HETEROCYCLES, Vol. 96, No. 6, 2018, pp. 1075 - 1079. © 2018 The Japan Institute of Heterocyclic Chemistry
Received, 22nd March, 2018, Accepted, 18th May, 2018, Published online, 23rd May, 2018
DOI: 10.3987/COM-18-13895

# NOVEL ANTIOXIDANT ISOLATED FROM *WARCUPIELLA SPINULOSA* JCM 2358, 7-HYDROXYCORDYLACTAM

**Ryosuke Shigemoto, Ken-Ichi Oinuma, Shunsuke Masuo, and Naoki Takaya***

Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan. E-mail: takaya.naoki.ge@u.tsukuba.ac.jp

**Abstract** – We identified a new compound, 7-hydroxycordylactam, together with the known compound, cordylactam, from cultured *Warcupiella spinulosa* JCM 2358. Their structures were elucidated using high-resolution mass spectrometry and NMR spectroscopy. The spectra indicated that these compounds have a γ-lactam-fused γ-pyrone skeleton. Analysis of their antioxidant activities showed that both 7-hydroxycordylactam and cordylactam scavenged 1,1-diphenyl-2-picrylhydrazyl radicals with an $IC_{50}$ of 340 μM.

Mammals incorporate oxygen and produce ATP via the mitochondrial electron-transport system to acquire the energy necessary for life activities.[1] Mitochondria generate reactive species such as superoxide anion ($\bullet O_2^-$) and hydroxyl (•HO) radicals that are derived from oxygen. These radicals are highly reactive, and oxidize biological compounds such as lipids, proteins and nucleic acids that lead to various diseases such as arteriosclerosis,[2] cancer[3] and myocardial infarction.[4] Natural antioxidants[5] such as α-tocopherol produced by many phototrophic plants and algae,[6] and polyphenols produced by plants and microbes are industrially important as food additives and supplements.

Filamentous fungi are produce an abundance of bioactive natural compounds.[7,8] While searching for bioactive metabolites from various fungal sources, we discovered that the ethyl acetate extract of *Warcupiella spinulosa* JCM 2358 cultured in yeast-malt sucrose (YMS) medium reduced the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Thus, the present study aimed to isolate, identify, and evaluate the antioxidant activities of the substance in the ethyl acetate extract of *Warcupiella spinulosa* JCM 2358 responsible for this activity.

We isolated and identified two antioxidants from *W. spinulosa* JCM 2358 culture. The fungus was cultured in 1.5 L of YMS medium, and then culture supernatants were acidified and extracted with ethyl acetate. The organic layer was fractionated by reverse-phase column chromatography, and fractions

containing antioxidant activities were further purified by preparative HPLC. This process yielded 6.0 and 6.8 mg of pure compounds **1** and **2**, which were subsequently analyzed by spectrometry.

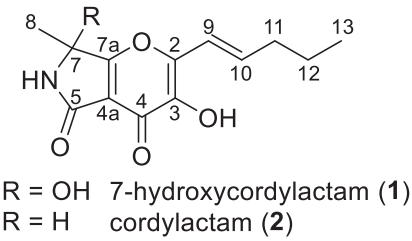


**Figure 1.** Structures of compounds **1** and **2**

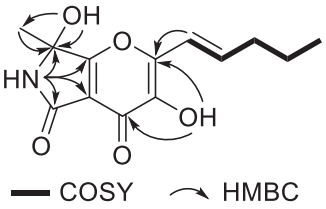


**Figure 2.** Key correlations of compound **1**

Compound **1** was yielded as an orange solid. High resolution-ESIMS analysis indicated a molecular formula of $C_{13}H_{15}NO_5$, based on a protonated molecular ion ($C_{13}H_{15}NO_5$ + H) at *m/z* = 266.1014. The UV spectrum of compound **1** showed absorption maxima of 215, 254 and 313 nm in methanol, which concurs with an extended conjugated system. Table 1 summarizes the $^1$H and $^{13}$C NMR spectra of compound **1** in DMSO-$d_6$. The $^{13}$C NMR spectrum showed two carbonyl groups ($\delta_C$ 163.7 and 169.0), five quaternary carbons ($\delta_C$ 81.8, 110.1, 142.1, 145.3 and 175.4), two methine ($\delta_C$ 117.7 and 135.9), two methylene ($\delta_C$ 21.4 and 34.5) and two methyl ($\delta_C$ 13.5 and 23.3) carbons. Three $D_2O$-exchangeable ($\delta_H$ 6.59, 8.62 and 9.68), two methine ($\delta_H$ 6.49 and 6.55), two methylene ($\delta_H$ 1.49 and 2.25), and two methyl ($\delta_H$ 0.92, 1.59) protons resonated in the $^1$H NMR spectrum. Heteronuclear multiple bond correlation (HMBC) spectra showed series of two- and three-bond correlations from 3-OH to C-2 and C-4, 7-OH to C-7 and C-8, 6 (NH) to C-4a, C-5, C-7 and C-7a, H-8 to C-7a, which revealed a γ-lactam-fused γ-pyrone skeleton with one methyl and two hydroxyl groups. Correlation spectroscopy (COSY) indicated successive cross peaks for aliphatic side chains at H-9/H-10, H-10/H-11, H-11/H-12 and H-12/13, and the HMBC spectra correlated H-9 with C-2, indicating an aliphatic side chain anchored at the C-2 carbon. The coupling constant of H-9 and H-10 ($J$ = 16.0 Hz) indicated that C-9 and C-10 have *E* geometry. Thus, we named compound **1** as 7-hydroxycordylactam based on the findings of structural analyses (Figure 1).

**Table 1.** $^{1}H$ and $^{13}C$ NMR spectral data for compounds **1** and **2** in DMSO-$d_6$

| Position | 7-Hydroxycordylactam (**1**) | | Cordylactam (**2**) | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$, multi., (*J* in Hz) | $\delta_C$ | $\delta_H$, multi., (*J* in Hz) |
| 2 | 145.3 | | 145.5 | |
| 3 | 142.1 | | 141.5 | |
| 4 | 169.0 | | 169.1 | |
| 4a | 110.1 | | 111.8 | |
| 5 | 163.7 | | 165.2 | |
| 7 | 81.8 | | 49.7 | 4.58, *dq* (7.0, 1.1) |
| 7a | 175.4 | | 177.3 | |
| 8 | 23.3 | 1.59, *s,* 3H | 17.2 | 1.38, *d* (7.0), 3H |
| 9 | 117.7 | 6.55, *d* (16.0) | 117.7 | 6.54, *d* (16.0) |
| 10 | 135.9 | 6.49, *dt* (16.0, 6.5) | 135.7 | 6.40, *dt* (16.0, 6.5) |
| 11 | 34.5 | 2.25, *m,* 2H | 34.5 | 2.24, *m*, 2H |
| 12 | 21.4 | 1.49, *m*, 2H | 21.4 | 1.48, *m*, 2H |
| 13 | 13.5 | 0.92, *t* (7.6), 3H | 13.5 | 0.92, *t* (7.4), 3H |
| 6 (NH) | | 8.62, *s* | | 8.29, *s* |
| 3-OH | | 9.68, *s* | | 9.57, *s* |
| 7-OH | | 6.59, *s* | | |

Compound **2** was obtained as a yellow solid, the UV spectrum of which revealed absorption maxima at 218, 250 and 310 nm in methanol, which was similar to that of compound **1**. High resolution-ESIMS indicated a molecular formula of $C_{13}H_{15}NO_4$ based on a protonated molecular ion at *m/z* = 250.1085 that differed from compound 1 in terms of the number of oxygen atoms. Table 1 summarizes the $^{1}H$ and $^{13}C$ NMR spectra analyzed in DMSO-$d_6$. The values for these chemical shifts were consistent with those for cordylactam.[9]

Cordylactam was originally isolated from the fungus, *Cordyceps* sp. BCC 12671, but its biological activity remains unknown.[9] We evaluated the antioxidant activities of 7-hydroxycordylactam and cordylactam by bleaching the stable DPPH free radical.[10] Both compounds dose-dependently reduced DPPH radicals at the micromolar level. The $IC_{50}$ of 7-hydroxycordylactam and cordylactam against the reduction of DPPH were 340 ± 9.1 and 340 ± 4.1 μM, respectively. These results indicated that the two cordylactam isolates acted as radical scavengers regardless of the presence of the hydroxyl group at C-7.

## EXPERIMENTAL

**General procedures.** Preparative HPLC proceeded using a 1260 infinity series instrument (Agilent Technologies, Palo Alto, CA, USA) equipped with a 250 × 10-mm Purospher Star RP-18 end-capped column (5 μm particle size; Merck Millipore, Billerica, MA, USA). Ultraviolet spectra were recorded using a DU 800 Spectrophotometer (Beckman Coulter, Brea, CA, USA). Mass spectra were analyzed using a SYNAPT G2 (Waters, Milford, MA, USA). Experiments involving $^{1}H$, $^{13}C$ and 2D-NMR (COSY, HSQC and HMBC) proceeded using an AVANCE III-600 Spectrometer (Bruker, Billerica, MA, USA). Optical rotation was recorded on a P-2200 (Jasco, Tokyo, Japan). IR spectra were determined on a FT/IR-300 (JASCO, Tokyo, Japan)

**Fermentation, extraction, isolation.** We obtained *W. spinulosa* JCM 2358 from the RIKEN BRC-JCM library and propagated it on potato dextrose (PD) medium (BD Biosciences, Franklin Lakes, NJ, USA) solidified with 1.5% agar at 28 °C for 7 - 14 days. The strain was then cultured in 500 mL Erlenmeyer flasks containing 100 mL YMS medium (10 g sucrose, 5 g peptone, 3 g yeast extract and 3 g malt extract in 1 L of distilled water) on a rotary shaker at 120 rpm and 28 °C for one week, and then transferred the contents to 5 L Erlenmeyer flasks containing 1.5 L YMS medium and incubated them under the same conditions. Cultures were filtered through Miracloth, acidified with 2 M HCl and extracted three times with the same volume of EtOAc. The organic layer was concentrated *in vacuo* to obtain a brown paste, which was dissolved in MeOH and applied to an open column packed with Wakogel 100C18 (Wako Pure Chemical Industries Ltd., Osaka, Japan) and separated by 20% aqueous MeCN. The eluate was separated by preparative HPLC using a mobile phase of 30% aqueous MeCN at a flow rate of 3.0 mL/min. Peaks at retention times of 7.5 and 10.2 min were collected and evaporated to obtain pure compounds **1** (6.0 mg) and **2** (6.8 mg).

**Scavenging DPPH radicals.** The antioxidant activities of compounds **1** and **2** were determined based on bleaching stable DPPH free radicals. The DPPH radical has an absorption at 520 nm, which disappears upon reduction by an antioxidant. Pure compounds were dissolved in 50% aqueous MeOH, then 100 μL was mixed with 50 μL of 200 μM 2-morpholinoethanesulfonic acid buffer (pH 6.0) and 800 μM DPPH in 96-well plates. Absorbance at 520 nm was determined after 20 min, and % inhibition activity was calculated. The $IC_{50}$ values indicated the sample concentration required to scavenge 50% of DPPH free radicals. The positive control was dibutylhydroxytoluene ($IC_{50}$ = 102 μM).

**7-Hydroxycordylactam (1):** Orange solid; $[\alpha]^{22.2}_{D}$ 8.75 (c 0.07, $CHCl_3$); IR (KBr) 3275, 3191, 2926, 1715, 1619, 1587, 1481, 1396, 1237, 1046, 972, 802 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ (log ε), 215 (4.63), 254 sh (3.71), 313 (4.09) nm; $^{1}H$ NMR, 600 MHz, DMSO; $^{13}C$ NMR, 150 MHz, DMSO (Table 1); HRESIMS, 266.1014 *m/z* (calcd for $C_{13}H_{15}NO_5$ + H, 266.1023 *m/z*)

**Cordylactam (2):** Yellow solid; $[\alpha]^{22.2}_{D}$ 8.67 (c 0.07, $CHCl_3$); IR (KBr) 3273, 2944, 2912, 1725, 1651, 1576, 1481, 1386, 1216, 1036, 961, 802 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ (log ε), 218 (4.23), 250 sh (3.98), 310 (4.15) nm; $^1H$ NMR, 600 MHz, DMSO; $^{13}C$ NMR, 150 MHz, DMSO (Table 1); HRESIMS, 250.1085 *m/z* (calcd for $C_{13}H_{15}NO_4$ + H, 250.1074 *m/z*)

## ACKNOWLEDGEMENTS

The authors thank Norma Foster for critical reading of the manuscript.

## REFERENCES

1. M. P. Murphy, *Biochem. J.*, 2009, **417**, 1.
2. W. Martinet, G. R. Y. de Meyer, A. G. Herman, and M. M. Kockx, *Eur. J. Clin. Invest.*, 2004, **34**, 323.
3. G.-Y. Liou and P. Storz, *Free Radic. Res.*, 2010, **44**, 479.
4. M. Hori and K. Nishida, *Cardiovasc. Res.*, 2008, **81**, 457.
5. M. S. Brewer, *Compr. Rev. Food. Sci. Food Saf.*, 2011, **10**, 221.
6. S. B. Nimse and D. Pal, *RSC Adv.*, 2015, **5**, 27986.
7. R. Chávez, F. Fierro, R. O. García-Rico, and I. Vaca, *Front. Microbiol*., 2015, **6**, 903.
8. J. Bérdy, *J. Antibiot*., 2012, **65**, 385.
9. M. Isaka, P. Chinthanom, P. Rachtawee, W. Somyong, J. J. Luangsa-ard, and N. L. Hywel-Jones, *Phytochem. Lett.*, 2013, **6**, 162.
10. O. P. Sharma and T. K. Bhat, *Food Chem.*, 2009, **113**, 1202.