



A Bioactive Depsidone from *Lachnum virgineum* (Hyaloscyphaceae)

Yoshihito Shiono*, Takuya Koseki, and Hiromasa Koyama

Department of Food, Life, and Environmental Science, Faculty of Agriculture, Yamagata University,
Tsuruoka, Yamagata 997-8555, Japan

Abstract – Norcolensoic acid (**1**) was isolated from both the fruiting bodies and the liquid culture of *Lachnum virgineum*. The structures of **1** was determined using spectroscopic methods. Compound **1** was isolated for the first time from this fungus. Compound **1** showed moderate antibacterial and phytotoxic activities.

Keywords – *Lachnum virgineum*, discomycetes, depsidone, norcolensoic acid

Introduction

Lachnum virgineum is an inedible discomycetes fungus which currently belong to the family Hyaloscyphaceae and embraces about 250 species.¹ *L. virgineum* produces tiny white fruiting bodies on rotting husks surrounding fruits (mast) of beech (*Fagus crenata*) in the early spring. For this study, the fruiting bodies of *L. virgineum* were collected at the foot of Mt. Gassan, Yamagata Prefecture, Japan. Phytochemical investigation of some species belonging to the family Hyaloscyphaceae revealed some biological active substances which included the glycosylated tetramic acid, virgineone, from *Lachnum virgineum*² and polysaccharide from *Luchnum* sp.³ However no reports on the previous phytochemical investigation from the fruiting body of *L. virgineum*. This paper describes the isolation, structure elucidation of the metabolite **1** isolated from the fruiting bodies and submerged fermentation of *L. virgineum*, and biological activity.

Experimental

General experimental procedures – Column chromatography (CC) was conducted on silica gel 60 (Kanto Chemical Co., Inc., Japan). TLC was carried out on Merck precoated silica gel plates (silica gel 60 F₂₅₄, 20 × 20 cm, Merck, Darmstadt, Germany), and spots were detected by spraying with 10% vanillin in H₂SO₄ followed by heating, or by UV irradiation. Semi-preparative HPLC was carried

out with Shimadzu pump and UV LC-10A detector (set at 210 nm) on Mightysil ODS column (250 × 6.0 mm i.d.; Kanto Chemical Co., Inc., Japan). Mass spectra were obtained with a Jeol JMS-700 instrument (JEOL, Tokyo, Japan), and ¹H- and ¹³C-NMR spectra were acquired with a Jeol EX-400 spectrometer (JEOL, Tokyo, Japan). Chemical shifts are given on a δ (ppm) scale with TMS as an internal standard.

Biological material – The fruiting bodies of *L. virgineum* were collected at the foot of Mt. Gassan, Yamagata Prefecture, Japan and characterized on the basis of carpophores morphology by one of authors (Y. S.). Spores were obtained through the suspension of sporulating basidiocarps of *L. virgineum* over potato-dextrose agar plates. Isolated spores were transferred to new plates by means of a sterile needle. Incubation was carried out at room temperature for 5 days. Several subculturing were carried out until pure culture was obtained. The mycelial culture thus obtained was used as inoculum in following liquid fermentation. A voucher specimen and this strain also have been deposited in the Faculty of Agriculture, Yamagata University, Yamagata, Japan.

Fermentation, Extraction and Isolation – The mycelium was grown in ten 500-ml Sakaguchi flasks containing 100 ml of a medium consisting of 40 g of glucose, 40 g of malt extract and 1.0 g peptone per 1 liter of water at 25 °C for 30 days on a rotary shaker at 120 rpm. After incubation period, 1.0 liters of culture broth was separated from the mycelia by filtration. The resulting filtrate was extracted with EtOAc. The EtOAc extract was concentrated *in vacuo* to give a residue (1.1 g), which was subject to silica gel column (10% stepwise elution with *n*-hexane/EtOAc) to yield fractions

*Author for correspondence

Yoshihito Shiono, Department of Food, Life, and Environmental Science, Faculty of Agriculture, Yamagata University, Tsuruoka, Yamagata 997-8555, Japan.

Tel: +81-235-28-2873; E-mail: yshiono@tds1.tr.yamagata-u.ac.jp

1 through 12 (Fr. 1-1 ~ Fr. 1-11). Purification of the eluate was monitored by the characteristic intense blue coloration with 10% vanillin in H₂SO₄ on TLC plates and by UV irradiation. Fr. 1-7 (0.6 g) was chromatographed on silica gel column (CHCl₃/EtOAc gradient) to obtain norcolensoic acid (**1**, 10.0 mg).

In our further phytochemical investigation, careful analysis of MeOH extract of the fruiting bodies of *L. virgineum* showed the presence of norcolensoic acid (**1**). For the isolation of **1**, the MeOH extract of the dried fruiting bodies (120 mg) was subjected to HPLC [Mightysil ODS column (4.6 mm i.d. × 60 mm, Kanto Co., Inc., Japan); flow rate, 0.8 ml min⁻¹; UV detection, 254 nm; solvent, MeOH/H₂O/CF₃COOH (80:20:0.1)] to give norcolensoic acid (**1**, 0.2 mg, *t_R* = 6.4 min), which showed ¹H NMR and MS data identical to those of **1**.

Norcolensoic acid (1) – Amorphous powder, UV (MeOH) λ_{max} nm (log ε): 220 (4.5), 250 (4.1), 256 (4.1), 262 (4.0); IR (KBr) ν_{max} cm⁻¹: 3420 (OH), 1765 (C=O), 1741 (C=O); ¹H-NMR (CD₃OD, 400 MHz): δ 6.66 (1H, s, H-9), 6.51 (1H, d, *J* = 2.1 Hz, H-2), 6.54 (1H, d, *J* = 2.1 Hz, H-4), 3.51 (2H, t, *J* = 8.0 Hz, H-2'), 2.68 (2H, t, *J* = 7.6 Hz, H-1''), 1.10-2.62 (overlapped signals, H-2', 3', 4', 2'', 3'', 4''), 0.82, 0.89 (each 3H, t, *J* = 7.0 Hz, Me-5', Me-5''); ¹³C-NMR (CD₃OD, 100 MHz): δ 14.7, 14.8 (C-5', 5''), 23.7 (C-4', 4''), 29.3, 32.2, 32.6, 33.1 (C-2', 3', 2'', 3''), 33.7(C-1''), 35.1(C-1'), 106.3 (C-4), 107.6 (C-9), 113.1 (C-1), 113.2 (C-11a), 116.5 (C-2), 139.7 (C-7), 143.3 (C-6), 149.4 (C-5a), 151.1 (C-9a), 160.5 (C-8), 163.9 (C-4a), 164.6 (C-3), 164.8 (C-10), 173.5 (C-12); FABMS *m/z* 427 [M-H]⁻; HRESITOFMS: [M+H]⁺*m/z* 429.1910 (calcd for C₂₅H₂₉O₇, 429.1908).

Antimicrobial activity – The minimal inhibition concentration (MIC) was determined by the agar dilution method, using nutrient agar for bacteria, Sabouraud agar for *Candida albicans* and PD agar for *Aspergillus clavatus*. Bioassay procedure was the same as article.⁴

Phytotoxic assay – Each sample was dissolved in 0.5 mL of MeOH and then poured on filter paper in a Petri dish (φ 40 × 10 mm). After sample-loaded paper had been air dried, 1 mL of distilled water was poured on the sample-loaded paper or intact filter paper (negative control). And then, lettuce seeds (n = 10 in each Petri dish) were put on the filter paper and were incubated in a growth chamber in the dark at 25 °C for 5 days. The length of the hypocotyl and the root were measured.

Result and Discussion

Norcolensoic acid (**1**) (Fig. 1) was found previously in

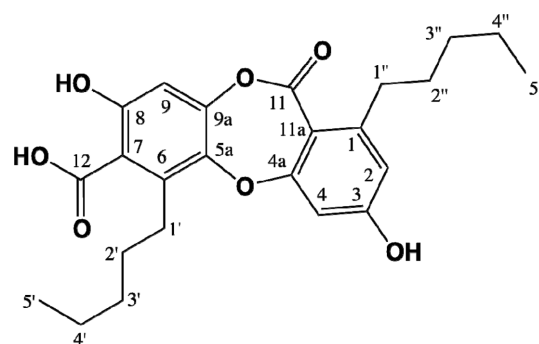


Fig. 1. The structures of **1** isolated from *Lachnum virgineum*.

an unidentified Australian lichen, *Lecanora* species, but in its report, no biological activity has yet been reported. Antimicrobial activity of **1** was evaluated against gram-positive and gram-negative bacteria, yeast, and fungus strains. Norcolensoic acid (**1**) showed antimicrobial activity against *Aspergillus clavatus* F 318a (MIC 50 µg/ml), *S. aureus* NBRC 13276 (MIC 25 µg/ml) and *P. aeruginosa* ATCC 15442 (MIC 25 µg/ml). At the concentration of 100 µg/ml, **1** was inactive against *Candida albicans*. Furthermore, we studied the potential phytotoxicity of **1** against the lettuce seedlings (*Lactuca sativa* L.). Aqueous solutions of **1**, ranging between 50 µg/ml and 500 µg/ml, were tested on seed germination, root and shoot length of the lettuce.

Norcolensoic acid (**1**) inhibited the root growth to 63% and 92% of the negative control at the concentration of 100 µg/ml and 300 µg/ml, respectively. While, at the highest concentration (500 µg/ml), **1** strongly exerted an inhibitory effect on the seed germination. To our knowledge, this is the first report of isolation of **1** from *L. virgineum* and biological activity of **1**. Depsidones, depsides and diphenyl ethers are a distinct class of lichen derived compounds, and showing interesting biologically activities such as antibiotic, antitumor, and antiviral.^{5,6,7} Lichens are well known for their ability to produce large amounts and a great variety of secondary metabolites. They are symbiotic organisms consisting of mycobiont and phytobiont partners which produce a large number of the characteristic secondary metabolites. Addition, the mycobiont is important for the production of these lichens secondary metabolites.⁸ In this study, we isolated norcolensoic acid (**1**) from the fruiting bodies and the liquid culture of discomycetes fungus *L. virgineum*. Compound **1** was originally isolated by Elix et al., from an unidentified Australian lichen *Lecanora* sp.⁹ It might be postulated that *L. virgineum* is closely related to lichen, due to the production of **1** in both species. Furthermore, it is a great interest from the

viewpoint of the ecological and biochemical significances that **1** was found to exhibit antimicrobial activity and phytotoxicity.

Dedication : This study is dedicated to the memory of Dr. Hiromasa Koyama, Professor at Faculty of Agriculture, University of Yamagata, who passed away in 2016.

References

- (1) Hosoya, T.; Sasagawa, R.; Hosaka, K.; Ho, S. G.; Hirayama, Y.; Yamaguchi, K.; Toyama, K.; Kakishima, M. *Mycoscience* **2010**, *51*, 170-181.
- (2) Ondeyka, J.; Harris G.; Zink, D.; Basilio, A.; Vicente, F.; Bills, G.; Platas, G.; Collado, J.; González, A.; de la Cruz, M.; Martin, J.; Kahn, J. N.; Galuska, S.; Giacobbe, R.; Abruzzo, G.; Hickey, E.; Liberator, P.; Jiang, B.; Xu, D.; Roemer, T.; Singh, S. B. *J. Nat. Prod.* **2009**, *72*, 136-141.
- (3) Chen, T.; Xu, P.; Zong, S.; Wang, Y.; Su, N.; Ye, M. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 1225-1232.
- (4) Shiono, Y.; Murayama, T.; Takahashi, K.; Okada, K.; Katohda, S.; Ikeda, M. *Biosci. Biotechnol. Biochem.* **2005**, *69*, 287-292.
- (5) Neamati, N.; Hong, H.; Mazumder, A.; Wang, S.; Sunder, S.; Nickolaus, M. C.; Milne, G. W. A.; Proska, B.; Pommier, Y. *J. Med. Chem.* **1997**, *40*, 942-951.
- (6) Yamamoto, Y.; Miura, Y.; Kinoshita, Y.; Higuchi, M.; Yamada, Y.; Murakami, A.; Ohigashi, H.; Koshimizu, K. *Chem. Pharm. Bull.* **1995**, *43*, 1388-1390.
- (7) Okuyama, E.; Umeyama, K.; Yamazaki, M.; Kinoshita, Y.; Yamamoto, Y. *Chem. Pharm. Bull.* **1995**, *61*, 113-115.
- (8) Schmitt, I.; Martin, M. P.; Kautz, S.; Lumbsch, H. T. *Phytochemistry* **2005**, *66*, 1241-1253.
- (9) Chester, D. C.; Elix, J. A. *Aust. J. Chem.* **1981**, *34*, 1507-1511.

Received November 16, 2017

Revised December 23, 2017

Accepted January 8, 2018