Arch Pharm Res Vol 26, No 7, 532-534, 2003

Archives of Pharmacal Research
http://apr.psk.or.kr

γ-Pyrone Derivatives, Kojic Acid Methyl Ethers from a Marine-Derived Fungus *Altenaria* sp.

Xifeng Li, Jee Hean Jeong[1], Kang Tae Lee[1], Jung Rae Rho[2], Hong Dae Choi[3], Jung Sook Kang[4], and Byeng Wha Son

Department of Chemistry, Pukyong National University, Busan 608-737, Korea, [1]R&D Center, Coreana Cosmetics Co. Ltd., Chungnam 330-830, Korea, [2]Marine Natural Products Laboratory, Korea Ocean Research & Development Institute, Ansan P. O. Box 29, Seoul 425-600, Korea, [3]Department of Chemistry, Dongeui University, Busan 614-714, Korea, and [4]College of Dentistry, Pusan National University, Busan 602-739, Korea

(Received April 25, 2003)

Kojic acid dimethyl ether (**1**), and the known kojic acid monomethyl ether (**2**), kojic acid (**3**) and phomaligol A (**4**) have been isolated from the organic extract of the broth of the marine-derived fungus *Altenaria* sp. collected from the surface of the marine green alga *Ulva pertusa*. The structures were assigned on the basis of comprehensive spectroscopic analyses. Each isolate was tested for its tyrosinase inhibitory activity. Kojic acid (**3**) was found to have significant tyrosinase inhibitory activity, but compounds **1**, **2**, and **4** were found to be inactive.

Key words: Marine fungus, *Altenaria* sp., Kojic acid, Kojic acid methyl ether, Phomaligol, Tyrosinase inhibitory activity

INTRODUCTION

Marine microorganisms, particularly marine-derived fungi, have recently gained prominence as an important source of biologically active secondary metabolites. Among marine fungi, those living in association with marine algae are a particularly promising source of novel natural products owing to the special ecological niche in which they exist.

Due to their very unusual living environment as compared with that of the terrestrial organisms, a great number of secondary metabolites with diverse structural features and interesting biological activities have been isolated from the marine organisms, some of which are of great interest to the new drug discovery (Schmitz, 2000; Faulkner, 2002; Pietra, 1997). Some of the marine natural products isolated have not only served as potential lead compounds for clinically useful drugs but have actually been used as chemical probes useful for basic studies in the fields of life science (Fenical *et al.*, 1996).

In the process of screening biologically active metabolites (Li *et al.*, 2003), kojic acid dimethyl ether (**1**) and monomethyl ether (**2**), related to kojic acid (**3**) (Kamal *et al.*, 1971; Li *et al*, 2003), and the known phomaligol A (**4**) (Pedras *et al.*, 1993) were isolated from the marine-derived algicolous fungus, *Altenaria* sp., which was separated from the green alga *Ulva pertusa*. We report here on the isolation and structural elucidation of these compounds.

Correspondence to: Byeng Wha Son, Department of Chemistry, Pukyong National University, Busan 608-737, Korea
Tel: 82+51-620-6378, Fax: 82+51-628-8147
E-mail: sonbw@pknu.ac.kr

MATERIALS AND METHODS

General experimental

The instruments used to obtain the physical data and the experimental conditions for chromatography were the same as those described in our previous paper (Li *et al.*, 2003).

Fungal isolation and culture

The marine-derived fungal strain (culture # MFA 898) was isolated from the surface of the marine green alga *Ulva pertusa* collected on Pyoseon Beach, Jeju Island in 2002 and identified as a *Altenaria* sp. based on fatty acid methyl ester analysis (Korean Culture Center of Microorganisms, Seoul, Korea) with similarity index 0.904. The fungus was cultured (1 L) for 30 days (static) at 29°C in SWS medium : soytone (0.1%), soluble starch (1.0%), and

seawater (100%).

Extraction and Isolation

The mycelium and broth were separated by filtration. The broth was extracted twice with EtOAc. The combined organic extract (117 mg) was subjected to silica gel flash column chromatography and elution was performed with CH_2Cl_2-MeOH (stepwise, 0-100% MeOH) to yield **5** fractions. These fractions were evaluated for tyrosinase inhibitory activity, and fraction **5** was active. The active component of fraction **5** was purified by assay guided isolation using silica gel column (CH_2Cl_2-MeOH = 10:1) and HPLC (YMC CDS-A, 10×250 mm) (MeOH) to yield kojic acid (**3**, 35 mg). Fraction **2** (31 mg), eluted with CH_2Cl_2-MeOH (20:1), was further chromatographed by silica gel column using *n*-hexane-EtOAc (1:1), followed by HPLC (ODS-A) using MeOH to yield phomaligol A (**4**, 5.0 mg). Fractions **3** (35 mg) and **4** (30 mg) were separated using a column of silica gel [(*n*-hexane-EtOAc = 1:5) and (CH_2Cl_2-MeOH = 10:1)] and further purified by HPLC (ODS-A) (MeOH) to afford kojic acid dimethyl ether (**1**, 7.0 mg) and kojic acid monomethyl ether (**2**, 5.0 mg), respectively.

Kojic acid dimethyl ether (1)

Colorless solid; IR (KBr): 1647, 1622 cm^{-1}; UV (MeOH): 216 (logε 3.57), 294 (3.68) nm; HREIMS *m/z* 170.0599 (calcd for $C_8H_{10}O_4$, 170.0579); LREIMS *m/z* 193 $[M+Na]^+$, 170 $M]^+$, 259 $[M-69]^+$, 191 $[M-137]^+$, 137 [3,7-dimethyl-2,6-octadienyl]$^+$, 69 [2-methyl-3-pentenyl]$^+$, 55 $[C_4H_7]^+$; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H 6.45 (1H, s, H-3), 7.56 (1H, s, H-6), 4.26 (2H, s, H_2-7), 3.77 (3H, s, 5-OCH_3), 3.44 (3H, s, 7-OCH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C 163.8 (C-2), 137.7 (C-3), 174.0 (C-4), 148.7 (C-5), 137.6 (C-6), 70.2 (C-7) 56.4 (5-OCH_3), 59.0 (7-OCH_3).

Kojic acid monomethyl ether (2)

Colorless solid; IR (KBr): 3437 (OH), 1738, 1638, 1603 cm^{-1}; UV (MeOH): 222 (logε 4.0), 283 (2.3) nm; HREIMS *m/z* 156.0419 (calcd for $C_7H_8O_4$, 156.0423); LREIMS *m/z* 156 $[M]^+$ (rel. int., 7), 138 (47), 125 (62), 95 (58), 83 (18), 69 (100); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H 6.29 (1H, s, H-3), 8.08 (1H, s, H-6), 4.29 (2H, d, *J* = 5.5 Hz, H_2-7), 3.64 (3H, s, 5-OCH_3), 5.68 (1H, t, *J* = 5.5 Hz, 7-OH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C 168.0 (C-2), 110.7 (C-3), 172.8 (C-4), 147.9 (C-5), 139.0 (C-6), 59.3 (C-7), 56.1 (5-OCH_3).

Kojic acid (3)

Colorless needles; IR (KBr): 3436, 1660, 1630, 1611 cm^{-1}; UV (MeOH): 269 (logε 4.0), 218 (4.2) nm; mp: 154-155 °C; LREIMS *m/z* 142 $[M]^+$ (rel. int., 100), 113 (56), 69 (54), 57 (47), 39 (50); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H 6.33 (1H, s, H-3), 8.02 (1H, s, H-6), 4.28 (2H, s, H_2-7), 9.06(1H, s, 5-OH), 5.67 (1H, s, 7-OH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C 168.0 (C-2), 109.8 (C-3), 173.9 (C-4), 145.7 (C-5), 139.2 (C-6), 59.4 (C-7). Identified by comparison of the IR, UV, NMR, and MS data with literature values (Li *et al.*, 2003).

Phomaligol A (4)

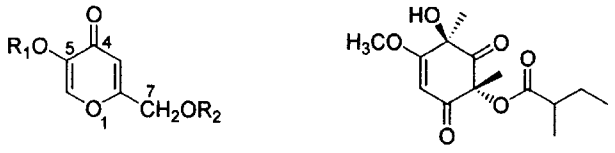
Isolated as a colorless oil which showed spectral data ($[\alpha]_D$, ^{1}H-NMR, ^{13}C-NMR, and LREIMS) virtually identical to those reported in the literature (Pedras *et al.*, 1993).

Tyrosinase inhibitory activity

Antityrosinase activity was measured by spectrophotometry following the method of Li (Li and Wu, 2002). The tyrosinase and tyrosine used for the bioassay were purchased from Sigma Chemical Co. The test substance was dissolved in 50% DMSO and the solution (40 μL) was dispensed into wells of a 96-well microtiter tray. Phosphate buffer solution (PBS, pH 6.8, 80 μL) and 0.05 M tyrosine (40 μL) in the same buffer were added to each well. The mixture was incubated with tyrosinase (250 U/mL, PBS, pH 6.8) at 37 °C for 10 min, and the absorbance of the resulting solution was measured at 475 nm with microplate reader (Packard Co., Spectra Count™). The inhibitory activity on tyrosinase was expressed as IC_{50}, which is a concentration of the tested compound required to give a 50% decrease of the absorbance from that of the control solution (OD at 475 nm without test substance).

RESULTS AND DISCUSSION

Kojic acid dimethyl ether (**1**) was isolated as a colorless solid. A molecular formula of **1**, $C_8H_{10}O_4$, which gave four double bond equivalents, was established by HRFABMS and ^{13}C-NMR data. The IR absorption spectrum of **1** showed bands characteristic of a γ-pyrone (1647 cm^{-1}) functionality. The ^{1}H- and ^{13}C-NMR spectra including DEPT showed two methoxy singlets, two aromatic methines, two aromatic quaternary carbons, one carbonyl carbon, and one oxygenated methylene. The overall NMR data, which were similar to kojic acid (**3**) (Buckingham *et al.*, 1994), indicated the presence of γ-pyrone, a methoxyl, and a methoxymethyl



1 $R_1 = R_2$ = Me
2 R_1 = Me, R_2 = H
Kojic acid (**3**), $R_1 = R_2$ = H

Phomaligol A (**4**)

Fig. 1. Structures of kojic acid methyl ethers (**1**, **2**), kojic acid (**3**), and phomaligol A (**4**)

groups. The presence of γ-pyrone chromophore was further supported by UV spectral data [216 nm (logε 3.57), 268 nm (3.68)].

The connection of the functional groups in **1** was achieved on the basis of 2D NMR (COSY, HMQC, HMBC) correlations which allowed all carbons and their respective protons to be assigned. Diagnostic HMBC correlations, observed from the 5-OCH_3 to C-5, from 7-OCH_3 to C-7, and from H_2-7 to C-2 and C-3, showed the location of two methoxyl groups and the connection of C-7 - C-2. On the basis of all of the foregoing evidence, the structure of **1** was proposed as the kojic acid dimethyl ether (**1**).

Kojic acid monomethyl ether (**2**) (Kamal *et al.*, 1971) was analyzed for $C_7H_8O_4$ by HRFABMS and ^{13}C-NMR methods. The general features of its UV, IR and NMR spectra closely resembled those of **1**, with an exception of the NMR signals for disappearance of one methoxyl signal from **1**. Detailed analyses of the ^{1}H- and ^{13}C-NMR spectra of **2**, including the results from DEPT, COSY, HMQC, and HMBC experiments, suggested **2** is the 7-demethylated derivative of **1**. This was further supported by a key HMBC correlation between 5-OCH_3 and C-5.

Although kojic acid dimethyl ether (**1**) has been reported as the synthetic products from kojic acid (**3**) (Poonia and Yadav *et al.*, 1978), compound **1** is the first example, to the best of our knowledge, from a natural source.

The isolated compounds (**1**, **2**, **3**, and **4**) were subjected to an evaluation of their antityrosinase activity. Among them, kojic acid (**3**) was found to have significant activity of IC_{50} 12.0 μM , but compounds **1**, **2**, and **4** were found to be inactive.

ACKNOWLEDGEMENTS

The authors thank Professor Ki Wan Nam, Department of Marine Biology, Pukyong National University for identification of the marine alga. NMR and Mass spectral data were kindly provided by the Korea Basic Science Institute, Taejeon. This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (02-PJ1-PG4-PT05-0002).

REFERENCES

Buckingham, J., Macdonald, F. M., and Bradley, H. M. (Eds.), *Dictionary of Natural Products*, Vol. 10, Chapman & Hall, London, p. 3037, (1994).

Faulkner, D. J., Marine natural products. *Nat. Prod. Rep.*, 19, 1-48 (2002).

Fenical, W., Marine biodiversity and the medicine cabinet. the status of new drugs from marine organisms. *Oceanography*, 9, 23-27 (1996) and references cited therein.

Kamal, A., Haider, Y., Akhtar, R., and Qureshi, A. A., Biochemistry of microorganisms. XVIII. Isolation of kamusol and structure of kojic acid, mannitol, kojic acid methyl ether, methyl 2,4,6-octatriene-1-carboxylate, and tetronic acid, metabolic products of *Aspergillus sulphureus*. *Pakistan J. Sci. Industrial Res.*, 65, 63-67 (1971).

Li, C. Y. and Wu, T. S., Constituents of the stigmas of *Crocus sativus* and their tyrosinase inhibitory activity, *J. Nat. Prod.*, 65, 1452-1456 (2002).

Li, X., Li, Y., Jeong, J. H., Lee, K. T., Choi, H. D., and Son, B. W., Screening of tyrosinase inhibiting activity from the marine-derived fungus. *Kor. J. Pharmacogn.*, 34, 138-141 (2003).

Li, Y., Li, X. F., Kim, D. S., Choi, H. D., and Son, B. S., Indolyl alkaloid derivatives, N_b-acetyltryptamine and oxaline from a marine-derived fungus. *Arch. Pharm. Res.*, 26, 21-23 (2003).

Pedras, M. S. C., Morales, V. M., and Taylor, J. L., Phomaligols and phomaligadiones: new metabolites from the blackleg fungus. *Tetrahedron*, 49, 8317-8322 (1993).

Pietra, F., Secondary metabolites from marine microorganisms: bacterial, protozoa, algae and fungi. Achievements and prospects. *Nat. Prod. Rep.*, 14, 453-464 (1997).

Poonia, N. S. and Yadav, B. P., Coordinative role of alkali cations in organic synthesis. Selective metylations of 5-hydroxy-2-hydroxymethyl-γ-pyrone. *J. Org. Chem.*, 43, 2842-2844 (1978).

Schmitz, F. J., Cited in *International Symposium on Natural Products Research, Proceedings of the Third Symposium on Pharmaceutical Science.* Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul, pp. 73-82 (2000).