Myrothenones A and B, Cyclopentenone Derivatives with Tyrosinase Inhibitory Activity from the Marine-Derived Fungus *Myrothecium* sp.

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Received January 7, 2005; accepted February 2, 2005

New 3-amino-5-ethenylcyclopentenones, myrothenones A (4) and B (5), were isolated together with known 6-n-pentyl-α-pyrene (1), trichodenone A (2), and cyclonerodiol (3) from the marine algicolous fungus of genus of *Myrothecium*. The structure and absolute stereochemistry of the new compounds were established by spectral interpretation and X-ray analysis. Compounds 1 and 4 exhibited a tyrosinase inhibitory activity with an IC50 value of 0.8 and 6.6 μM, respectively, which are more active than kojic acid (IC50 7.7 μM) currently being used as a functional personal-care compound.

Key words marine-derived fungus; *Myrothecium* sp.; myrothenone; 6-n-pentyl-α-pyrene; trichodenone A; tyrosinase inhibitory activity

Marine microorganisms such as bacteria and fungi inhabit virtually any environment in the sea, and they are the source of greatest diversity in the sea.1,2) The source of increasing interests, these microbes have been shown to produce novel substances with utilities in fine chemicals, drugs and cosmetics, and functional personal-care products.3)

Tyrosinase is known as a key enzyme implicated in the metabolism of melanin in melanocytes, and it is involved in skin-coloring and local hyperpigmentation such as melasma, ephelis, and lentigo.4)

Therefore, the anti-tyrosinase active compounds have been of great concern as functional personal-care products for skin-whitening effects and for preventive and therapeutic effects on the local hyperpigmentation diseases.

In our screening aimed at identifying tyrosinase inhibitors of microbial origin, we investigated tyrosinase inhibiting activity from the fungal extracts, and a significant activity was observed in three strains (MFA58, 581 and 898). The anti-tyrosinase active component of two strains (MFA581 and 898) was purified by assay-guided isolation to yield the known tyrosinase active component of two strains (MFA581 and 898). The anti-tyrosinase activity of microbial origin, we investigated tyrosinase inhibiting activity, and it 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 compounds 1 (150 mg), 2 (3.5 mg), 3 (17.0 mg), 4 (8.0 mg), and 5 (8.5 mg).

Myrothenone B (5) was isolated as a colorless oil, which was analyzed for C11H10O3 (4 unsaturation) by HR-FAB-MS and 13C-NMR methods. The IR absorption spectrum of 5 showed bands characteristic of a hydroxyl and amino groups (3390 cm−1 and enone (1630 cm−1) functionality.

The 1H- and 13C-NMR spectra, including distortionless enhancement by polarization transfer (DEPT), showed one monosubstituted double bond, one trisubstituted double bond, one primary amine, one oxygenated quaternary carbon, one carbonyl carbon, and one diastereotopic methylene.

The overall NMR data indicated the presence of a trisubstituted enone, a 3-hydroxy-3,4-dialkyl-1-buten, and an amino group. The presence of a 3-aminoenone chromophore was further supported by UV spectral data [203 nm (log e 3.7), 268 nm (log e 4.0)] and by the characteristic double bond carbon signals [δ 95.8 (d), C-2; 174.7 (s), C-3] located considerably upfield and downfield, respectively.13)

The connection of functional groups in 5 was achieved on the basis of 2D NMR, 1H-detected heteronuclear multiple-quantum coherence (HMQC) and heteronuclear multiple-bond coherence (HMBC), which allowed all carbons and their respective protons to be assigned. Diagnostic HMBC from H-2 to C-4 and C-5, and from H2-4 to C-1, C-2, and C-6, from H-6 to C-1, C-4, and C-5, and from H2-7 to C-5 and C-6 showed the connections of C1–C5 and C3–C4 in 5. On the basis of all the foregoing evidence, the structure of myrothenone B was proposed as the 3-amino-5-ethenyl-5-hydroxy-2-cyclopenten-1-one (5).

To substantiate our presumption and to establish the absolute stereochemistry of myrotheneone B (5), the molecular structure with its absolute configuration of N-(p-bromobenzoyl)myrotheneone B (5a) was determined by the X-ray crystal structure analysis (Fig. 1).15,16)

Myrothenone A (4) was cultured (10 l) in a seawater-based medium.11) The filtered broth was extracted with EtOAc to afford crude extracts (0.8 g). The extract revealed a significant anti-tyrosinase activity, and it 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 compounds 1 (150 mg), 2 (3.5 mg), 3 (17.0 mg), 4 (8.0 mg), and 5 (8.5 mg).

Myrothenone B (5) was isolated as a colorless oil, which was analyzed for C11H10O3 (4 unsaturation) by HR-FAB-MS and 13C-NMR methods. The IR absorption spectrum of 5 showed bands characteristic of a hydroxyl and amino groups (3390 cm−1 and enone (1630 cm−1) functionality.

The 1H- and 13C-NMR spectra, including distortionless enhancement by polarization transfer (DEPT), showed one monosubstituted double bond, one trisubstituted double bond, one primary amine, one oxygenated quaternary carbon, one carbonyl carbon, and one diastereotopic methylene.

The overall NMR data indicated the presence of a trisubstituted enone, a 3-hydroxy-3,4-dialkyl-1-buten, and an amino group. The presence of a 3-aminoenone chromophore was further supported by UV spectral data [203 nm (log e 3.7), 268 nm (log e 4.0)] and by the characteristic double bond carbon signals [δ 95.8 (d), C-2; 174.7 (s), C-3] located considerably upfield and downfield, respectively.13)

The connection of functional groups in 5 was achieved on the basis of 2D NMR, 1H-detected heteronuclear multiple-quantum coherence (HMQC) and heteronuclear multiple-bond coherence (HMBC), which allowed all carbons and their respective protons to be assigned. Diagnostic HMBC from H-2 to C-4 and C-5, and from H2-4 to C-1, C-2, and C-6, from H-6 to C-1, C-4, and C-5, and from H2-7 to C-5 and C-6 showed the connections of C1–C5 and C3–C4 in 5. On the basis of all the foregoing evidence, the structure of myrothenone B was proposed as the 3-amino-5-ethenyl-5-hydroxy-2-cyclopenten-1-one (5).

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Bond distances and angles were all normal. The C-1, C-2, C-3, and C-4 atoms were in a plane with the maximum deviation of 0.004 Å, and C-5 is displaced toward beta-orientation from the plane of the other four [diagonal angle: 15.1 (0.5)]. Therefore, the cyclopentenone of 5 shows a typical envelope geometry.

The absolute configuration of asymmetric center for 5 has been unambiguously determined to be 5R by refinement of the Flack parameter.17)

Myrothenone A (4),18 which is a formamide derivative of 5, was obtained as a very unstable colorless oil, and the HR-FAB-MS and 13C-NMR methods established the molecular formula to be C8H9NO3. The general features of its UV , IR obtained at a low temperature (50 °C). The NMR signals of FAB-MS and 13C-NMR methods established the molecular structure of 5.

Detailed analyses of the 1H- and 13C-NMR spectra of 4, including the results from DEPT, HMOC, and HMBC experiments, suggested the metabolite (4) is a formamide derivative of 5. The key HMBC correlation from H-9 to C-3 was critical in establishing the location of aldehyde of 4 as shown.

On the basis of these data, the structure of myrothenone A was proposed as the 5-ethenyl-3-formamido-5-hydroxy-2-cyclopenten-1-one (4). In order to clarify the structure of 4, we synthesized 4 from myrothenone B (5) and formic acid using 1,3-dicyclohexylcarbodiimide (DCC) as a base.20,21) The spectral data of synthetic compound were identical to those of myrothenone B (5) in all aspects. Accordingly, the absolute stereostructure of myrothenone A was determined as 5(R)-5-ethenyl-3-formamido-5-hydroxy-2-cyclopenten-1-one (4).

Vinylcyclopentenone analogues have been reported as the artifact22) and the conjugated adduct,23) but natural products of this class having amino group (4, 5) are very rare examples.

6-n-Pentyl-α-pyrone (1) and myrothenone A (4) exhibited a tyrosinase inhibitory activity with ED50 value of 0.8 and 6.6 μM, respectively, which are more active than kojic acid (ED50, 7.7 μM) currently being used as a functional personal-care compound.

The further biological evaluation of 1, 2, 3, 4, and 5 is in progress.

Acknowledgements X.L. acknowledges the support of the Brain Korea 21 (BK21) (F020). Circular dichroism (CD) and Mass spectral data were kindly provided by the Korea Basic Science Institute. This work was supported by MarineBio21 (Ministry of Maritime Affairs and Fisheries, Korea).

References and Notes
10) The fungal strain was identified as Myrothecium sp. based on fatty acid methyl ester analysis (Korean Culture Center of Microorganisms, Seoul, Korea), similarity index 0.697.
11) The fungus was cultured for 30 d (static) at 29 °C in SWS medium: soytone (0.1%), soluble starch (1.0%), and seawater (100%).

12) Myrothenone B (5) was isolated as a colorless oil which showed: 

\[ \alpha_D^{20} = +35° \ (c=0.6, \ MeOH) \]; IR (KBr) 203 nm (log \varepsilon 3.7), 268 (4.0); LR-EI-MS \( m/z 139 \ [M]^- \) (rel. int. 87), 122 \([M-OH]^+ \) (13), 110 (84), 94 (33), 83 (83), 67 (32), 55 (100); HR-FAB-MS \( m/z 140.0710 \ [M-H]^+ \) (Calcd for \( C_7H_{10}NO_2, 140.0712 \)); see Table 1 for NMR spectral data.


14) \( p \)-Bromobenzoyl chloride (0.3 ml) (0.2 mmol) was added to a solution of myrothenone B (5) (20 mg) (0.5 mmol) in pyridine at 0 °C under N\(_2\) atmosphere, then the mixture was stirred for 12 h at 60 °C. The reaction mixture was then poured into water and extracted with EtOAc. The EtOAc extract was washed with brine, then dried over MgSO\(_4\). Removal of the solvent under reduced pressure from the EtOAc extract gave a product, which was purified by column chromatography (n-hexane–EtOAc = 20 : 1) to furnish \( p \)-bromobenzoate derivative (5a, 18 mg) (Fig. 1).

15) The Crystallographic data were, \( C_{14}H_{12}BrNO_3 \), MW = 322.16, \( T = 298(2) \ K, \lambda = 0.71069 \ \text{Å}, \) monoclinic, \( P2_1, a = 5.6300(10) \), \( b = 5.685(3), c = 20.715(3) \ \text{Å}, \beta = 94.850(10)^\circ, \) \( V = 660.6(2) \ \text{Å}^3, \) \( Z = 2, \) \( d_{calc} = 1.619 \text{Mg m}^{-3}, \) \( F(000) = 324, \) the final \( R \) and \( wR \) values with 3375 Friedel pair reflections \( \langle I > 2\sigma(I) \rangle \) were 0.0502 and 0.0541, respectively. An absolute structure parameter, \( \chi = -0.02(1) \). Crystallographic data have been deposited with the Cambridge Crystallographic Data Center (deposit No. CCDC 256884).


18) Myrothenone A (4) was isolated as a colorless oil which showed: 

\[ \alpha_D^{20} = +61° \ (c=0.6, \ MeOH) \]; IR (KBr) 3419, 3240, 1683, 1603, 1554, 1524, 1410, 1189, 1103, 1093, 1005 cm\(^{-1}\); UV (MeOH) 203 nm (log \varepsilon 3.7), 272 (4.2); LR-EI-MS \( m/z 167 \ [M]^- \) (rel. int. 34), 139 \([M-\text{CO}]^- \) (50), 122 \([M-\text{CO}+\text{OH}]^- \) (10), 111 (43), 94 (54), 84 (43), 68 (72), 55 (100); HR-FAB-MS \( m/z 190.0482 \ [M+Na]^+ \) (Calcd for \( C_8H_9NO_3Na, 190.0480 \)); see Table 1 for NMR spectral data.


21) Formic acid (0.5 ml) (12.7 mmol) and DCC (5 mg) was added to a solution of myrothenone B (5) (10 mg) (0.5 mmol) in pyridine at 0 °C under N\(_2\) atmosphere, then the mixture was stirred for 3 h. The reaction mixture was then poured into water and extracted with EtOAc. The EtOAc extract was washed with brine, then dried over MgSO\(_4\). Removal of the solvent under reduced pressure from the EtOAc extract gave a product, which was purified by column chromatography (n-hexane–EtOAc = 20 : 1) to furnish myrothenone A (8 mg). Synthetic compound was shown to be identical with myrothenone A (4) by TLC, \( \alpha_D \), 1H- and 13C-NMR.
