chemistry calculation. * Corresponding author. Tel.: +82-51-620-6378; fax: +82-51-628-8147;

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Table 1. NMR spectral data for terreusinone $(1)^{a,b}$

Position	$\delta_{\rm H}$ (mult., J)	$\delta_{\rm C}$ (mult)	HMBC (H to C)
1	12.19 (s)		2, 3, 3a, 8, 8a
2		143.4 (s)	
3	6.28 (s)	104.4 (d)	2, 3a, 4, 8a
3a		126.0 (s)	
4/8		174.0 (s)	
8a		131.3 (s)	
1′	4.24 (dd, 6.0, 5.0)	71.5 (d)	2, 3, 2', 4'
1′-OH	5.18 (d, 5.0)		2, 1', 2'
2'	1.91 (ggd, 6.8,	33.8 (d)	2, 1', 3', 4'
	6.6, 6.0)		
3'	0.86 (d, 6.8) ^c	18.1 (q) ^d	1', 2', 4'
4′	0.76 (d, 6.6) ^c	18.7 $(q)^d$	1', 2', 3'

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

^b Assignments aided by DEPT, COSY, TOCSY, HMQC and HMBC. ^{c,d} Interchangeable in each column.

molecule (Table 1). These data suggested that **1** is a dimer, having a high symmetry in the molecule. The ¹H and ¹³C NMR data for **1**, including the results from COSY, TOCSY, DEPT, HMQC, and HMBC experiments, showed the presence of a dipyrroloquinone or a pyridone dimer, which was further supported by the UV spectral data [355 nm (log ε 3.57), 277 nm (log ε 3.83)], and 1-hydroxyisobutyl groups (Table 1). The connection of the functional groups in **1** was achieved on the basis of HMBC correlations. Key HMBC correlations from H-1' to C-2 and C-3, from H-2' to C-2, from 1'-OH to C-2, and from H-1 to C-8/4 were critical in establishing the one half of the plain structure of **1** as shown.

Based on all of the foregoing evidence, four kinds of isomers **1a–d** were assumed as possible structure of **1** (Fig. 1). Among them, 4-pyridone dimer (**1c** and **1d**) and 2-pyridone dimer were excluded from the HMBC

correlation of H-1 to C-8/4 and the downfield shift of the carbonyl carbon signal ($\delta_{\rm C}$ 174.0, C-4/8),⁷ respectively. The unsymmetrical dipyrroloquinone structure (**1a**) was deduced as the terreusinone from the typical chemical shift of the carbonyl carbon ($\delta_{\rm C}$ 174.0), relative to the symmetrical dipyrroloquinone (**1b**) ($\delta_{\rm C}$ 179.3, 168.3).⁸

To confirm the framework and dimeric orientation of 1, the gauge-independent atomic orbital (GIAO)⁹ based ¹³C NMR chemical shifts calculations, which have emerged as a powerful tool for structure assignments of natural products,¹⁰ were carried out on the four proposed isomers 1a-d (Fig. 1). The geometries of the four proposed isomers were fully optimized with C_2 symmetry at the B3LYP/6-31G** level of theory. The vibrational frequency analysis performed at the same level gave no imaginary frequencies, ensuring that they are true energy minium structures. Based on the optimized geometries, the NMR calculations were performed at the B3LYP/6-311G+(2d,p) level of theory. Meanwhile, the same procedure was applied on TMS to calculate the relative chemical shifts of these four isomers. All the calculations were performed using the Gaussian 98 program.¹¹

The plots were made using experimental data versus theoretical values, and then were analyzed with linear regression. It was found that the correlation coefficient (R^2) for the ¹³C NMR spectra with the isomer **1a** is 0.9963, which is higher than that for the other isomers (0.9942, 0.9285, and 0.9557 for **1b**, **1c**, and **1d**, respectively). The computational results are summarized in Table 2. As all the isomers have C_2 symmetry structures, only a half of ¹³C NMR chemical shifts data have been obtained in the calculation results. In line with the experimental data, nine ¹³C NMR chemical shifts were observed for isomers **1a**, **1c** and **1d**, respectively. Comparing to isomers **1a**, **1c** and **1d**, ten ¹³C NMR chemical shifts were obtained from isomer **1b**, in which the



Figure 1. Possible isomers of terreusinone (1).

Table 2. Experimentally observed ¹³C NMR data for 1 and values obtained by quantum chemistry calculations (ppm)^a

Carbon no. ^b	Exptl values	Calcd values		Carbon no. ^c	Calcd values	
		Isomer 1a	Isomer 1b		Isomer 1c	Isomer 1d
2	143.4	143.1	142.0	6	149.7	147.0
3	104.4	111.3	112.0	5	131.1	135.3
3a	126.0	130.7	132.2	3	124.6	146.8
4/8	174.0	180.1	173.3, 186.1	4	166.3	167.3
8a	131.3	138.5	137.2	2	177.8	158.1
1′	71.5	79.2	79.1	7	79.9	80.1
2'	33.8	40.6	40.6	8	43.6	42.7
3'	18.1	15.2	15.2	9	13.8	13.5
4′	18.7	20.2	20.2	10	20.5	20.2

^a Calculated by B3LYP/6-311+G (2d,p)//B3LYP/6-31G (d,p) method.

^b Carbon number for isomers 1a and 1b.

^c Carbon number for isomers 1c and 1d.

electronic environment of the C-4 was different from C-8. So, isomer **1b** was excluded. From Table 2, it can be seen that the chemical shifts deduced by the B3LYP/ 6-311+G(2d,p) program for isomer **1a** are closer to the experimental ¹³C NMR data, while the corresponding values for the other isomers are more distant from the experimental values. As a result, it became clear that the isolated natural product has a greater probability of adopting the structure and orientation of isomer **1a**.

The relative configuration at the C-1' position of **1** was deduced from ROESY spectral data which showed the correlations of H-1 to H-1', and H-3 to 1'-OH, indicative of their *cis* orientations, respectively.

To determine the absolute stereostructure at C-1' of 1, we prepared benzoylated analogue from 1^{12} and have examined it using the CD exciton chirality method.¹³ The CD spectrum of benzoate derivative of 1 demonstrated occurrence of the coupling between CD excitons of the dipyrroloquinone and the C-1' benzoyloxy chromophore.¹³ From the sign of the first Cotton effect at 252 nm ($\Delta \varepsilon$ -7.7), the C-1' configuration in 1 has been assigned *R*, as depicted in Figure 2.

This conclusion was further supported by applying Horeau's method¹⁴ to **1**, where the recovered α -phenylbutyric acid showed $[\alpha]_D = +4.7^{\circ}$ (*c* 0.5, benzene).¹⁵





Based on the above evidence, the absolute stereostructure of terreusinone was determined to be 2,6-bis[(1*R*)-1-hydroxyisobutyl]-1*H*,5*H*-pyrrolo[2,3-*b*]indole-4,8-dione (1). Terreusinone (1), having a dipyrroloquinone moiety, showed a UV-A absorbing activity with ED₅₀ value of 70 μ g/mL, which is stronger than oxybenzone currently being used as sunscreen.

Further biological evaluation of terreusinone (1) is in progress.

Acknowledgements

CD and 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).

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- 4. The fungal strain was identified as an *Aspergillus terreus* based on fatty acid methyl ester analysis (Korean Culture Center of Microorganisms, Seoul, Korea), similarity index 0.839.
- 5. The fungus was cultured for 30 days (static) at 29°C in SWS medium: soytone (0.1%), soluble starch (1.0%), and seawater (100%).
- 6. Terreusinone (1) was isolated as a yellowish solid which showed: mp 230°C<(decomposed); [*α*]_D=+47° (*c* 0.3, MeOH); IR (KBr) 3435, 3216, 1626, 1471, 1399, 1384, 1164 cm⁻¹; UV (MeOH) 355 nm (log ε 3.57), 277 (3.83), 247 (4.08), 204 (3.73); HREIMS *m/z* 330.1563 [M]⁺ (calcd for C₁₈H₂₂N₂O₄, 330.1580); LREIMS *m/z* 331 [M+H]⁺ (rel. int. 3), 330 [M]⁺ (11), 312 [M-H₂O]⁺ (13), 294

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- 12. A solution of 1 (10 mg) in 0.5 mL pyridine was treated with benzoyl chloride (0.1 mL) and the whole solution was stirred under a nitrogen atmosphere at rt for 10 min. The mixture was poured into ice water and the whole was extracted with EtOAc. The organic phase was washed with satd aq. NaHCO₃ and brine, and dried over MgSO₄. The product, obtained by removing the solvent under reduced pressure, was purified by SiO₂ column chromatography (elution with *n*-hexane–EtOAc 3:1) to furnish the benzoate (12 mg) as a red solid. The following data recorded the benzoate: ¹H NMR (400 MHz, DMSO-d₆) δ 12.76 (1H, s, H-1), 6.46 (1H, s, H-3), 5.73 (1H, d, J=7.0 Hz, H-1'), 2.32 (1H, qqd, J=7.0, 7.0, 6.7)

Hz, H-2'), 0.97 (3H, d, J=6.7 Hz, H-3'), 0.87 (3H, d, J=7.0 Hz, H-4'), 8.05 (2H, d, J=7.5 Hz, H-2"), 7.55 (2H, t, J=7.5 Hz, H-3"), 7.68 (1H, d, J=7.5 Hz, H-4"); LREIMS m/z 520 [M-H₂O]⁺ (rel. int. 2), 398 [M-H₂O-C₆H₅COOH]⁺ (2), 294 [M-(C₆H₅COOH×2)]⁺ (100), 277 (18), 146 (15), 105 (37), 91 (13).

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- 15. (\pm) - α -Phenylbutyric anhydride (15 mg) was added to a solution of 1 (8 mg) in pyridine, and the mixture was stirred under a nitrogen atmosphere for 48 h at rt. The reaction mixture was partitioned into an EtOAc-aq. satd NaHCO₃ mixture. The organic phase was dried under vacuum and the residue was chromatographed by silica gel (*n*-hexane–EtOAc = 5:1) to afford the ester (4.0 mg). The aq. NaHCO₃ phase was acidified with aq. 2N HCl and extracted with EtOAc. Work-up of the EtOAc extract in the usual manner afforded the recovered acid, which was purified by HPLC (ODS-A, MeOH- $H_2O =$ 10:1) to furnish α -phenylbutric acid (5 mg) of $[\alpha]_{\rm D} = +4.7^{\circ}$ (c 0.5, benzene). The following data were recorded for the ester: ¹H NMR (400 MHz, CDCl₃) δ 9.06 (1H, br.s, H-1), 6.35 (1H, dd-like, J=2.8, 2.2 Hz, H-3), 5.48 (1H, d, J=7.5 Hz, H-1'), 2.11 (1H, m, H-2'), 0.94 (3H, br.d, J=6.6 Hz, H-3'), 0.85 (3H, dd, J=6.7, 1.5 Hz, H-4'), 7.26 $(5H, m, Ph-\alpha)$, 3.50 $(1H, m, H-\alpha)$, 1.60 $(2H, br.s, H-\beta)$, 0.88 (3H, t, J = 7.5 Hz, H- γ); IR (KBr) 3193, 1736, 1635, 1463, 1261, 1078, 1019 cm⁻¹; HREIMS m/z 623.3122 $[M+H]^+$ (calcd for C₃₈H₄₃N₂O₆, 623.3121); LREIMS m/z623 [M+H]⁺ (rel. int. 3), 622 [M]⁺ (11).