

ORIGINAL ARTICLE

# *N*-methylphenylalanyl-dehydrobutyryne diketopiperazine, an *A*-factor mimic that restores antibiotic biosynthesis and morphogenesis in *Streptomyces globisporus* 1912-B2 and *Streptomyces griseus* 1439

Bohdan Matselyukh<sup>1</sup>, Fatemeh Mohammadipanah<sup>2</sup>, Hartmut Laatsch<sup>2</sup>, Jürgen Rohr<sup>3</sup>, Olga Efremenkova<sup>4</sup> and Volodymyr Khilya<sup>5</sup>

The cell-free extracts of a landomycin E-producing strain, *Streptomyces globisporus* 1912-2, were shown to contain a low-molecular-weight compound that, like *A*-factor, restored the landomycin E and streptomycin biosynthesis and sporulation of the defective mutants *S. globisporus* 1912-B2 and *S. griseus* 1439, respectively. The compound was purified by thin layer chromatography and HPLC. It had an absorption maximum at  $\lambda_{\max} = 245$  nm and a molecular mass of  $m/z$  244. On the basis of NMR spectroscopy (<sup>1</sup>H, <sup>13</sup>C, HSQC, HMBC, COSY and NOE) the chemical structure of the compound was elucidated as 6-benzyl-3-eth-(*Z*)-ylidene-1-methyl-piperazine-2,6-dione ((*L*)-*N*-methylphenylalanyl-dehydrobutyryne diketopiperazine (MDD)). The sequences of *arpA* genes in *S. globisporus* 1912-2 and *S. griseus* NBRC 13350 are highly conserved. An explanation for the observed biological activity of MDD was proposed.

*The Journal of Antibiotics* (2015) 68, 9–14; doi:10.1038/ja.2014.86; published online 9 July 2014

## INTRODUCTION

Communication systems (quorum sensing) among Gram-positive and Gram-negative bacteria involve diffusible signal molecules ( $\gamma$ -butyrolactones, *N*-acyl homoserine lactones, diketopiperazines (DKPs), furans and other molecules) interacting with specific cell surface-associated or intracellular receptors, thereby inducing genes that control biofilm formation, production of antibiotics and virulence factors, morphogenesis of cells and other functions.<sup>1–5</sup>

*A*-factor-dependent cascade regulation is one of the important systems of secondary metabolism and morphogenesis regulation in streptomycetes.<sup>6,7</sup> The *A*-factor belongs to the family of  $\gamma$ -butyrolactones, small signaling molecules that establish communication between the neighboring hyphae of streptomycetes in nanomolar concentrations.<sup>3,8</sup> It binds to the receptor protein *ArpA*, causing its dissociation and consequently activation of transcription of the genes of the *AdpA* regulon, which control aerial mycelium formation as well as the biosynthesis of antibiotics and pigments. The protein *AdpA* binds to 37 regions of the DNA, activating 72 genes of streptomycetes, many of whose functions

have not yet been assigned.<sup>9</sup> A variety of  $\gamma$ -butyrolactones from streptomycetes with different chemical structures is known. Each receptor protein in various *Streptomyces* species is highly specific for its cognate  $\gamma$ -butyrolactone.<sup>6,10</sup>

*Streptomyces globisporus* 1912 produced the red-orange antitumor antibiotic landomycin E, a member of the angucycline family. Landomycin E induces apoptosis in cancer cells, including those resistant to doxorubicin.<sup>11</sup> The biosynthesis of landomycin E is encoded by the *Ind*-cluster, which consists of 27 structural and 3 regulatory genes.<sup>12</sup> The wild-type strain *S. globisporus* 1912 and many of its mutants, including mutant 1912-2, produce compounds, with  $R_f$  0.4 and 0.8, respectively, which were found to be able to restore the biosynthesis of landomycin E and sporulation of the antibiotically inactive mutant 1912-B2.<sup>13</sup> A preliminary description of the compound with  $R_f$  0.4 and its relation to the compound with  $R_f$  0.8 was published earlier.<sup>14</sup> The subject of the present paper was the structure elucidation of the compound with  $R_f$  0.4 as a new DKP, and also description of its biochemical and biological properties.

<sup>1</sup>Department of Genetics of Microorganisms, D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, Kyiv, Ukraine; <sup>2</sup>Department of Chemistry, Institute for Organic and Biomolecular Chemistry, University of Göttingen, Göttingen, Germany; <sup>3</sup>Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, USA; <sup>4</sup>Sector of Screening of Natural Compounds, G.F. Gause Institute of New Antibiotics, Russian Academy of Medical Sciences, Moscow, Russia and <sup>5</sup>Department of Organic Chemistry, Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

Correspondence: Professor B Matselyukh, Department of Genetics of Microorganisms, D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, Zabolotnoho Street 154, Kyiv D 03680, Ukraine.

E-mail: bohdan.matselyukh@gmail.com

Received 22 February 2014; revised 13 May 2014; accepted 22 May 2014; published online 9 July 2014

## MATERIALS AND METHODS

### Strains and media

The objects of the investigation were the sporulating strains *S. globisporus* 1912-2 and *S. griseus* 773, the producers of landomycin E and streptomycin, respectively, and their antibiotically inactive mutants *S. globisporus* 1912-B2 and *S. griseus* 1439, defective in biosynthesis of the (L)-N-methylphenylalanyl-dehydrobutyryne diketopiperazine (MDD) and the A-factor, respectively.<sup>13</sup> The A-factor-deficient strain 1439 was obtained by nitrosomethylbiuret treatment of *S. griseus* 32-12.<sup>15</sup> The streptomycin-sensitive and -resistant strains of *S. levoris* 165 were used for the detection of streptomycin production. The streptomycetes were grown on corn-soy medium consisting of corn meal 20 g, soy meal 10 g, NaCl 5 g, agar 10 g in 1 l distilled water, pH 7, with sterilization at 1 bar overpressure for 30 min. Cultures of the strains 773 and 1912-2 produced the A-factor and MDD during 5–6 days of growth on minimal solid medium at 28 °C, containing asparagine 1 g, glycine 1 g, K<sub>2</sub>HPO<sub>4</sub> 0.5 g, MgSO<sub>4</sub> 0.2 g, NaCl 4 g, glycerol 15 g, agar 10 g, trace elements (FeSO<sub>4</sub>, CuCl<sub>2</sub>, MnSO<sub>4</sub>, CaCl<sub>2</sub>, 10.0 mg each) in 1.0 l distilled water, pH 7.2, with sterilization at 0.75 bar overpressure for 30 min. The same solid medium in ten Petri dishes was used for examination of production of the MDD and A-factor by the 1912-B2 and 1439 strains, respectively, after adding the corresponding cross-regulators (1 µg of the MDD or 100 ng of the A-factor in 20 ml of the medium) and cultures growing for 6 days.

### Purification of the A-factor and MDD

The minimal solid medium of the grown cultures was cut into cubic pieces and extracted with chloroform-acetone (2:1) to obtain the A-factor ((2S)-isocaprolyl-(3R)-oxymethyl-γ-butyrolactone) from strain 773 and the so far unknown inducer MDD of landomycin E biosynthesis, respectively, from strain 1912-2.

The crude extracts were evaporated to dryness using a rotary evaporator and the residues were dissolved in ethanol. The regulators were separated by thin layer chromatography on silica gel 60 F254 (Merck, Darmstadt, Germany) sheets with benzene-ethyl acetate-acetone-ethanol (4:2:1:0.5). The UV-absorbing zones were triturated with ethanol, and the silica gel was removed by centrifugation at 10 000 r.p.m. The compound MDD was further purified by HPLC using an HPLC/MS (Agilent Technologies, Waldbronn, Germany) system with UV detector and a Zorbax Hypersyl ODS reversed phase column. This way, 0.5 mg of A-factor and 5.0 mg of the MDD were obtained from 5.0 l of the corresponding agar culture. The output of A-factor corresponds to similar results obtained earlier by other authors.<sup>16</sup> For comparison, the A-factor was also chemically synthesized in the Institute of Bioorganic Chemistry of the Russian Academy of Sciences. Concentrations of the A-factor and of MDD were determined by HPLC.

### Biological activity

The biological activity of the purified A-factor and of MDD was examined using test strains 1439 and 1912-B2. Strain 1439 was inoculated on Petri dishes with solid corn-soy or minimal medium as a central stripe, and small paper discs with the A-factor (100.0 ng) and MDD (1.0 µg), respectively, were put near the ends of the last. Strain 1912-B2 was inoculated evenly on a solid medium using a glass spatula, and paper discs with the same concentration of the A-factor and MDD, respectively, were placed on the surface. The results of the action of both factors on antibiotic biosynthesis and morphogenesis were registered on the second and fifth day of incubation of the strains 1912-B2 and 1439, respectively. The purified MDD and the chemically synthesized A-factor were also added to fresh lawns of 1439 and 1912-B2 solid cultures in 10 Petri dishes (1.0 µg per 20 ml and 100.0 ng per 20 ml, respectively). After 6 days of incubation at 28 °C the agar cultures were extracted using a mixture of chloroform and acetone (2:1), and the resulting extracts were analyzed by TLC. Identification of MDD and the A-factor from spots detected under UV light on TLC aluminum sheets was performed by spectrophotometry and HPLC.

**Absorption and NMR spectra.** NMR spectra of MDD were measured at 125.707 MHz (<sup>13</sup>C NMR) and 300.141 MHz (<sup>1</sup>H NMR) on Varian INOVA 500 and Mercury 300 spectrometers, respectively. The absorption spectra of the ethanol solutions of the A-factor and MDD were recorded on a Beckman

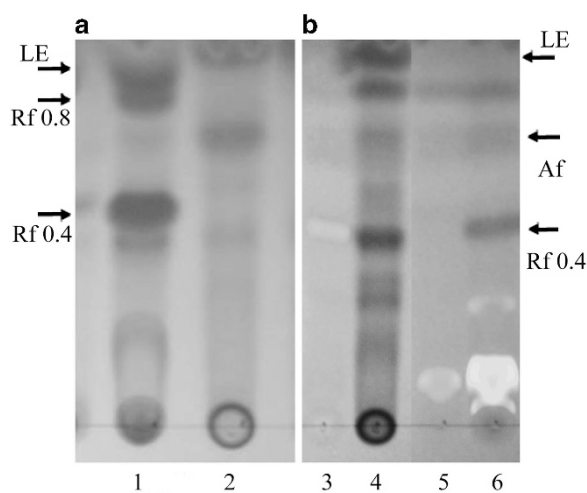
DU-8 spectrophotometer. LC/MS was performed using a liquid chromatograph, Agilent Technologies 1200, with a single quadrupole detector, model G19, and a G1978A ion source in ESI mode was used. The separations were performed on a Zorbax Hypersyl ODS column (12.0 × 125.0 mm<sup>2</sup>, 3 µm) with hexane:ethanol (98:2) at a flow rate of 0.17 ml min<sup>-1</sup>, and with dual detection at 210–400 nm. The chromatograms were analyzed with the Chemstation software.

**Configuration.** To determine the absolute configuration of the NMePhe unit, 0.4 mg of MDD was hydrolyzed by heating in 0.2 ml 6 N HCl for 24 h at 110 °C. After cooling, the solution was evaporated to dryness and the residue re-dissolved in H<sub>2</sub>O (50 µl). To the resulting hydrolysate solution or to a solution of the reference amino acids (L-NMePhe and D-NMePhe, respectively, 50 µl; 50 mM each), a solution of FDAA (Marfey's reagent, N-(2,4-dinitro-5-fluorophenyl)-L-alanineamide, 100 µl of 1% (w/v) solution in acetone) was added. After addition of NaHCO<sub>3</sub> solution (20 µl; 1 M), the mixture was incubated for 1 h at 40 °C. The reaction was stopped by addition of HCl (10 µl; 2 M), the solution was evaporated to dryness, and the residue was re-dissolved in acetonitrile (1 ml). An aliquot of this solution (20 µl) was analyzed by HPLC (Phenomenex Luna C18 (National Analytical Corporation, Maharashtra, India), 250 4.6 mm, 5 µm; solvents: A, H<sub>2</sub>O + 0.05% HCOOH; B, MeOH + 0.05% HCOOH; linear gradient from 10% B in A at t = 0 min to 90% B in A within 20 min; 25 °C; 300 µl min<sup>-1</sup>). The configuration of NMePhe was determined by comparing the chromatograms with derivatives of commercially available D- and L-N-methyl phenylalanine. The FDAA derivative obtained from the regulator MDD had a retention time of 15.9 min and thus indicated L-N-methyl phenylalanine as its chiral component, in comparison with the derivatives of authentic L-NMePhe (15.9 min), and D-NMePhe (11.6 min).

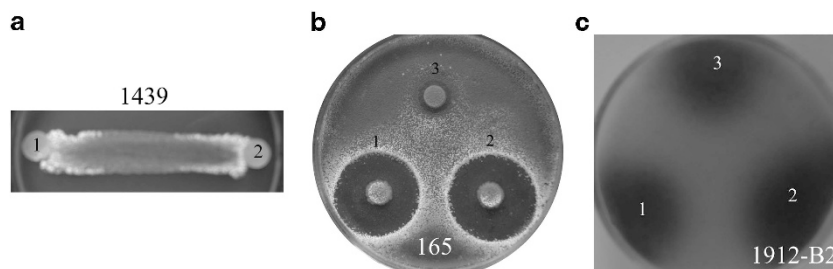
## RESULTS

### Biological activity of the compound with R<sub>f</sub> 0.4 compared to the A-factor

Two UV-absorbing compounds were produced by donor strain 1912-2 (Figure 1a, 1, arrows), which were missing in the mutant recipient strain 1912-B2 (Figure 1a, 2). A labile compound with R<sub>f</sub> 0.8



**Figure 1** Thin layer chromatogram in UV light of cell-free extracts of the agar cultures: (a) *S. globisporus* 1912-2 (1) and its inactive mutant 1912-B2 (2), grown on minimal medium; the arrows show localization of landomycin E and new compounds with R<sub>f</sub> 0.8 and R<sub>f</sub> 0.4 ((L)-N-methylphenylalanyl-dehydrobutyryne diketopiperazine (MDD)), respectively. (Reprinted with permission from Matselyukh et al.<sup>14</sup> ©2012 National Academy of Sciences of Ukraine). (b) Mutants 1912-B2 (3, 4) and *S. griseus* 1439 (5, 6) grown on minimal medium after addition of A-factor (4) and purified MDD (6); the arrows show localization of landomycin E, A-factor and MDD.



**Figure 2** Biological activity of (L)-*N*-methylphenylalanyl-dehydrobutyryne diketopiperazine (MDD): (a) restoration of sporulation of *S. griseus* 1439 by A-factor (1) and MDD (2); (b) restoration of streptomycin biosynthesis in *S. griseus* 1439 by A-factor (1) and MDD (2). Agar blocks cut out from sporulating (1, 2) and non-sporulating (3) areas of an agar culture of strain 1439 (a) were put on the lawn of a streptomycin-sensitive culture *S. levoris* 165. Zones of growth inhibition were generated by streptomycin. (c) Restoration of landomycin E biosynthesis in *S. globisporus* 1912-B2 by A-factor (1), MDD (3) and a compound with  $R_f$  0.8 (2). (Reprinted with permission from Matselyukh *et al.*<sup>14</sup> ©2012 National Academy of Sciences of Ukraine).

disappeared after 6 days of culture growth and transformed into a stable compound with  $R_f$  0.4. We then found that the TLC-purified compound with  $R_f$  0.4, consequently called MDD, restored production of landomycin E and sporulation in a 1912-B2 mutant and displayed a biological activity similar to that of the A-factor: MDD restored sporulation (Figure 2a, 1) as well as streptomycin (Figure 2b, 1) and A-factor production (Figure 1b, 6) in *S. griseus* 1439. For comparison, we also tested an extracted and TLC-purified A-factor from a sporulating and streptomycin-producing culture of *S. griseus* 773, as well as a chemically synthesized A-factor: Both restored sporulation as well as MDD (Figure 1b, 4) and landomycin E (Figure 2c) biosynthesis in *S. globisporus* 1912-B2. However, the concentration of MDD needs to be one order of magnitude higher than the A-factor concentration to reach the above-described biological effects.

### Structure elucidation of the new DKP

HPLC and LC/ESIMS of the purified MDD gave a clear molecular ion signal at  $m/z$  245 (M + H)<sup>+</sup> (Figure 3). (+)-ESI-HRMS afforded the molecular formula of C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub> (found 267.11036, calcd. 267.11041 for C<sub>14</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub>Na). In the <sup>13</sup>C and <sup>1</sup>H NMR spectra (Table 1 and Figure 4), respectively, two acid/amide carbonyls were seen, one OH/NH signal at low field, diastereotopic methylene protons of an ABX system, and a conspicuous CH-CH<sub>3</sub> signal with the methine signal at  $\delta_{CH}$  111.6. A search in AntiBase<sup>17</sup> delivered a C=CH-CH<sub>3</sub> fragment as the only plausible substructure. In the HMBC spectrum, the latter CH signal gave a cross peak with the carbonyl at  $\delta$  158.7, resulting in an  $\alpha,\beta$ -unsaturated acid/amide (see also Figure 4). A second spin system consisted of a monosubstituted benzene unit, connected with a CH<sub>2</sub>-CH-CO fragment. The methine proton in this fragment coupled with both carbonyls. A search in AntiBase with these substructures and 13 or 14 carbon atoms resulted in the DKPs *cyclo*(Phe, dehydroAbu) and *cyclo*(NMe-Tyr, dehydroAbu) as the only results. It was clear therefore that the compound is derived from *cyclo*(Phe, dhydroAbu), where one of the nitrogen atoms is methylated. As the NMe signal and the olefinic methine H-3' coupled with the same carbonyl C1', both must be neighbors of the latter. Correspondingly, the NMe singlet gave an HMBC signal with C-2. This resulted in *cyclo*(NMePhe, dhAbu) (dhAbu = dehydroaminobutyric acid), which was confirmed by all further correlations (Figure 5). The olefinic methyl gave an NOE signal with the NH singlet and the double bond was therefore believed to have (*Z*) configuration, which resulted in the structure shown in Figure 5. To determine the absolute configuration of the NMePhe unit, a sample of compound was hydrolyzed and derivatized with

FDAA (Marfey's reagent, *N*-(2,4-dinitro-5-fluorophenyl)-L-alaninamide). The configuration of NMePhe was determined by comparing the chromatograms with derivatives of commercially available D- and L-*N*-methyl phenylalanine. The FDAA derivative obtained from the compound had a retention time of 15.9 min and thus indicated L-*N*-methyl phenylalanine, by comparison with the derivatives of authentic L-NMePhe (15.9 min) and D-N-MePhe (11.6 min). Thereby, the molecular structure of the new compound of antibiotic biosynthesis and morphogenesis in *Streptomyces* has been elucidated as L-6-benzyl-3-eth-(*Z*)-ylidene-1-methyl-piperazine-2,5-dione (MDD). This compound had been obtained by synthesis previously; however, details on the structure confirmation or the NMR data were not available.<sup>18</sup>

### Receptor protein ArpA

A-factor is known to bind to the regulatory domain of the receptor protein ArpA *S. griseus* NBRC 13350.<sup>3</sup> Production of ArpA-like protein by *S. globisporus* 1912-2 was not studied. High-molecular-weight pure genomic DNA of 1912-2 strain ( $A_{260}/A_{280} = 2.1$ ) was prepared according to the Kirby procedure.<sup>19</sup> Illumina Paired End sequencing and *de novo* genome assembly was carried out in BaseClear B.V. (Leiden, Netherlands). The genome of 1912-2 consists of 7.124511,0 nucleotides and more than 1000 contigs. The following sequence of an *arpA* gene was identified with BLAST (National Center of Biotechnological Information, Bethesda, MD, USA; <http://www.ncbi.nlm.nih.gov/blast>):

```
5'-ATGGCGAAGCAGGCTCGCGGGTCCAGACGTGGCGGTCG
ATCGTGGATGCCGCGGCGAGCGTCTTCGACACTACGGCTACG
AGCGTGCCGCCATCTCGGAGATTCTCGCCGCGCCAAGGTC
ACCAAGGGGGCCCTGTACTTCCACTTCGCCCTCCAAGGAGGCCA
TCGCGCAGGGGATCATGGACGAGCAGACCTCCACGGTTCGAGT
TCGAGCAGGAGGGGTCGCCGCTCCAGTCCCTGGTGGACGGG
GGCCAGCAGTTCGCTTTCGCCCTGCGCCACAACGCGATGGCCC
GGGCCGACCAGGCTCTCCATCGAGGGCGTCTTCTCCGCGC
GGACCGCATCCGTGGGGCGACTGGATCGACGCGACGGCCCGG
ATGCTGGAGCTGGGGCAGGAGCGCGGGCAGGTGTTCCCGCAG
ATTGACCCGATGGTGTTCGGCCAAAATCATCGTTGCCTCGTTTAC
CGGTATCCAGCTGGTCTCGGAGGCCGACACCGGGCGGGCCGA
TCTGCGCGAGCAGGTTCGCGGAGATGTGGCGCCACATCTGC
CGTGCATCGCTCACCCCGGTGTCATGCCCCACATCAAGCCCGA
GGGCCGGTTCGATCTGGCGGCCAGGCGCGTGAGAAGGCGGA
GCGGGAGGAGCAGGAGGCGAGGGTTGCCGCGGAGGCCAAGG
GGGCCGGCTCCGATCCCGCGACGGACACGGGCTCCAGGGCCG
GCGGGGCGGGCTTCGGGGCGGTGGATCCCTCGCGGTGCGC
GAGCCGGGCAGGCCGACGACGAGGGTGACGAGGAGCCTGCG
GGCGCGGGGTCGCGGC-3'.
```

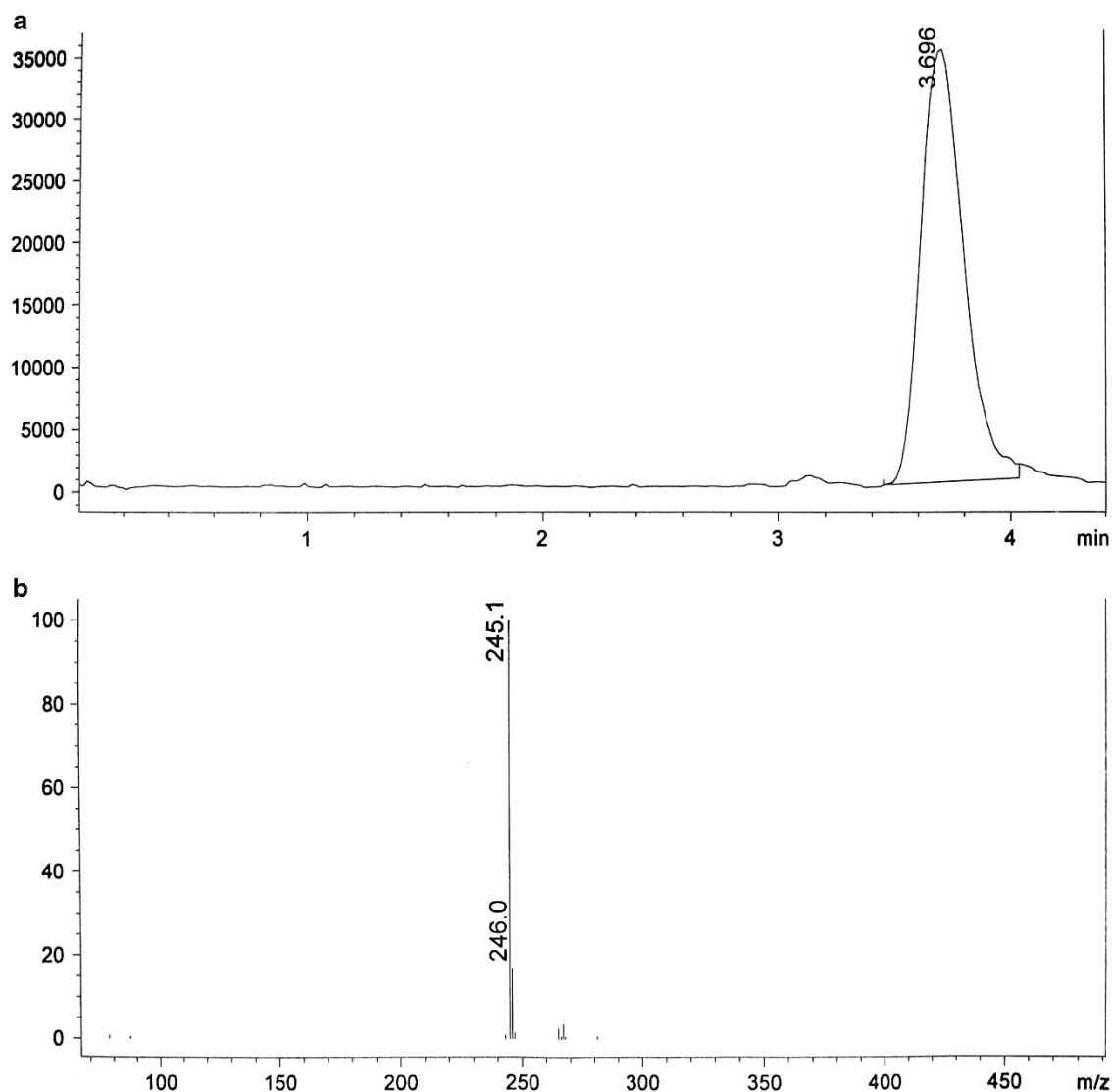


Figure 3 HPLC (a) and HPLC/MS (b) of (L)-N-methylphenylalanyl-dehydrobutyryne diketopiperazine from strain 1912-2.

Table 1 Results of NMR assignments ( $^1\text{H}$ ,  $^{13}\text{C}$ , HSQC, HMBC, COSY, NOE)

	$^{13}\text{C}$ (125 MHz)	Atom type	$^1\text{H}$ (300 MHz)	HMBC	COSY	NOESY
1	165.4	C <sub>q</sub>	-			
1'	158.7	C <sub>q</sub>	-			
4	134.8	C <sub>q</sub>	-			
5, 9	129.6	2 CH	7.02 (m)	7	6/8	
6, 8	128.0	2 CH	7.20 (m)	4	5/9	
2'	127.2	C <sub>q</sub>	-			
7	126.8	CH	7.20 (m)			
3'	111.6	CH	5.38 (q, J)		1'	4'
2	62.7	CH	4.36 (m)	1, 1', 3, 4	3	
3	36.7	CH <sub>2</sub>	3.15, 3.01, ABX, J <sub>AB</sub> , J <sub>AX</sub> , J <sub>BX</sub>	1, 2, 4, 5/9	2	
NMe	32.2	CH <sub>3</sub>	2.97 s	1', 2		-
4'	10.6	CH <sub>3</sub> NH	1.41 d, J 9.74 s br	2', 3'		3', NH

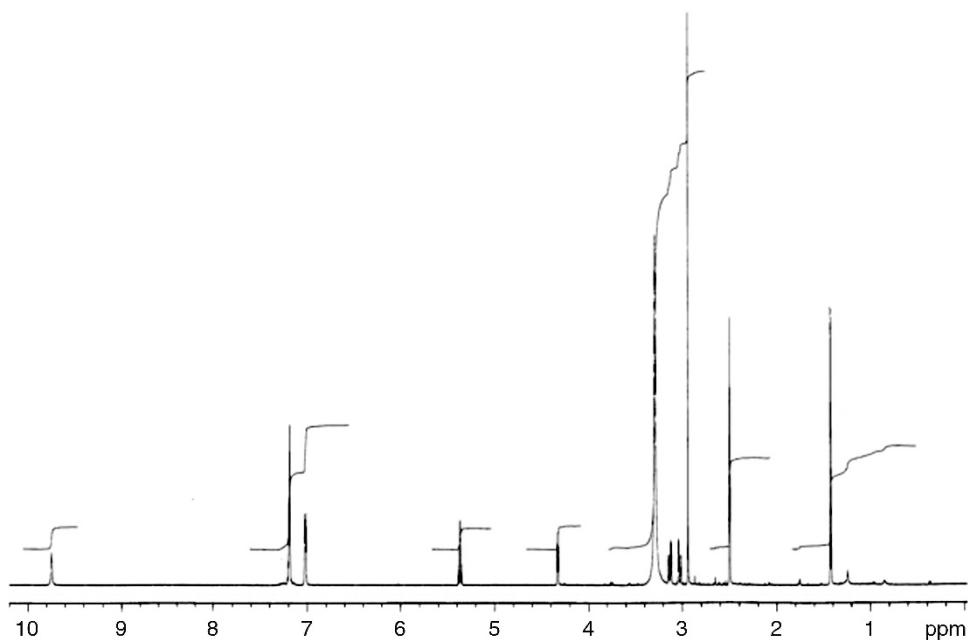
The sequence of the *arpa* gene was found in contig 777 (length = 2545), containing 831 nucleotides, 92% of which have identity with the sequence of the SGR\_3731 gene encoding the ArpA protein in *S. griseus* NBRC 13350. The high identity between the *arpa* of 1912-2 and SGR\_3731 genes explains the biological activity found for the A-factor in the mutant strain *S. globisporus* 1912-B2. Strain 1912-2 does not produce the A-factor. It may contain a mutation in the *afsA* gene encoding A-factor synthase. Sequencing data gave evidence for such a possibility, because *afsA* of *S. griseus* NBRC 13350 and *afsA* of *S. globisporus* 1912-2 are only 83% identical, significantly less than the corresponding *arpa* genes (92% identity). There are eight gaps among 945 nucleotides of *afsA* genes and six of them are present in a short 20-nucleotide sequence, which may indicate the localization of mutational changes in this region of the *afsA* 1912-2 gene:

```

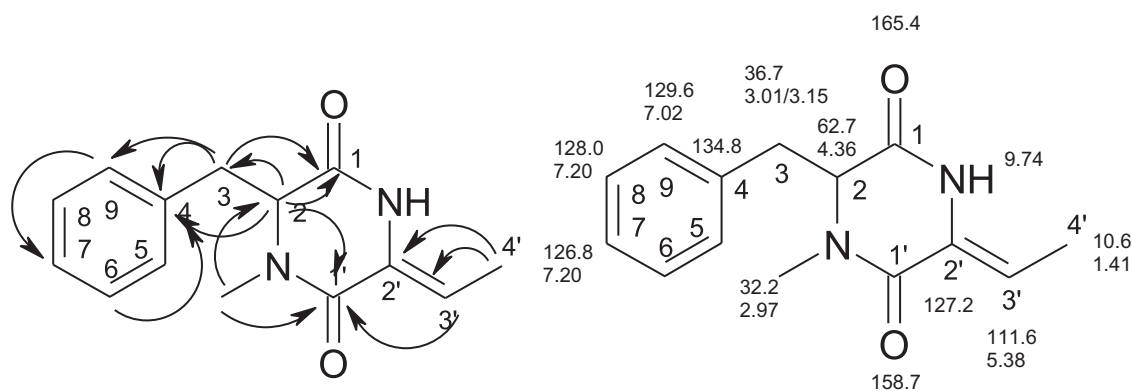
570 -CCG- -GCCTCGCCGGCGGG 587 afsA S. griseus NBRC 13350
      |||  |||  |||  |||  |||  |||
      GCCGCTGCC-CGC-GCCGG - afsA S. globisporus 1912-2
  
```

## DISCUSSION

The molecular structure of a new compound that is able to restore the antibiotic biosynthesis and morphogenesis in *S. globisporus* 1912-B2



**Figure 4**  $^1\text{H}$  NMR spectrum of (L)-*N*-methylphenylalanyl-dehydrobutyryne diketopiperazine from strain 1912-2.



**Figure 5** HMBC correlations observed for L-6-benzyl-3-eth-(*Z*)-ylidene-1-methyl-piperazine-2,5-dione [(L)-*N*-methylphenylalanyl-dehydrobutyryne diketopiperazine].

and *S. griseus* 1439 has been elucidated as the new DKP MDD. This DKP has not previously been described, although DKPs with similar structures were found as products of streptomycetes and other bacterial species. For instance, L-Phe subunits of other DKPs were paired with L-Pro,<sup>20–22</sup> L-Ser<sup>23</sup> or L-Leu.<sup>24</sup> Dehydrobutyryne was found previously only in a pair with L-Trp.<sup>25</sup> DKPs are widely produced by microorganisms and exhibit a variety of biological activities, including antibacterial, antifungal, antiviral, anti-prion, antitumor and immunosuppressive.<sup>26</sup> They also participate in bacterial communication systems as imitators of signalling molecule actions.

The most probable explanation of the biological activity of MDD may be its imitation of the A-factor action. According to the literature data, DKPs may mimic the action of *N*-acyl homoserine lactones by interaction with LuxR regulatory proteins and the resulting complex can bind to *lux*-boxes, specific gene promoter sequencing that affect the expression of quorum sensing target

genes.<sup>1,4,20</sup> ArpA and LuxR proteins belong to the TetR protein superfamily. MDD may interact with the regulatory domain of the receptor protein ArpA in *S. griseus* 1439 and *S. globisporus* 1912-B2, imitating A-factor action. However, the concentration of MDD must be ~10 times higher than that of the A-factor to achieve the same level of landomycin E and streptomycin production in the corresponding cultures. A possible reason for this lower activity of MDD may be its lower specificity in comparison with A-factor in interaction with receptor protein ArpA.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

#### ACKNOWLEDGEMENTS

This work was supported by the National Academy of Sciences of the Ukraine (0111U000606).



- 1 Degrassi, G. *et al.* Plant growth-promoting *Pseudomonas putida* WCS358 produces and secretes four cyclic dipeptides: cross-talk with quorum sensing bacterial sensors. *Curr. Microbiol.* **45**, 250–254 (2002).
- 2 Dunn, A. K. & Handelsman, J. Toward an understanding of microbial communities through analysis of communication networks. *Antonie van Leeuwenhoek* **81**, 565–574 (2002).
- 3 Takano, E.  $\gamma$ -Butyrolactones: *Streptomyces* signalling molecules regulating antibiotic production and differentiation. *Curr. Opin. Microbiol.* **9**, 287–294 (2006).
- 4 Ryan, R. P. & Dow, M. Diffusible signals and interspecies communication in bacteria. *Microbiology* **154**, 1845–1858 (2008).
- 5 Sidda, J. & Corre, C. Gamma-butyrolactone and furan signaling systems in *Streptomyces*. *Methods Enzymol.* **517**, 71–87 (2012).
- 6 Ohnishi, Y., Yamazaki, H., Kato, J.-y., Tomono, A. & Horinouchi, S. AdpA, a central transcriptional regulator in the A-factor regulatory cascade that leads to morphological development and secondary metabolism in *Streptomyces griseus*. *Biosci. Biotechnol. Biochem.* **69**, 431–439 (2005).
- 7 Horinouchi, S. Mining and polishing of the trove in the bacterial genus *Streptomyces*. *Biosci. Biotechnol. Biochem.* **71**, 283–299 (2007).
- 8 Kleiner, E. M. *et al.* The structure of A-factor, a bioregulator from *Streptomyces griseus*. *Bioorg. Khim.* **2**, 1142–1147 (1976).
- 9 Hara, H., Ohnishi, Y. & Horinouchi, S. DNA microarray analysis of global gene regulation by A-factor in *Streptomyces griseus*. *Microbiology* **155**, 2107–2210 (2009).
- 10 Anisova, L. N. *et al.* Regulators of the development in *Streptomyces coelicolor* A3(2). *Izv. Akad. Nauk. SSSR Biol.* **1**, 98–107 (1984).
- 11 Korynevska, A. *et al.* Mechanisms underlying the anticancer activities of the angucycline landomycin E. *Biochem. Pharmacol.* **74**, 1713–1725 (2007).
- 12 Ostash, B. *et al.* Identification and characterization of the *Streptomyces globisporus* 1912 regulatory gene *indYR* that affects sporulation and antibiotic production. *Microbiology* **157**, 1240–1249 (2011).
- 13 Matselyukh, B. P., Tymoshenko, S. G., Bambura, O. I. & Kopejko, O. P. Screening and characterization of regulators of landomycin E biosynthesis. *Mikrobiol. Zh.* **73**, 16–20 (2011).
- 14 Matselyukh, B. P., Mohammadipanah, F., Laatsch, H., Rohr, J. & Efremenkova, O. Purification and structure elucidation of the by-product of new regulator of antibiotic production and differentiation of *Streptomyces*. *Mikrobiol. Z.* **74**, 66–73 (2012).
- 15 Borisova, L. N., Ivkina, N. S. & Rapoport, I. A. Chromatographic study of the mutants of *Actinomyces streptomycini* induced by nitrosomethylbiuret. *Dokl. Akad. Nauk. SSSR* **171**, 728–732 (1996).
- 16 Pliner, S. A. *et al.* Isolation and primary characteristics of A-factor. *Bioorg. Chim.* **1**, 70–76 (1975).
- 17 Laatsch, H. *AntiBase, A Database for Rapid Dereplication and Determination of Microbial Natural Products* (Wiley-VCH, Weinheim, Germany. See <http://www-ser.gwdg.de/hlaatsch/>, 2013)
- 18 Li, W.-R. & Peng, S.-Z. Rational design and synthesis of unsaturated 2,5-dioxopiperazine derivatives as potential protein tyrosine kinase inhibitors. *Tetrahedron Lett.* **39**, 7373–7376 (1998).
- 19 Kieser, T., Bibb, M., Buttner, M., Chater, K. & Hopwood, D. *Practical Streptomyces Genetics* (The John Innes Foundation, Norwich, 2000).
- 20 Holden, M. T. G. *et al.* Quorum sensing cross talk: isolation and chemical characterization of cyclic dipeptides from *Pseudomonas aeruginosa* and other Gram-negative bacteria. *Mol. Microbiol.* **33**, 1254–1266 (1999).
- 21 Gu, Q., Fu, L., Wang, Y. & Lin, J. Identification and characterization of extracellular cyclic dipeptides as quorum-sensing signal molecules from *Shewanella baltica*, the specific spoilage organism of *Pseudosciaena crocea* during 4 °C storage. *J. Agric. Food Chem.* **61**, 11645–11652 (2013).
- 22 Hamza, A. A., Ali, H. A., Clark, B. R., Murphy, C. D. & Elobied, E. A. Cyclo (L-phenylalanyl, L-prolyl). Diketopiperazines from a newly isolated *Streptomyces sudanensis*. A4.4.E3. *J. Biotechnol. Pharm. Res.* **4**, 1–7 (2013).
- 23 Cho, J. Y. *et al.* Isolation and structural determination of the antifouling diketopiperazines from marine-derived *Streptomyces praecox* 291-11. *Biosci. Biotechnol. Biochem.* **76**, 1116–1121 (2012).
- 24 Lautru, S., Gondry, M., Genet, R. & Pernodet, J. L. The albonoursin gene cluster of *S. noursei* biosynthesis of diketopiperazine metabolites independent of nonribosomal peptide synthetases. *Chem. Biol.* **9**, 1355–1364 (2002).
- 25 Avendano, C., Cabezas, N., de la Cuesta, E. & Gonzalez, J. F. Reinvestigation of tryptophan-dehydrobutyryne diketopiperazine structure. *ARKIVOC* **ix**, 30–38 (2005).
- 26 Martins, M. & Carvalho, I. Diketopiperazines: biological activity and synthesis. *Tetrahedron* **63**, 9923–9932 (2007).