Human African trypanosomiasis, also known as sleeping sickness, is a potentially fatal parasitic disease transmitted by the bite of the tsetse fly that plagues many regions of Africa. Although the number of people being infected with the disease has declined due to better diagnosis and treatment, an estimated 30,000 people are currently infected with 7,000 new infections in 2012 without treatment, sleeping sickness is fatal. Moreover current therapies often have unpleasant side effects.

During the course of our screening program for compounds that treat the disease, which is caused by the parasite Trypanosoma brucei, we had reported on various natural products, such as microbial and fungi metabolites and plant products, for the study of metabolites of Ophiocordyceps coccidiicola NBRC 100683. Their structures were identified by the analysis of high resolution-electron ionization (HR-EI)-MS and HR-FAB-MS, and 1H- and 13C-NMR spectra, including extensive two dimensional (2D)-heteronuclear NMR experiments, and comparison with literature data for destruxin A (1), destruxin B (2), destruxin E chlorohydrin (3) and helvolic acid (4). Compounds 1–4 showed in vitro antitrypanosomal activity against Trypanosoma brucei brucei with IC50 values of 0.33, 0.16, 0.06 and 5.08 µg/mL, respectively.

Key words Trypanosoma brucei brucei; depsipeptide; nortriterpenoid; Ophiocordyceps coccidiicola

© 2016 The Pharmaceutical Society of Japan
Table 1. In Vitro Anti-trypanosomal Activity against Trypanosoma brucei brucei GUTat3.1 and Cytotoxicity in MRC-5 Cells of Compounds 1–4

| Compound                | IC\(_{50}\) (µg/mL) | Anti-tryp activity (T. b. b. GUTat 3.1) | Cytotoxicity (MRC-5) | Selectivity index (MRC-5/Try) |
|-------------------------|----------------------|----------------------------------------|----------------------|-------------------------------|
| Destruxin A (1)         | 0.33                 | 4.63                                   | 4.63                 | 14.0                          |
| Destruxin B (2)         | 0.16                 | 3.75                                   | 3.75                 | 24.3                          |
| Destruxin E chlorohydrin (3) | 0.061              | 0.36                                   | 0.36                 | 5.9                           |
| Helvolic acid (4)       | 5.08                 | >100                                   | >100                 | 19.7                          |
| Suramin (positive control) | 1.58               | >100                                   | >100                 | >63                           |

Fig. 1. Structures of 1–4

In conclusion, this paper describes the identification of entomopathogenic fungal metabolites produced by O. coccidicola, possessing in vitro anti-trypanosomal activity. To the best of our knowledge, this is the first report for the study of metabolites of O. coccidicola. Among them, destruxins 1–3 are belonging to cyclo depsipeptides. We had reported other anti-trypanosomal cyclo depsipeptides, cardinalisamides A–C, from the insect pathogenic fungus Cordyceps cardinalis NBRC 103832. 2 Helvolic acid (4) is an antibacterial nortriterpenoid that is an inhibitor of the protein synthesis through elongation factor G (EF-G) on the bacterial ribosome. Although fusidic acid (analog of 4) was reported to show anti-protozan activities against malaria, babesia and so on, this is the first report for anti-trypanosomal activity of 4.

Experimental

General Experimental Procedures Optical rotations were recorded on a JASCO P-1030 polarimeter. IR spectra were measured on a Shimazu FTIR-8400S instrument. NMR spectra were obtained on a Varian UNITY 600 NMR spectrometer. The chemical shifts were given in δ (ppm), and coupling constants were reported in Hz. HR-MS spectra were ob-
on silica gel (Kanto Kagaku silicagel 60N, 40–50 mesh) was used for column chromatography, and silica gel 60F-254 (Merk) for TLC. HPLC was performed on a JASCO-PU 1580 instrument with a COSMOSIL C18 P-MS II (250×20 mm).

**Material, Extraction and Isolation** The fungus *O. coccidicola* NBRC 100683 was identified by Dr. Ban of the Nite Biological Resource Center (NBRC). A voucher specimen (O. coccidicola NBRC 100683 and IU-3) is deposited at the NBRC Culture Collection. IU-3 was cultured for 2 weeks at 25°C in potato sucrose medium (15 L culture). The fungus from the culture (15 L) of IU-3 was separated from the mycelia by filtration and subsequently absorbed on Diaion HP-20 resin. And then, the absorbed fraction was eluted with MeOH. The MeOH eluted fraction was evaporated to an aqueous concentration.

The EtOAc-soluble portion (1.1 g) was repeatedly chromatographed on silica gel (Kanto Kagaku silicagel 60N, 40–50µm) by n-hexane and an increasing ratio of CHCl₃. Further purification was carried out by repeated preparative HPLC on an ODS C-18 column (COSMOSIL MS-II 20 250×250 mm for 1–3, COSMOSIL AR-II 20 20×250 mm for 4), using 55–80% MeOH in H₂O (flow rate; 3 mL/min, room temperature; 23°C and isocratic) to afford four compounds, destruxin A (1) (60% MeOH, 90 min) (51.4 mg), destruxin B (2) (60% MeOH, 39.6 min) (37.2 mg), destruxin E chlorohydrin (3) (55% MeOH, 54.8 min) (46.8 mg) and helvolic acid (4) (80% MeOH, 22 min) (9.1 mg).

**Trypanosome** The bloodstream forms of *T. brucei brucei* strain GUTat 3.1 parasites were used for experimentation, as described previously. In brief, 95 µL of parasite suspension was incubated with 5 µL of drug solution for 72 h and Alamar Blue was used for parasite survival determination to calculate IC₅₀ values.

**In Vitro Assay** The *in vitro* antitypanosomal assays using *T. brucei brucei* strain GUTat 3.1 have been described previously. In brief, 95 µL of parasite suspension was incubated with 5 µL of drug solution for 72 h and Alamar Blue was used for parasite survival determination to calculate IC₅₀ values. Cytotoxicity assay against human diploid embryonic cell line MRC-5 was carried out as previously described.

**Acknowledgments** We are indebted to Dr. M. Tanaka for measurements of the 600 MHz NMR spectra, Faculty of Pharmaceutical Sciences of Tokushima Bunri University. This work was supported by a Grant from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan-Senryaku project (No. S0891078).

**Conflict of Interest** The authors declare no conflict of interest.

**Supplementary Materials** The online version of this article contains supplementary materials.

**References**

1. Home page of World Health Organization: WHO: [http://www.who.int/mediacentre/factsheets/fs259/en/](http://www.who.int/mediacentre/factsheets/fs259/en/), 2015.
2. Umeyama A., Takahashi K., Grudniewska A., Shimizu M., Hayashi S., Kato M., Okamoto Y., Suenaga M., Ban S., Koizuma T., Ishiyama A., Iwatsuki M., Otoguro K., Omura S., Hashimoto T., *J. Antibiot.*, 67, 163–166 (2014).
3. Umeyama A., Ohta C., Shino Y., Okada M., Nakamura Y., Hamagiki T., Imagawa H., Tanaka M., Ishiyama A., Iwatsuki M., Otoguro K., Omura S., Hashimoto T., *Tetrahedron*, 70, 8312–8315 (2014).
4. Inahashi Y., Iwatsuki M., Ishiyama A., Namatame M., Nishihara-Tsukashima A., Matsunaga M., Hirose T., Sunakaza T., Yamada H., Otoguro K., Takahashi Y., Omura S., Shiomi K., *J. Antibiot.*, 64, 303–307 (2011).
5. Iwatsuki M., Kinoshita Y., Niitsu M., Hashida J., Mori M., Ishiyama A., Namatame M., Nishihara-Tsukashima A., Nonaka K., Masuma R., Otoguro K., Yamada H., Shiomi K., Omura S., *J. Antibiot.*, 63, 331–333 (2010).
6. Kobayashi Y., Shimizu D., *Bull. Natl. Sci. Mus., Tokyo*, 5, 69–85 (1960).
7. Imai S., *Transactions of the Sapporo Natural History Society*, 14, 101–106 (1936).
8. Sung G. H., Hywel-Jones N. L., Sung J. M., Luangsrd-ard J. J., Shrestha B., Spatafora J. W., *Stud. Mycol.*, 60, 5–59 (2007).
9. Home page of Index Fungorum: [http://www.indexfungorum.org/](http://www.indexfungorum.org/).
10. Gupta S., Roberts D. W., Renwick J. A. A., *J. Chem. Soc., Perkin Trans. 1*, 1989, 2347–2357 (1989).
11. Lee S.-Y., Kinoshita H., Ihara F., Igarashi Y., Nihira T., *J. Biosci. Bioeng.*, 105, 476–480 (2008).
12. Otoguro K., Kohana A., Manabe C., Ishiyama A., Uji H., Shiomi K., Yamada H., Omura S., *J. Antibiot.*, 54, 658–663 (2001).
13. Otoguro K., Ishiyama A., Namatame M., Nishihara A., Furusawa T., Masuma R., Shiomi K., Takahashi Y., Yamada H., Omura S., *J. Antibiot.*, 61, 372–378 (2008).