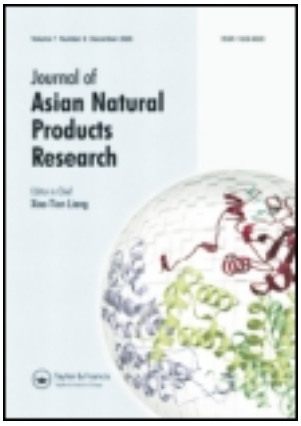

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

A new spirocyclic compound from the liquid culture of entomogenous fungus Isaria cateniannulata

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Version of record first published: 29 Oct 2012.

To cite this article: Xiao-Long Yang , Zhuang-Zhuang Li , Su Zhang , He Yu , Hua-Jie Zhu & Du-Qiang Luo (2012): A new spirocyclic compound from the liquid culture of entomogenous fungus Isaria cateniannulata , Journal of Asian Natural Products Research, 14:11, 1093-1096

To link to this article: http://dx.doi.org/10.1080/10286020.2012.707650

Journal of Asian Natural Products Research
Vol. 14, No. 11, November 2012, 1093–1096

NOTE

A new spirocyclic compound from the liquid culture of entomogenous fungus *Isaria cateniannulata*

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(*Received 15 March 2012; final version received 24 June 2012*)

A new spirocyclic compound named (2*S*, 5*S*, 7*S*)-3α-hydroxyl-exogonic acid (**1**) was isolated from the liquid culture of entomogenous fungus *Isaria cateniannulata*. The structure and relative stereochemistry of **1** were elucidated by extensive spectroscopic analysis and by comparison of its NMR data with those of known compound. Compound **1** showed weak inhibitory activity against HeLa with IC_{50} value of 80.5 μg ml^{-1}.

Keywords: entomogenous fungus; *Isaria cateniannulata*; exogonic acid; antitumor activity

1. Introduction

The genus *Isaria* is insect pathogens including Homoptera, Lepidoptera, and Coleoptera, which have been the source of a wide range of bioactive compounds [1]. Representative secondary metabolites are sphingoid ISP-I as the potent immunosuppressants which was isolated from *Isaria sinclairii* ATCC 24400 [2], hanasanagin as the antioxidative pseudodipeptide which was produced by the fruiting bodies of *Isaria japonica* [3], and three types of insecticidal cyclodepsipeptides (including isariins, isarolides, isafelins, and isaridins) which were isolated from *Isaria cretacea*, *Isaria felina*, and *Isaria* sp. [4–10].

As a continuing research on bioactive metabolites from special ecological environmental fungi of China, the chemical constituents of the liquid culture of entomogenous fungus *Isaria cateniannulata* were investigated. This report describes the isolation, structure elucidation, and biological activity of one new spirocyclic compound.

2. Results and discussion

Compound **1** was obtained as white powder. The molecular formula of **1** was determined to be $C_{10}H_{16}O_5$ on the basis of HR-APCI-MS $[M + H]^+$ (calcd for $[M + H]^+$*m/z* 217.0965, found 217.0960) with three degrees of unsaturation. The IR spectrum displayed absorption bands of hydroxyl (3440 cm^{-1}), methyl (1457 cm^{-1}), and carbonyl (1783 cm^{-1}) groups. Its ^{13}C NMR (DEPT) spectrum exhibited 10 carbon resonances, assigned as one carbonyl carbon (δ_C 176.8), four methylene groups (δ_C 41.6, 36.6, 32.0, and 35.5), three oxygenated methine groups (δ_C 84.3, 77.0, and 76.6), one methyl group (δ_C 21.7), and one highly oxygenated sp^3 quaternary carbon atom (δ_C 115.5). The ^{1}H NMR spectrum

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ISSN 1028-6020 print/ISSN 1477-2213 online

http://dx.doi.org/10.1080/10286020.2012.707650
http://www.tandfonline.com

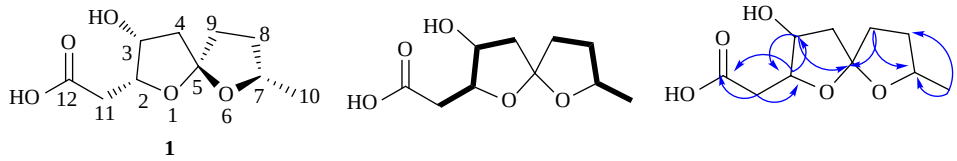

Figure 1. The structure, $^{1}H-^{1}H$ COSY and selected HMBC correlations of **1.**

displayed signals of three oxygenated methine protons at δ 5.16 (1H, m), 4.77 (1H, m), 4.22 (1H, m), and one methyl proton at δ 1.27 (3H, d, $J = 6.1$ Hz). The connectivity of the protons and C-atoms was established by the HSQC, ^{1}H and ^{13}C NMR spectra. The cross-peaks between H-11 and H-2/H-3/H-4, H-7 and H-8/H-9 were observed in the $^{1}H-^{1}H$ COSY spectrum (Figure 1). It allowed establishment of two H-atom systems: one at C-11 through C-2, C-3 to C-4, and the other at C-7 through C-8 to C-9. By careful analysis of NMR data, we found that compound **1** is very similar to the known compound exogonic acid except for one additional hydroxyl group [11], which was further supported by the following key correlations observed in the HMBC spectra (Figure 1): from H-2 to C-3 and C-12, from H-3 to C-2 and C-5, from H-9 to C-5 and C-7, from H-10 to C-7 and C-8, and from H-11 to C-2 and C-12. The additional hydroxyl group was located at C-3 in **1** deduced from the following key correlation in the HMBC spectra (Figure 1): from H-3 to C-2 and C-5, along with the $^{1}H-^{1}H$ COSY correlations of H-2/H-3/H-4. Finally, considering the information mentioned above, the gross structure of **1** was determined as 3-hydroxyl-exogonic acid (**1**) (Figure 1).

Professor William Kitching systematically reported the absolute stereochemistry of four possible *E,E*, *E,Z*, *Z,E*, and *Z,Z* diastereomers of natural methyl exogonate, and the most diagnostic features of the spectrum are the CH_3CH doublets ($J = 6.1$ Hz) assigned as follows: δ_H 1.05 (*Z,E*); 1.06 (*E,E*); 1.25 (*E,Z*); and 1.13 (*Z,Z*) [11]. Although the results indicated that natural exogonic acid was predominantly the *E,E* and *Z,Z* diastereomers, the chemical shift of the key feature methyl group proton is 1.27 ppm (3H, d, $J = 6.1$ Hz), which indicated that compound **1** belonged to the *E,Z* form. On further careful comparison of the chemical shifts of **1** with those of exogonic acid, the absolute stereochemistry of C-2, C-5, and C-7 in **1** was finally determined to be 2*S*, 5*S*, and 7*S*. The NOE correlations (Figure 2) of H-2/H-3 and H-4β (2.54), and H-4α (2.33)/H-11 along with no observed NOE correlations between H-3 and H-11 indicated that the hydroxyl group is α-oriented.

The cytotoxic activity of **1** was evaluated against HeLa cell line. The results indicated that **1** showed weak activity against HeLa tumor cell line with IC_{50} value of 80.5 μg ml^{-1}.

3. Experimental

3.1 General experimental procedures

Optical rotation spectra were recorded on JASCO P-1020 spectropolarimeter. IR spectra were recorded on a Perkin-Elmer (Shimadzu, Tokyo, Japan) 577 spectrometer. UV spectra were recorded on Shimadzu (Perkin-Elmer, Waltham, MA, USA) UV-2401PC spectrometer (Shimadzu, Kyoto, Japan), λ_{max} (log ε) in nm.

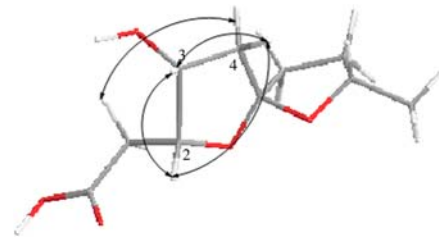

Figure 2. The key NOESY correlations of **1.**

1D and 2D NMR spectra were recorded on a Bruker AM-600 spectrometer (Bruker, Faellanden, Switzerland) with TMS as an internal standard, δ in ppm, J in Hz. FT-MS spectra were recorded on a Bruker apex-ultra 7.0 T spectrometer (Bruker, Faellanden, Switzerland). Column chromatography (CC) was carried out over silica gel (200~300 mesh; Qingdao Haiyang Chemical Co. Ltd., Qingdao, China). TLC was carried out on precoated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co. Ltd., Qingdao, China) and Sephadex LH-20 gel (25~100 μm, GE Healthcare Co. Ltd., Uppsala, Sweden).

3.2 Fungal material and cultivation conditions

I. cateniannulata was bought from the Agricultural Culture Collection of China in April 2011, and assigned the accession number ACCC37782. The fungal strain was cultured on slants of potato dextrose agar (PDA) at 28°C for 7 days, and then inoculated into a 500-ml Erlenmeyer flask containing 100 ml of PDA medium (20.0 g of glucose, 200.0 g of potato (peeled), 3.0 g of KH_2PO_4, 1.5 g of $MgSO_4$, 0.1 g of citric acid, and 10.0 mg of thiamine hydrochloride in 1 liter of deionized H_2O). The final pH of the media was adjusted to 6.5 before sterilization. After 7 days of incubation at 28°C on rotary shakers at 150 rpm, 25 ml of culture liquid were transferred as seed into each 1000-ml Erlenmeyer flask containing 250 ml of PDA medium, and static fermentation was carried out on a rotary shaker for 25 days.

3.3 Extraction and isolation

The culture broth (40 liters) was extracted three times with ethyl acetate, and the organic layer was concentrated *in vacuo* to yield a brown oily residue (12.6 g). This residue was subjected to silica gel CC with a gradient elution of petroleum ether–acetone [100:0, 98:2, 95:5, 90:10, 80:20, and 50:50 (v/v)] to obtain six fractions. Fr. 5 (3.1 g) eluted with petroleum ether–acetone (80:20) was further fractionated by silica gel CC using petroleum ether–acetone gradient elution (from 40:1 to 1:1, v/v) to obtain five fractions. Fr. 5-2 (290 mg) eluted with petroleum ether–acetone (30:1, v/v) was further purified by repeated CC [silica gel; petroleum ether–acetone 35:1 (v/v)], Sephadex LH-20 (CH_3OH), and preparative thin layer chromatography (TLC) (petroleum ether–acetone, 2:1) to afford compound **1** (3 mg).

3.4 Cytotoxicity assay

Cytotoxic activity was evaluated against HeLa cell by the 3-(4,5-di-methyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [12]. HeLa cell line was grown in RPMI-1640 medium Grand Island Biological Company (GIBKO) Grand Island, New York, United States supplemented with 10% heat-inactivated bovine serum, 2 nM l-glutamine, 10^5 IU l^{-1} penicillin, 100 mg l^{-1} streptomycin, and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4. Cells were kept at 37°C in a humidified 5% CO_2 incubator. An aliquot (180 μl) of HeLa cell suspension at a density of 1500 cells ml^{-1} was pipetted into 96-well microtiter plates. Subsequently, 180 μl of the sample (in DMSO) at different concentrations was added to each well and incubated for 72 h at the above conditions in a CO_2 incubator. MTT solution (20 μl of 5 mg l^{-1} in RPMI-1640 medium) was added to each well and further incubated for 4 h at 37°C. After addition of 100 μl dimethyl sulfoxide (DMSO) and incubation for 1 h, the cells were lysed to liberate the formed formazan crystals. Optical density (OD) was read on a Multiscan plate reader at a wavelength of 570 nm. DMSO control well, in which the sample was absent, was included in the experiment in order to eliminate the influence of DMSO. The inhibitory rate of cell proliferation was calculated by the

Table 1. NMR data for compound **1**[a] and two diastereomers of exogonic acid.[b]

	1		*E,E*−exogonic acid	*Z,Z*−exogonic acid
	δ_H	δ_C	δ_C	δ_C
2	4.77 (m)	77.0 (d)	73.9 (d)	74.3 (d)
3	5.16 (m)	84.3 (d)	30.0 (t)	30.5 (t)
4α	2.33 (dd, 1.8, 15.0)	41.6 (t)	40.4 (t)	42.2 (t)
4β	2.54 (dd, 1.8, 15.0)	41.6 (t)	40.4 (t)	42.2 (t)
5		115.5 (s)	115.0 (s)	115.1 (s)
7	4.22 (m)	76.6 (d)	75.2 (d)	76.3 (d)
8	2.10, 1.73 (m)	32.0 (t)	31.9 (t)	32.4 (t)
9	2.10 (m)	36.6 (t)	35.5 (t)	35.9 (t)
10	1.27 (d, 6.1)	21.7 (q)	20.9 (q)	22.8 (q)
11	2.54, 2.89 (dd, 6.0, 18.0)	35.5 (t)	34.9 (t)	35.3 (t)
12		176.8 (s)	176.2 (s)	176.5 (s)

[a] Compound **1** was measured at 150 MHz in CD_3OD.
[b] Exogonic acid was measured in $CDCl_3$.

following formula:

$$\text{Growth inhibition}\,(\%) = \left[\frac{\text{OD}_{\text{control}} - \text{OD}_{\text{treated}}}{\text{OD}_{\text{control}}}\right] \times 100\%.$$

The cytotoxicity of samples on HeLa cell was expressed as IC_{50} values and calculated by the LOGIT method.

3.5 (2S, 5S, 7S)-3α-hydroxyl-exogonic acid (1)

White powder. $[\alpha]_D^{20} = -52.8°$ ($c = 0.02$, MeOH). UV (MeOH) λ_{max} ($\lg \varepsilon$): 203 (3.48), 248 (3.50), 323 (2.33) nm. IR (KBr) ν_{max}: 3440 (OH), 1783 (C = O), 1457 (CH_3) cm^{-1}. For NMR spectral data, see Table 1. HR-APCI-MS $[M + H]^+ m/z$ 217.0960 (calcd for $C_{10}H_{17}O_5$, 217.0965).

Acknowledgments

This work was supported by the programs for the Natural Science Foundation of Hebei Province (C2012201047), New Century Excellent Talents in University (NCET-09-0112), the Key Project of Chinese Ministry of Education (209010), the Key Applied Basic Research Programs of Hebei Province (0996030917D), and the National Natural Science Foundation of China (31071701 and 31171885).

References

[1] R. Haritakun, P. Srikitikulchai, P. Khoyaiklang, and M. Isaka, *J. Nat. Prod.* **70**, 1478 (2007).
[2] T. Fujita, K. Inoue, S. Yamamota, T. Ikumoto, S. Sasaki, R. Toyama, K. Chiba, Y. Hoshino, and T. Okumoto, *J. Antibiot.* **47**, 208 (1994).
[3] A. Sakakura, K. Suzuki, H. Katsuzaki, T. Komiya, T. Imamura, Y. Aizono, and K. Imai, *Tetrahedron Lett.* **46**, 9057 (2005).
[4] L.C. Vining and W.A. Taber, *Can. J. Chem.* **40**, 1579 (1962).
[5] W.A. Wolstenholme and L.C. Vining, *Tetrahedron Lett.*, **26**, 2693 (1972).
[6] R. Baute, G. Deffieux, D. Merlet, M.A. Baute, and A. Neveu, *J. Antibiot.* **34**, 1261 (1981).
[7] G. Deffieux, D. Merlet, R. Baute, G. Bourgeois, M.A. Baute, and A. Neveu, *J. Antibiot.* **34**, 1266 (1981).
[8] Y.X. Guo, Q.H. Liu, T.B. Ng, and H.X. Wang, *Peptides* **26**, 2384 (2005).
[9] V. Sabareesh, R.S. Ranganayaki, S. Raghothama, M.P. Bopanna, H. Balaram, M.C. Srinivasan, and P. Balaram, *J. Nat. Prod.* **70**, 715 (2007).
[10] G. Ravindra, R.S. Ranganayaki, S. Raghothama, M.C. Srinivasan, R.D. Gilardi, I.L. Karle, and P. Balaram, *Chem. Biodivers.* **1**, 489 (2004).
[11] E.N. Lawson, J.F. Jamie, and W. Kitching, *J. Org. Chem.* **57**, 353 (1992).
[12] M.C. Alley, D.A. Scudiero, A. Monks, M.L. Hursey, M.J. Czerwinski, and D.L. Fine, *Cancer Res.* **48**, 589 (1988).