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Redoxcitrinin, a Biogenetic Precursor of Citrinin from Marine Isolate of Fungus *Penicillium* sp.

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Abstract A chemical analysis of the fermentation of the marine-derived fungus *Penicillium* sp. led to the isolation of a biogenetic precursor of citrinin, redoxcitrinin (**1**), together with polyketide mycotoxins, phenol A (**2**), citrinin H2 (**3**), 4-hydroxymellein (**4**), citrinin (**5**), and phenol A acid (**6**). The structures of compounds **1–6** were determined on the basis of physicochemical data analyses. Among them, compounds **1–3** exhibited a potent radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) with IC_{50} values of 27.7, 23.4, and 27.2 μM, respectively.

Keywords: Marine-derived fungus, *Penicillium* sp., redoxcitrinin, biogenetic precursor, citrinin, polyketide

Exploitation of the marine environment has been intriguingly successful in recent years in the search for structurally unusual and biologically highly active natural products [4]. To avoid depletion of marine resources and to enable access to large quantities of interesting compounds, there has been a particular interest in the marine microorganisms that are culturable. We have been studying fungi isolated from marine sources for their potential of providing new bioactive compounds [11]. In a combined approach of biological and chemical screening, we are gaining a thorough understanding of the secondary metabolite pattern of the marine-derived fungi. A marine isolate of the fungus *Penicillium* sp. (MFA446) was selected from our screening program for further studies, because of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of its broth extract. We report here the isolation and structure elucidation of redoxcitrinin (**1**) [3], together with phenol A (**2**) [5, 8, 9], citrinin H2 (**3**) [8, 9], 4-hydroxymellein (**4**) [6], citrinin (**5**) [5], and phenol A acid (**6**) [5] as well as the biological activity of these compounds.

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Fungal Isolation and Culture

The fungal strain, *Penicillium* sp., was isolated from the surface of the marine green alga *Ulva pertusa* collected in Bijin Island, Gyeongnam, Korea and identified based on morphological evaluation and fatty acid methyl ester analysis (Korean Culture Center of Microorganism, Seoul, Korea, a similarity index of 0.65). A voucher specimen was deposited at Pukyong National University with the code MFA446. The isolate was cultured for three weeks (static) at 29°C in SWS medium that consisted of soytone (0.1%), soluble starch (1.0%), and seawater (100%) (20× 1 l).

Extraction and Isolation

The mycelium and broth were separated by filtration, and the whole broth was extracted with EtOAc (20 l) to afford crude extract (1.2 g). The broth extract showed mild DPPH scavenging activity, and the active components were purified by assay-guided isolation.

The broth extract (1.2 g) was subjected to Si gel flash chromatography. Elution was performed with *n*-hexane-EtOAc (stepwise, 0–100% EtOAc) to yield five fractions. Fractions 2–5 on medium-pressure liquid chromatography (MPLC) (ODS) by elution with H_2O-MeOH (gradient) afforded crude compounds **1**, **2** and **3**, **4** and **5**, and **6**, respectively, which were further purified by HPLC (YMC, ODS-A) utilizing a 30 min gradient program of 50% to 100% MeOH in H_2O to furnish **1** (5.1 mg), **2** (31.5 mg), **3** (6.7 mg), **4** (8.4 mg), **5** (3.8 mg), and **6** (3.9 mg), respectively.

Redoxcitrinin (**1**): a colorless solid; $[\alpha]_D$ +26° (*c* 0.2, EtOH); UV (MeOH), λmax (log ε) 301 (6.29), 342 (5.93) (sh) nm; IR (KBr) ν_{max} 3,392, 1,708, 1,607, 1,418, 1,276, 1,220, 1,160 cm^{-1}; LREIMS *m/z* 236 $[M]^+$ (85), 218 $[M-H_2O]^+$ (70), 203 $[M-H_2O-CH_3]^+$ (19), 193 $[M-COCH_3]^+$ (100), 176 (74), 165 (46), 161 (44), 147 (24), 91 (38); HREIMS *m/z* 236.1047 $[M]^+$ (calcd for $C_{13}H_{16}O_4$, 236.1049). See Table 1 for NMR spectral data.

Phenol A (**2**), citrinin H2 (**3**), and phenol A acid (**6**) were obtained as colorless oils, and 4-hydroxymellein (**4**) and citrinin (**5**) were obtained as colorless solids. Compounds **2-6** showed spectral data virtually identical to those reported in the literature [3, 5, 6, 8, 9].

Structural Elucidation and Bioactivity

Citrinin (**5**), a polyketide mycotoxin isolated from fungi, *Penicillium* or *Aspergillus* species, is a well-known contaminant of a number of agricultural products, and it has been demonstrated to possess nephrotoxic activity in addition to a number of other chronic toxic effects [5]. The known compounds **2–6** were identified by a spectroscopic analysis (^1H and ^{13}C NMR, LREIMS, and $[\alpha]_D$) and in comparison to literature data (Fig. 1) [3, 5, 6, 8, 9].

Redoxcitrinin (**1**), $[\alpha]_D$ +26° (*c* 0.2, EtOH), was obtained as a colorless solid that yielded a molecular formula of $C_{13}H_{16}O_4$ by the HREIMS [$(M)^+$ *m/z* 236.1047 (dev −0.2 mmu)] and ^{13}C NMR methods. The IR spectrum of **1** exhibited bands characteristics of hydroxyl (3,392 cm^{-1}), carbonyl (1,708 cm^{-1}), and aryl (1,607 cm^{-1}) functionalities. The ^1H and ^{13}C NMR data for compound **1**, including the results from COSY, DEPT, HMQC, and HMBC experiments, showed the presence of a hexasubstituted benzene ring possessing two hydroxyl, two methyl, an aldehyde, and a

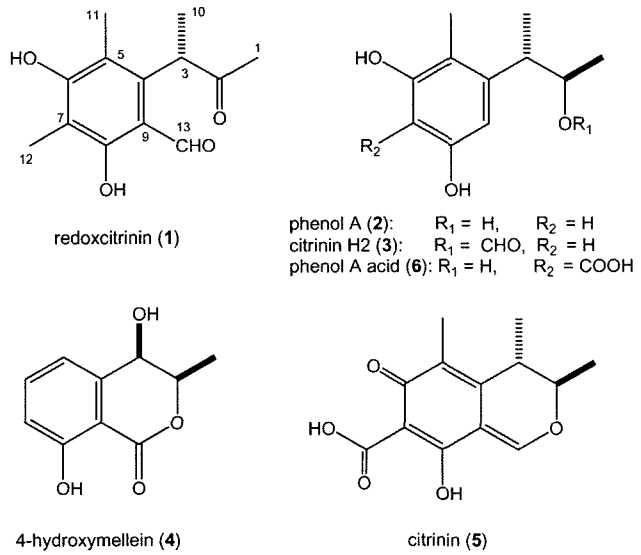

Fig. 1. Polyketide metabolites of *Penicillium* sp.

Table 1. NMR spectroscopic data (^1H: 400 MHz; ^{13}C: 100 MHz, DMSO-d_6) for Redoxcitrinin (**1**).

Position	δ_C, mult.	δ_H (*J* in Hz)	HMBC (H to C)
1	28.1, CH_3	1.96, s	3
2	208.6, qC		
3	47.1, CH	4.42, q (7.0)	2, 4, 5, 9, 10
4	141.8, qC		
5	116.5, qC		
6	162.0, qC		
7	109.4, qC		
8	162.0, qC		
9	111.4, qC		
10	16.4, CH_3	1.32, d (7.0)	2, 3, 4
11	11.8, CH_3	1.98, s	4, 5, 6
12	8.0, CH_3	2.03, s	6, 7, 8
13	193.9, CH	9.95, s	
8-OH		13.01, s	7, 8, 9

2-butanon-3-yl groups (Table 1). The EIMS spectrum of **1** showed two prominent fragment ions corresponding to the parent molecule with losses of an acetyl and a 2-butanon-3-yl groups [*m/z* 193 (M^+-$COCH_3$), 165 (M^+-$CH(CH_3)COCH_3$)]. Pentasubstituted benzaldehyde was further supported by UV data [301 nm (log ε 6.29), 342 (5.93) (sh)].

The connectivity and assignment of the carbon and proton resonances for **1**, which led to the planar structure for this metabolite, were made by interpretation of HMBC data. Diagnostic HMBC correlations, from H_3-1 to C-3, from H-3 to C-2, C-4, C-5, C-9, and C-10, from H_3-10 to C-2, C-3, and C-4, from H_3-11 to C-4, C-5, and C-6, from H_3-12 to C-6, C-7, and C-8, and from 8-OH to C-7, C-8, and C-9, showed the connection of the C3-C4 bond as well as the position of the 5,7-dimethyl, 6,8-dihydroxyl, and 9-aldehyde groups in **1** (Table 1). On the basis of all the foregoing evidence, we propose that the structure of redoxcitrinin is 3-(3,5-dihydroxy-2,4-dimethyl-6-formyl)buten-2-one (**1**). Despite the structural diversity among compounds **1–3**, **5**, and **6**, it is likely that they all derive from common or similar biogenetic intermediates, presumably as part of a polyketide origin [2, 7]. Accordingly, the configuration of C-3 in **1** was supposed to be 3*S* (Fig. 1).

Compound **1** has not been previously described from a natural source. Redoxcitrinin (**1**) has only been reported as a synthetic product obtained during biosynthetic studies of citrinin (**5**) [1, 2, 7]. Compounds **2** and **3** have been reported not only as the secondary metabolites of *Penicillium citrinum* [8], but also as a byproduct of the thermal and alkaline decompositions of citrinin (**5**), respectively [5, 9].

The antioxidant activity was assessed on the basis of the radical scavenging effect on the DPPH free-radical [10–12]. Compounds **1-3** exhibited mild radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) with IC_{50} values of 27.7, 23.4, and 27.2 μM, respectively, which

were less active than the positive control, L-ascorbic acid (IC_{50}, 20 μM).

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