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Dahurelmusin A, a Hybrid Peptide–Polyketide from *Elymus dahuricus* Infected by the *Epichloë bromicola* Endophyte

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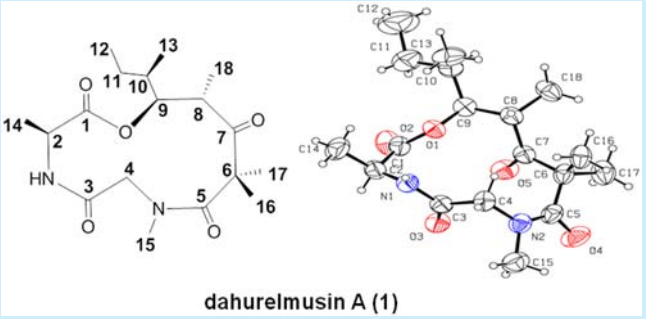
Supporting Information

ABSTRACT: One novel hybrid peptide–polyketide, dahurelmusin A (**1**), was isolated from *Elymus dahuricus* infected by the *Epichloë bromicola* endophyte. Comprehensive spectroscopic analysis revealed that **1** is the first example of hybrid peptide–polyketide possessing an unprecedented 5-hydroxy-2,2,4,6-tetramethyl-3-oxooctanoic acid moiety. The single-crystal X-ray diffraction analyses allowed the absolute configuration assignment of this compound. Compound **1** also exhibited significant insecticidal activities against *Rhopalosiphum padi* and *Brevicoryne brassicae* with LC_{50} values of 0.092 and 0.251 mM, respectively.



As one of the cool season grasses, *Elymus dahuricus* (*E. dahuricus*) is widely planted in subtropical and temperate regions.[1] The stems and leaves of *E. dahuricus* are abundant natural resources in China and are used for high-quality herbage purposes, indicating that the grass may have low toxicity to dairy animals and be environmentally biodegradable. The frequency of infection of *E. dahuricus* by *Epichloë* spp. ranges from 20 to 100% in China.[2−5] However, until now, the metabolites of *Epichloë bromicola* (*E. bromicola*) infected *E. dahuricus* and its insecticidal activities have not been investigated. As part of a program with the goal of screening of *Epichloë* spp. infected forage for insecticides, we report herein the isolation and identification of metabolites from an extract of *E. bromicola*-infected *E. dahuricus*. The isolated metabolites were further evaluated for their insecticidal activities against *Rhopalosiphum padi* (*R. padi*) and *Brevicoryne brassicae* (*B. brassicae*).

Compound **1** was obtained as an optically active colorless crystal (Figure 1). The ^{13}C NMR and DEPT spectra, together with the molecular ion peak in the HRESIMS, indicated a molecular formula of $C_{18}H_{30}O_5N_2$, requiring five degrees of unsaturation. The IR spectrum of compound **1** showed absorption bands of carbonyl groups (1719, 1701, and 1687 cm^{-1}). The ^1H NMR and DEPT spectra of metabolite **1** revealed the presence of an *N*-H group at δ_H 6.89 (d, J = 10.0 Hz); an oxygenated methine proton at δ_H 5.19 (d, J = 9.2 Hz); two methine protons at δ_H 3.67 (m) and 4.60 (m); a set of methylene protons at δ_H 2.92 and 4.96 (d, J = 13.2 Hz); another set of methylene protons at δ_H 1.06 and 1.41 (m); an *N*-methyl group at δ_H 3.16; two tertiary methyl signals at δ_H 1.62 and 1.76; three secondary methyl groups at δ_H 0.91 (d, J = 6.8 Hz), 1.02 (d, J = 7.2 Hz), and 1.32 (d, J = 7.2 Hz); and a triplet methyl group at δ_H 0.94 (t, J = 7.2 Hz). The ^{13}C NMR and DEPT spectra of compound **1** resolved 18 carbon signals, corresponding to four carbonyl carbons (one ketone carbon), one quaternary carbon, four methine carbons (one oxygenated carbon), two methylene carbons, and seven methyl carbons (Table 1). Accordingly, a ring was required for **1** to fulfill the unsaturation requirement.

The presence of characteristic signals for two amino carbons ($\delta_{C/H}$ 54.9/2.92, 4.96; 49.9/4.60), one *N*-H group (δ_H 6.89), one *N*-methyl group ($\delta_{C/H}$ 36.5/3.16), and two carbonyl/amide carbonyl carbons (169.8, 170.0) in the NMR spectra of **1** was indicative of a peptide. The $^1H-^1H$ COSY spectrum of metabolite **1** revealed a spin-coupling system of the H_3-14, H-2, and *N*-H proton, and the HMBC correlation of H_3-14 (δ_H

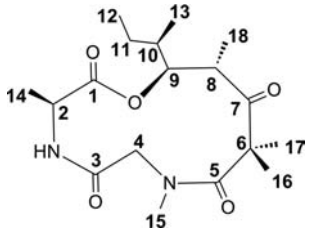


Figure 1. Structure of dahurelmusin A (**1**).

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Table 1. ^{1}H and ^{13}C NMR Data of 1[a]

	1	
	δ_H mult (J in Hz)	δ_C
1		169.5
2	4.60 m	49.9
3		169.8
4	2.92 d (13.2)	54.9
	4.96 d (13.2)	
5		170.0
6		58.3
7		210.0
8	3.67 m	42.1
9	5.19 d (9.2)	77.8
10	1.64 m	36.6
11	1.06 m	26.9
	1.41 m	
12	0.94 t (7.2)	12.7
13	0.91 d (6.8)	30.6
14	1.32 d (7.2)	21.2
15	3.16 s	36.5
16	1.62 s	22.8
17	1.76 s	25.8
18	1.02 d (7.2)	15.5
NH	6.89 d (10.0)	

[a] ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz), TMS, measured in $CDCl_3$.

1.32) with C-1 led to the construction of the alanine. Furthermore, the HMBC correlations of H_2-4 (δ_H 2.92, 4.96) with C-3 and *N*-methyl (δ_H 3.16) with C-4 and C-5 suggested that metabolite **1** also contained an *N*-methylglycine fragment. In addition, the $^{1}H-^{1}H$ COSY spectrum of metabolite **1** revealed a spin-coupling system of H_3-12, H_2-11, H-10, H-9, H-8, and H_3-18, another spin-coupling system of H-10 and H_3-13; the HMBC correlations of H_3-16/17 (δ_H 1.62, 1.76) with C-5, C-6, C-7, and H_3-18 (δ_H 1.02) with C-7 indicated that metabolite **1** had a 5-hydroxy-2,2,4,6-tetramethyl-3-oxooctanoic acid (HTMOO) residue. The amino acid units and HTMOO residue of **1** were then connected through the analysis of HMBC correlations (Figure 2). The connection between alanine and *N*-methylglycine was demonstrated by correlations from H-4 (δ_H 2.92, 4.96) with C-2. The *N*-methyl (δ_H 3.16) was correlated with C-5 (δ_C 170.0) of the HTMOO unit, and the H-9 (δ_H 5.19) of the HTMOO unit was correlated with C-1 (δ_C 169.5) in the HMBC spectrum. Therefore, the linkage of alanine and *N*-methylglycine to the HTMOO was established to satisfy the unsaturation requirement and create a 12-membered cyclic lipodepsipeptide. Accordingly, the whole planar structure of metabolite **1** was elucidated.

The stereochemistry of metabolite **1** was determined by analysis of NOESY data and Marfey's method.[6] The NOESY correlations of H-9/H_3-18, H-10/H-8, and H_3-13/H_3-14 indicated that these protons were cofacial (Figure 2). A detailed analysis of the structure of amino acid units of **1** clearly suggested that only the alanine has chirality. Thus, the stereochemistry of alanine was partly determined by acid hydrolysis, derivatization with Marfey's reagent, and then HPLC analysis. Finally, the configuration of alanine was identified as L-alanine.

A single crystal of metabolite **1** was obtained from acetone after recrystallization. The structure and relative configuration of metabolite **1** was further confirmed by single-crystal X-ray

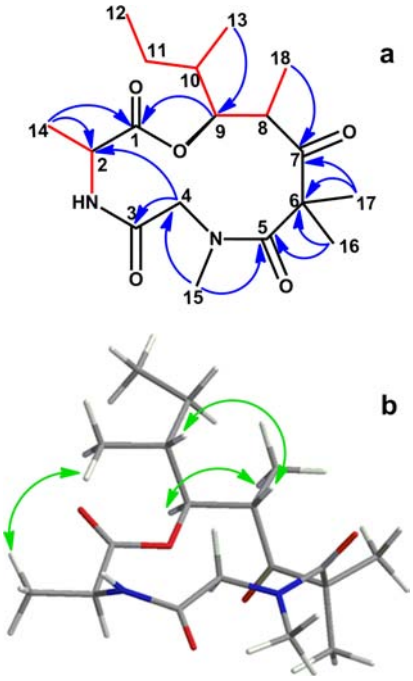

Figure 2. Key (a) $^{1}H-^{1}H$ COSY (—), HMBC (H → C), and (b) NOESY correlations of **1**.

diffraction analysis with Cu Kα radiation (Figure 3). The −0.1 value of the Flack parameter permitted the establishment of the

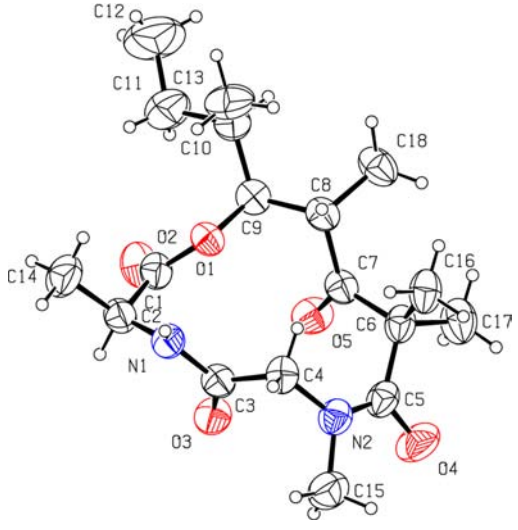

Figure 3. X-ray crystallographic structure of **1**.

absolute configuration of metabolite **1**.[7,8] Therefore, the overall stereochemistry of **1** was assigned as 3*S*,5*S*,6*S*,12*R* and named dahurelmusin A.

Comprehensive spectroscopic analysis revealed that **1** contains the peptide portion and the HTMOO unit, which were derived from nonribosomal peptide synthetase pathways and polyketide synthase origin, respectively.[9] Metabolite **1** incorporated a unique HTMOO unit; no metabolites have been reported to possess this structural feature. Additionally, this was the first time that metabolite **1**, as a 12-membered cyclic hybrid peptide−polyketide, was obtained from the extract of a grass infected by *Epichloë* spp. endophyte. Microtermolide A, a cyclic hybrid peptide−polyketide, is derived from

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microorganisms; it was isolated from the fungus *Streptomyces* sp.[10] Accordingly, dahurelmusin A (**1**) is very likely derived from *E. bromicola* living within *E. dahuricus*, and the obtained metabolite was first isolated from the *Elymus* genus.

The insecticidal activity of compound **1** was then assayed against *R. padi* because *R. padi* is one kind of phytopageous on *Elymus dahuricus*. The LC_{50} value of compound **1** against *R. padi* was 0.251 ± 0.006 mM. The result suggested that compound **1** displayed obvious insecticidal effects against *R. padi*. To further investigate the possibility of utilizing compound **1** as a broad-spectrum insecticide, we tested its insecticidal activity against *B. brassicae* with an LC_{50} value of 0.092 ± 0.007 mM.[11] The results showed that compound **1** has the potential to be exploited as a natural broad-spectrum insecticidal agent resistant to aphids. This is the first report that hybrid peptide−polyketides from *E. bromicola* infected *E. dahuricus* exhibit insecticidal activities against *R. padi* and *B. brassicae*. Therefore, compound **1** could be considered as a lead compound for the development of a new kind of industrial insecticide to suppress aphids. These results will provide a foundation for researchers to find better insecticides from the *Epichloë* spp. infected forage.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b03568.

Experimental procedures; crystallographic data of **1**; 1D and 2D NMR, UV, CD, IR, and HRESIMS of dahurelmusin A (**1**) (PDF)

X-ray crystallographic data of compound **1** (CIF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Chen, Z. H.; Wang, Z.; Yun, X. J.; Li, X. F.; Gao, H. W. *Afr. J. Biotechnol.* **2011**, *10*, 14862−14867.

(2) Leuchtmann, A.; Bacon, C. W.; Schardl, C. L.; White, J. F.; Tadych, M. *Mycologia* **2014**, *106*, 202−215.

(3) Nan, Z. B.; Li, C. J. *Proceedings of the 4th International Neotyphodium/ Grass Interactions Symposium*; University Paderborn: Paderborn, 2000; pp 41−49.

(4) Nan, Z. B.; Li, C. J. *Acta Ecol. Sin.* **2004**, *24*, 605−616.

(5) Zhang, Y. P.; Nan, Z. B. *J. Agron. Crop Sci.* **2007**, *193*, 377−386.

(6) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591−596.

(7) Flack, H. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **1983**, *39*, 876−881.

(8) Sheldrick, G. M. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **2008**, *64*, 112−122.

(9) Bishara, A.; Rudi, A.; Aknin, M.; Neumann, D.; Ben Califa, N.; Kashman, Y. *Org. Lett.* **2008**, *10*, 4307−4309.

(10) Carr, G.; Poulsen, M.; Klassen, J. L.; Hou, Y.; Wyche, T. P.; Bugni, T. S.; Currie, C. R.; Clardy, J. *Org. Lett.* **2012**, *14*, 2822−2825.

(11) Chinese Patent Appl. 201610051377.9.

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