УДК: 577. 181. 4: 579. 87

B. Matselyukh[1]*, F. Mohammadipanah[2], H. Laatsch[2], J. Rohr[3], O. Efremenkova[4], V. Khilya[5]

[1]Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, 154 Acad. Zabolotny St., Kyiv, D 03680, Ukraine
[2]Institute for Organic and Biomolecular Chemistry, University of Goettingen, 2 Tammannstrasse, Goettingen, D-37077, Germany
[3]College of Pharmacy, University of Kentucky, 789 S.Limestone St, Lexington, KY 40536-0596, USA
[4]G.F. Gause Institute of New Antibiotics, Russian Academy of Medical Sciences, 11 Bol`shaya Pirogovskaya St., Moscow, 11902, Russia
[5]Taras Shevchenko Kyiv National University, 64 Volodymyrs`ka St., Kyiv, 01601, Ukraine

PURIFICATION AND STRUCTURE ELUCIDATION OF THE BY-PRODUCT OF NEW REGULATOR OF ANTIBIOTIC PRODUCTION AND DIFFERENTIATION OF *STREPTOMYCES*

Streptomyces globisporus 1912, a producer of the antitumor antibiotic landomycin E, forms the new low-molecular signaling molecule N-methylphenylalanyl-dehydrobutyrine diketopiperazine (BDD) and its complex and unstable by-product which restore, like the A-factor in Streptomyces griseus 773, landomycin E and streptomycin biosynthesis, and sporulation of the defective mutants S. globisporus 1912-B2 and S. griseus 1439, respectively. Here, we report the purification and structure elucidation of two compounds with R_f 0.8 by HPLC, LC/MS and ^{1}HMR analysis. These compounds have m/z 338 and 384, accordingly, and each of them is presented by two stereoisomers containing BDD in their structure. A hypothesis explaining the composition and regulatory properties of these unstable compounds is presented.

K e y w o r d s: Streptomyces, new diketopiperazine, signaling molecules, antibiotic biosynthesis, morphogenesis

**Corresponding author*

Soil mycelial bacteria of genus *Streptomyces* produced more than 70 % of known antibiotics widely used in medicine, veterinary and agriculture, and therefore streptomycetes belong to the important group of industrial microorganisms [24]. The genomes of streptomycetes contain many regulatory genes in comparison with simpler procaryotic bacteria coding 965 regulatory proteins [2]. The regulatory proteins belong to the families SARP and LAL, bld- andA-factor-depending regulatory cascades, and two-component systems of signals transfer AfsK-AfsR, AbsA1-AbsA2, AfsQ1-AfsQ2, DraR-K, and CutRS. Regulation of antibiotic production and complex multicellular development is of the cascade character including the basic and global levels [3, 4, 18, 22].

The biosynthesis of antibiotics is under control of small extracellular signaling molecules (bacterial hormones) presented by γ-butyrolactones (A-factor, SCB1, SCB2, SCB3, virginia butanolides), γ-butenolides (avenolide, SRB1, SRB2) and others [1, 5, 6, 10, 11, 22, 23]. The A-factor binds to the receptor protein ArpA, repressor of *adpA* gene, causing its dissociation and loss of bond with *adpA* promoter. AdpA then activates a number of genes of AdpA regulon controlling sporulation and biosynthesis of the secondary metabolites. The protein AdpA binds to 37 regions of DNA, activating 72 genes of streptomycetes [7].

Streptomyces globisporus 1912 is a producer of red-orange antitumor antibiotic landomycin E from the angucycline family. The molecular mechanism of landomycin E activity is based itself on the induction of apoptosis in cancer cells [12]. Very important property of antitumor activity of landomycin E is overcoming of the resistance of cancer cells to doxorubicin and the blockade of cells in G1 phase of cell cycle by the mediation of the reactive oxygen [20]. The biosynthesis of landomycin E is controlled by 32 genes of *lnd*-cluster. Only three regulatory genes are known at present to control landomyin E biosynthesis. Gene *lndI* from SARP family encodes regulatory protein LndI, gene *prx* specifies putative proteinase Prx, and gene *lndYR* determines a GntR-like regulator of the YtrA subfamily, controlling the transcription of transport system genes [19].

Wild type strain *Streptomyces globisporus* 1912 and many its derivatives produced low-molecular signaling molecule, restoring biosynthesis of landomycin E and sporulation by antibiotic inactive and asporogenic mutant 1912-B2 in nanomolar concentration [17]. The molecular structure of this new regulator with R_f 0.4 was elucidated as N-methulphenylalanyl-dehydrobutyrine diketopiperazine (BDD) [16]. The paper object is investigation of the structure of a complex compound with R_f 8.0

possessing regulatory property like above mentioned regulator and synthesizing by active culture 1912-2 during first 3 days of growth as BDD by-product.

Materials and Methods. *Strains and media.* The object of investigation were the sporulating strains *S. globisporus* 1912 and *S. griseus* 773, the producers of landomycin E and streptomycin, respectively, and their positive regulators, and derived from their asporogenic and antibiotically inactive mutants *S. globisporus* 1912-B2 and *S. griseus* 1439 defective in biosynthesis of the mentioned regulators [10, 17].

For antibiotic biosyntesis and sporulation, the streptomycetes were grown on corn-soy medium (g/L): corn meal 20.0, soy meal 10.0, NaCl 5.0, agar 15.0, distilled water 1.0 L, pH 7.0; sterilization at 1.0 bar overpressure for 30 min. Cultures of the strains 773 and 1912-2 were grown on minimal solid medium during 5-6 days at 28 °C (g/L): asparagine 1.0, glycine 1.0, K_2HPO_4 0.5, $MgSO_4$ 0.2, NaCl 4.0, glycerol 15.0, agar 10.0, trace elements ($FeSO_4$, $CuCl_2$, $MnSO_4$, $CaCl_2$) 10.0 mg each, pH 7.2, sterilization at 0.75 bar overpressure for 30 min. The agar cultures were extracted with chloroform-acetone (2:1) to obtain the A-factor [(2S)-isocapryloyl-(3R)-oxymethyl-γ-butyrolacton] from strain 773 and the so far unknown regulator of landomycin E biosynthesis, respectively, from strain 1912-2.

Purification of the regulators. The crude extracts were evaporated to dryness at a vacuum rotary evaporator and the residues dissolved in ethanol. The metabolites were separated by means of thin layer chromatography on Sorbfil sheets (Kuban`, Russia) and Silica gel 60 F254 (Merck) with benzene-ethyl acetate-acetone-ethanol (4:2:1:0.5). The UV absorbing zones were triturated with ethanol, and the silica gel was centrifuged off at 10,000 rpm. The regulator was further purified by HPLC.

Biological activity. The biological activity of the purified metabolites was examined using mutant strains 1439 and 1912-B2. The strain 1439 was inoculated on Petri dishes with solid corn-soy medium as a central line or cross, and small paper discs with the regulators (10 µl) were put near the end of the lines. Alternatively, holes cut into the medium and filled with a solution of 10 µl regulator in ethanol, were used instead of paper discs. The strain 1912-B2 was inoculated evenly on a solid medium by glass spatula, and discs with the regulators were placed on the surface. The results of the influence of regulators on the antibiotic biosynthesis and morphogenesis were registered on the second and fifth day of incubation of the strains 1912-B2 and 1439, respectively.

Absorption, MS, and NMR spectra. The absorption spectra of ethanol solutions of the regulator were registered by means of the Beckman DU-8 spectrophotometer. Liquid chromatography/mass spectrometry (LC/MS) of unknown regulators was performed by means of Waters Micromass ZQ 2000 LC/MS system and liquid chromatograph Agilent Technologies 1200 with a single quadrupole detector. The conditions of separation were: Zorbax Hypersyl ODS reversed phase column with hexane:ethanol (98:2) as solvent at a flow rate of 0.17 ml min^{-1}, and with detection at 210 to 400 nm. The chromatograms have been developed with Chemstation software. ESI-HRMS spectra were acquired on a Fourier-transform ion cyclotron resonance mass spectrometer APEX IV(Bruker Daltonik), and argon was used as collision gas for CID-MS/MS under high-resolution conditions. Samples were introduced via a syringe pump. The NMR spectra of regulator were measured at 125.7 MHz (^{13}C NMR) and 300 MHz (^{1}H NMR) on Varian INOVA 500 and Mercury 300 spectrometers, respectively.

Results and Discussion. *Discovery of the regulator of landomycin E biosynthesis.* A number of highly active and landomycin E defective mutants were obtained by means of mutagenesis induced in initial wild type strain *S. globisporus* 1912 by UV and nitrosoguanidine. Examination of antibiotic inactive mutants by phenotypic complementation test revealed the ability of some donor strains to restore landomycin E biosynthesis by recipient 1912-B2 mutant during the growth in close proximity on corn-soy medium. It can be concluded that the recipient strain produced landomycin E under the influence of external metabolites from the donor. Metabolites of both strains were extracted from agar cultures and separated by thin layer chromatography.

Chromatographic mobility and absorption spectra of regulator. Two UV absorbing zones were produced by donor strain, which were missing in the recipient (Fig. 1). Both fractions purified by TLC displayed biological activity similar to that of A-factor. They restored sporulation and streptomycin production by *S. griseus* strain 1439 (Fig. 2a, 2b). The A-factor extracted and purified by TLC

from a sporulating and streptomycin producing culture of *S. griseus* 773 restored landomycin E biosynthesis by the strain *S. globisporus* 1912-B2 in a similar way (Fig. 2c). Despite of the similarity of biological activity, A-factor and regulator of landomycin E biosynthesis distinctly differed in the chromatographic mobility and absorption spectra, indicating their different chemical structures. Fractions 1 and 2 have the R_f values of 0.4 and 0.8, correspondingly, and their different absorption spectra in ethanol are presented in Fig. 1 (c1, c2).

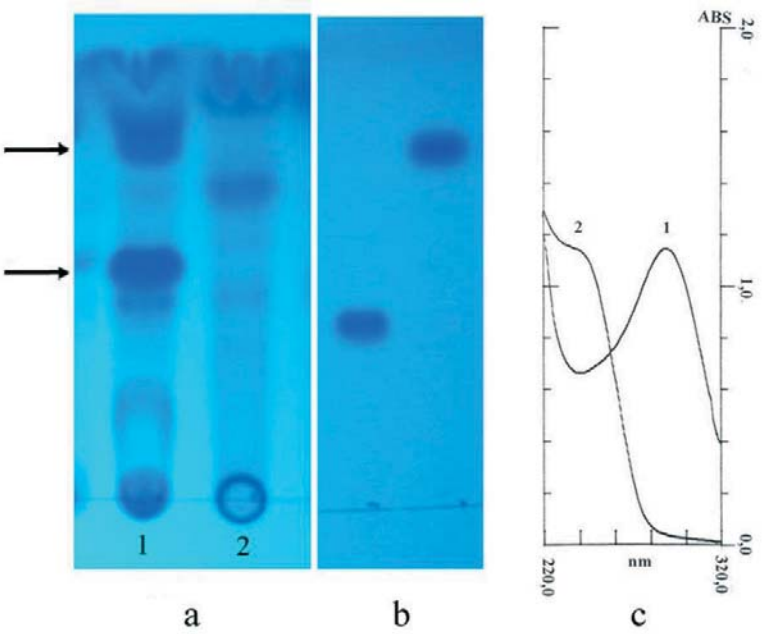


Fig.1. Thin layer chromatogram in UV light of the cell-free extracts of the agar cultures of landomycin E producing strain *S. globisporus* 1912-2 (a, 1) and its inactive mutant 1912-B2 (a, 2); b) TLC of the purified fractions of regulator, indicated by arrows (a, 1); c) The absorption spectra in UV light of the regulators having R_f 0.8 (1) and R_f 0.4 (2).

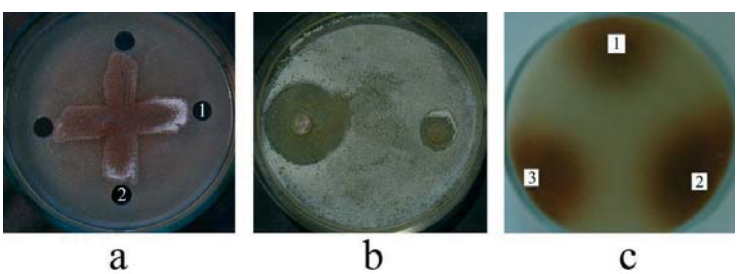


Fig.2. Restoration of sporulation (a), streptomycin (b) and landomycin E (c) biosynthesis by the strains *S. griseus* 1439 (a, b) and *S. globisporus* 1912-B2 (c) under influence of exogenous A-factor (1), and regulator with R_f 0.4 (2), and R_f 0.8 (3); b) blocks from sporulating (left) and non sporulating (right) area of agar culture 1439 (a) on the lawn of streptomycin sensitive test-culture.

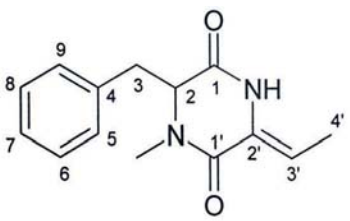
Structure elucidation of regulator with R_f 0.8. Chemical structure of the regulator with R_f 0.4 was elucidated as N-methylphenylalanyl-dehydrobutyrine diketopiperazine (BDD) (Fig. 3) [16]. HPLC of the purified regulator with R_f 0.8 showed 4 nearly distributed peaks with R_t 15.617 (1), 15.898 (2), 16.246 (3) and 16.695 min (4) (Fig. 4, Table). Absorption maxima of the peaks 1 and 2 are the same, 294 nm, and λ_{max} of the peaks 3 and 4 is equal 270 nm. LC/MS showed that peaks 1 and 2 produced the same fragments with *m/z* 244 and 338, and peaks 3 and 4 in turn gave similar fragments with *m/z* 244, 338 and 384. These results indicated the presence of two compounds with *m/z* 338 and 384, each of which was supposedly present as two stereoisomers. The same molecular mass of both compounds were identified by Waters Micromass ZQ 2000 LC/MS system (Fig. 5). Each isomer of

these compounds contained in their structure the same fragment with *m/z* 244 typical for the regulator BDD. This assumption was confirmed by the ^{1}H NMR spectrum, where the compound with R_f 0.8 had some common signals with BDD (Fig. 6). To these signals belong 2CH groups at 7.0-7.2, guartet at 5.6-5.8 and CH_2 groups at 3.1. Study of the dynamics of the production of these compounds showed its complete transformation into BDD during 4-6 days of culture growth (Fig. 7, track 4). The process of transformation can be reduced to 3 days in the presence of phenylalanine (20.0 mg/L) in the minimal medium (Fig. 7, track 5). Threonine increased the production of the regulator to a lower degree than phenylalanine (Fig. 7, tracks 6, 7). The effect of both amino acids is summarized in Fig. 7, track 8. So, phenylalanine as part of the molecular structure of BDD is the main limiting factor of its production. Deficiency of this amino acid during the first three days of growth of the culture in the minimal medium resulted in formation of two compounds with stereoisomeric structures, which are transformed to stable regulator BDD after addition to the medium of phenylalanine or biosynthesis this amino acid during culture growth.

Table

LC/MS analysis of the regulator (R_f 0.80)

Rt peak (min)	λ_{max} (nm)	Frag.70, ESI_Pos (m/z)	Frag.70, APCI_Pos (m/z)
15.617	294	244, 338	244, 338
15.898	294	244, 338	244, 338
16.246	270	207, 244, 338, 384	244, 338, 384
16.695	270	207, 244, 338, 384	244, 338, 384



6-Benzyl-3-eth-(Z)-ylidene-1-methyl-piperazine-2,5-dione

Fig.3. Chemical structure of L-6-benzyl-3-eth-(Z)-ylidene-1-methyl-piperazine-2,5-dione [(L)-N-methylphenylalanyl-dehydrobutyrine-diketopiperazine, BDD).

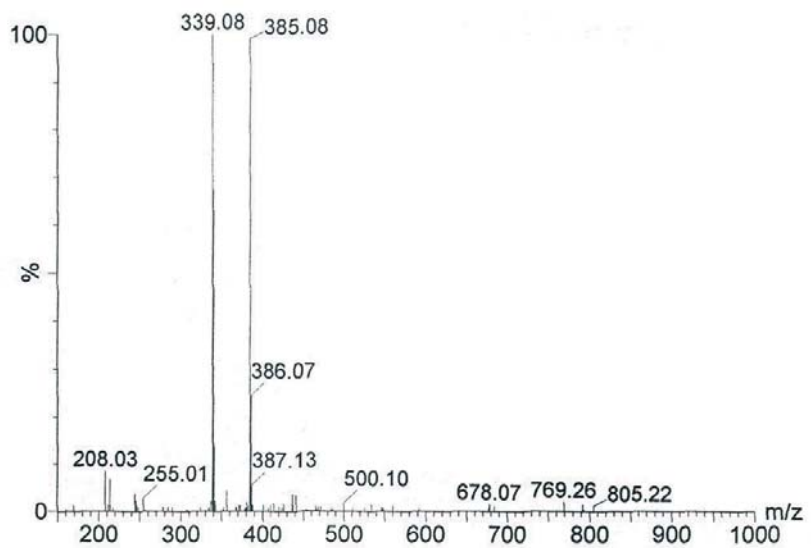


Fig.4. LC/MS spectrum of the regulator with R_f 0.8 and Rt 16.042.

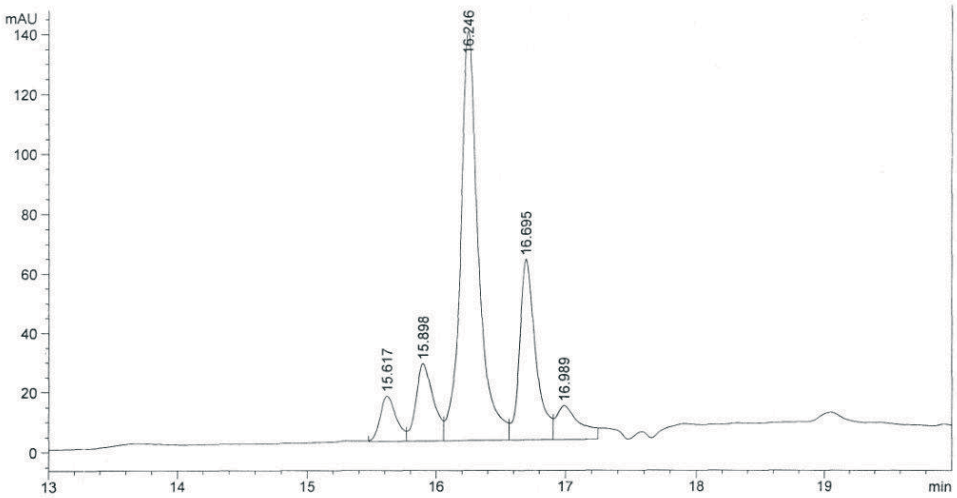
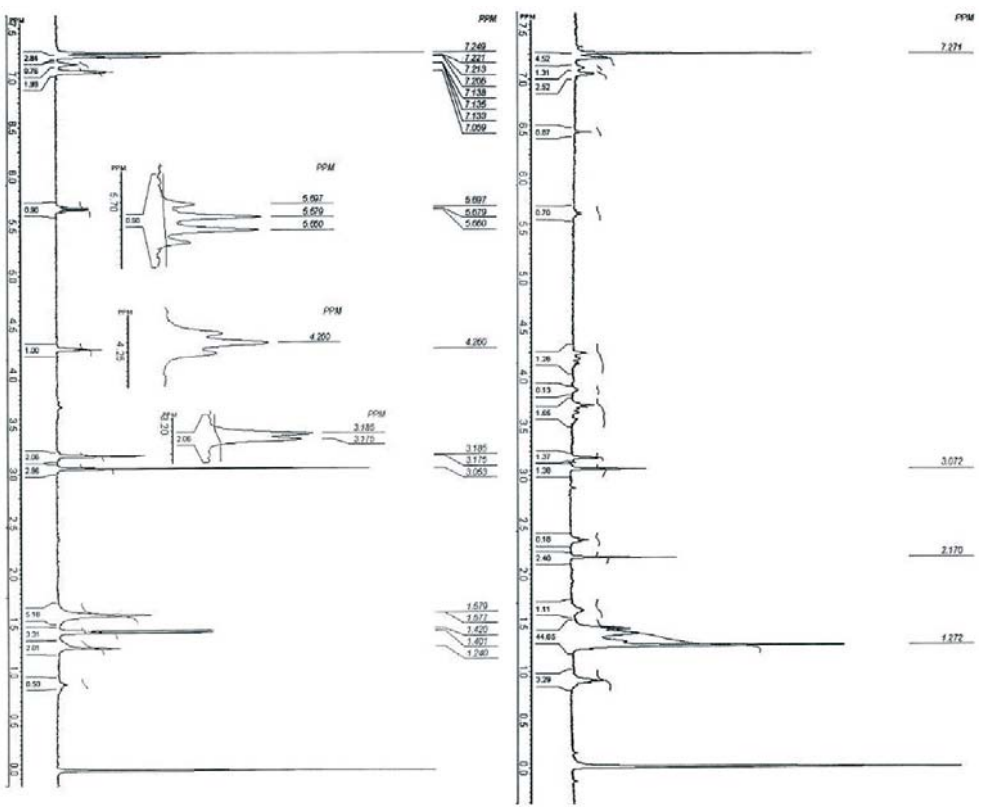


Fig.5. HPLC of the regulator with R_f 0.8.



a **b**

Fig.6. ^{1}H NMR spectra of the regulators with R_f 0.4 (a) and R_f 0.8 (b).

One can propose the following speculation of obtained results. The compound with m/z 338 consists of the regulator BDD (m/z 244) and -Phe-OH group (m/z 93) at C4` benzene ring after joining HO-Phe-OH. Water solution of phenol and its anion form have λ_{abs} = 270 nm and 290 nm, correspondingly, that agreed with absorption maxima of four peaks of isomers (Table). The compound with m/z 338 can connect $(CH_3)_2$-CH- (m/z 43) resulting in formation of compound with m/z 384. Fragmentation of this compound gives intermediate with m/z 207 (Table). Speculation about its

formation may be the following. Regulator BDD (m/z 244) loses phenyl cycle (244 – 65 = 179) and then connects C_2H_5 (179 + 28 = 207).

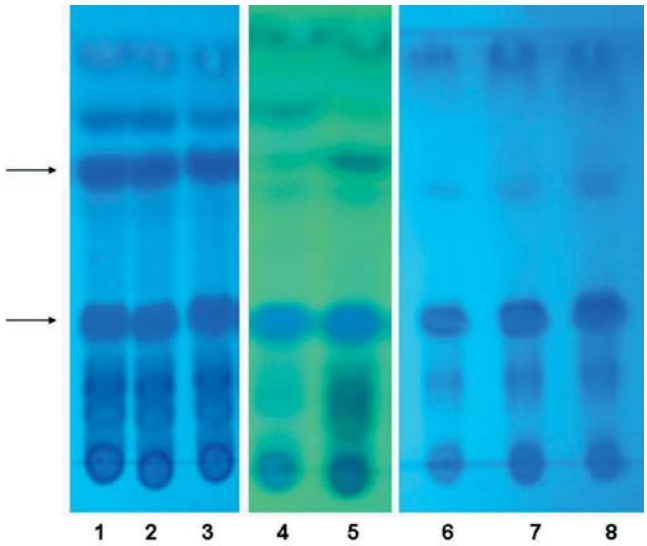


Fig.7. Thin layer chromatogram in UV light of the cell free extracts of the test-culture 1912-B2 grown during three (1, 2, 3, 5), four (6, 7, 8) and six (4) days on the solid minimal medium (1, 4, control) after addition 20 mg/L tyrosine (2), tryptophan (3), phenylalanine (5, 7), threonine (6) and threonine + phenylalanine (8). The arrows indicate the strips of regulator with R_f 0.4 (low) and R_f 0.8 (high).

So, both unstable compounds with m/z 338 and 384 are the by-products with higher mass transforming to stable regulator BDD (m/z 244) after 6 days of culture growth or after 3 days after addition phenylalanine to the minimal medium.

On the basis of obtained results one can suppose that regulatory properties of the mentioned complex compounds belong to BDD component, the new diketopiperazine derivative, as an essential part of their molecular structure. The hypothesis explaining the regulatory activity of BDD includes the inactivation of the corresponding sensor kinase as a part of two-component global regulatory system by this new diketopiperazine. The cognate response regulatory protein without phosphorylation cannot interact with regulatory region of DNA and repress the corresponding genes of the specific pathway of antibiotic biosynthesis.

The regulators AfsR and AfsQ1 from the two-component regulatory systems of *S. coelicolor* A3(2) and *S. lividans* are known to restore the A-factor biosynthesis and antibiotics production in corresponding defective mutants [8, 9, 21]. It is also known that some diketopiperazines are the potential inhibitors of tyrosine kinase that also supported our hypothesis 13, 14, 15].

Б. Мацелюх[1], Ф. Мохаммадіпанаг[2], Г. Лаатш[2], Ю. Рор[3], О. Єфременкова[4], В. Хиля[5]

[1]Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України, Київ
[2]Інститут органічної і біомолекулярної хімії, Університет ім. Георг-Августа, Геттінген
[3]Коледж фармації, Університет Кентуккі, Лексінгтон
[4]Інститут нових антибіотиків ім. Г.Ф. Гаузе РАМН, Москва
[5]Національний університет ім. Т.Г. Шевченка, Київ

ОЧИСТКА І З`ЯСУВАННЯ СТРУКТУРИ ПОБІЧНОГО ПРОДУКТУ НОВОГО РЕГУЛЯТОРА БІОСИНТЕЗУ АНТИБІОТИКІВ І ДИФЕРЕНЦІАЦІЇ *STREPTOMYCES*

Р е з ю м е

Streptomyces globisporus 1912, продуцент протипухлинного антибіотика ландоміцину Е, синтезує низькомолекулярну сигнальну молекулу N-метилфеніліланіл-дегідробутирін дікетопіперазін (BDD) і її

комплексний і нестабільний побічний продукт, які відновлюють, подібно до А-фактора *Streptomyces griseus* 773, біосинтез ландоміцину Е і стрептоміцину та споруляцію у дефектних мутантів *S. globisporus* 1912-Б2 і *S. griseus* 1439 відповідно. Тут ми повідомили про очистку і з`ясування структури двох сполук з R_f 0.8 за допомогою ВЕРХ, РХ/МС і ^{1}Н ЯМР аналізів. Ці сполуки мають m/z 338 і 383 відповідно і кожна із них представлена двома стереоізомерами, які включають BDD у свою структуру. Запропоновано гіпотезу для пояснення складу і регуляторних властивостей даних нестабільних сполук.

Ключові слова: *Streptomyces,* нові похідні дікетопіперазинів, біосинтез антибіотиків, морфогенез.

Б. Мацелюх[1], Ф. Мохаммадипанаг[2], Г. Лаатш[2], Ю. Рор[3], О. Єфременкова[4], В. Хиля[5]

[1]Институт микробиологии и вирусологии им. Д.К. Заболотного НАН Украины, Киев
[2]Институт органической и биомолекулярной химии, Университет им. Георг-Августа, Геттинген
[3]Колледж фармации, Университет Кентукки, Лексингтон
[4]Институт новых антибиотиков им. Г.Ф. Гаузе РАМН, Москва
[5]Национальный университет им. Т.Г. Шевченко, Киев

ОЧИСТКА И ВЫЯСНЕНИЕ СТРУКТУРЫ ПОБОЧНОГО ПРОДУКТА НОВОГО РЕГУЛЯТОРА БИОСИНТЕЗА АНТИБІОТИКОВ И ДИФФЕРЕНЦИАЦИИ STREPTOMYCES

Р е з ю м е

Streptomyces globisporus 1912, продуцент противоопухолевого антибиотика ландомицина Е, образует низкомолекулярную сигнальную молекулу N-метилфенилаланил-дегидробутирин дикетопиперазин (BDD) и ее комплексный и нестабильный побочный продукт, восстанавливающие, подобно А-фактору *Streptomyces griseus* 773, биосинтез ландомицина Е и стрептомицина и споруляцию у дефектных мутантов *S. globisporus* 1912-Б2 и *S. griseus* 1439 соответственно. Здесь мы сообщили об очистке и исследовании структуры двух соединений с R_f 0.8 с помощью ВЭЖХ, ЖХ/МС и ^{1}Н ЯМР анализов. Эти соединения имеют m/z 338 и 383 соответственно и каждое из них представлено двумя стереоизомерами, включающими BDD в свою структуру. Предложено гипотезу для объяснения состава и регуляторных свойств этих нестабильных соединений.

Ключевые слова: *Streptomyces,* новые производные дикетопиперазинов, биосинтез антибиотиков, морфогенез.

1. *Arakawa K., Tsuda N., Taniguchi A., Kinashi H.* The butanolide signaling molecules SRB1 and SRB2 induce lankacidin and lankamycin production in *Streptomyces rochei* //Chem. BioChem. – 2012. – **13**, N10 – P. 1447–1457.
2. *Bentley S.D., Chater K.F., Cerdeno-Tarraga A-M., Challis G.L., Thompson N.R., James K.D. et al*. Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2) // Nature. – 2002. – **417** – P. 141–147.
3. *Bibb M.* Regulation of secondary metabolism in streptomycetes // Curr. Opin. Microbiol. – 2005. – **8**. – P. 208–215.
4. *Bibb M., Hesketh A.* Analyzing the regulation of antibiotic production in *Streptomycetes //* Methods Enzymol. – 2009. – **458**. – P. 93–116.
5. *Efremenkova O.V., Anisova L.N., Bartoschevych Y.E.* Regulators of differentiation of *Actinomycetes //* Antibiot. Med. Biotechnol. – 1985. – **9**. – P. 687–707.
6. *Hara O., Beppu T.* Mutants blocked in streptomycin production in *Streptomyces griseus* – the role of A-factor // J. Antibiot. – 1982. – **35**. – P. 349–358.
7. *Hara H., Ohnishi Y., Horinouchi S.* DNA microarray analysis of global gene regulation by A-factor in *Streptomyces griseus* // Microbiology. – 2009. – **155**. – P. 2107–2210.
8. *Horinouchi S., Hara O., Beppu T.* Cloning of a pleiotropic gene that positively controls biosynthesis of a A-factor, actinorhodin, and prodogiosin in *Streptomyces coelicolor* A3(2) and *Streptomyces* lividans // J. Bacteriol. – 1983. – **155**, N3. – P. 1238–248.
9. *Ishizuka H., Horinouchi S., Kieser H., Hopwood D., Beppu T.* A putative two-component regulatory system involved in secondary metabolism in *Streptomyces* spp. // J. Bacteriol. – 1992. – **174**. – P. 7585–7594.
10. *Khokhlov A.S., Tovarova I.I., Borisova L.N., Pliner S.A., Schevchenko L.A., Kornitskaya E.Y., Ivkina N.S., Rapoport I.A.* A-factor responsible for the biosynthesis of streptomycin by a mutant strain of *Actinomyces streptomycini* //Dokl. Akad. Nauk SSSR. – 1967. – **177**. – P. 232–1235.

11. *Kitani S., Miyamoto K., Takamatsu S., Herawati E., Iguchi H., Nishitomi K. et al.* Avenolide, a *Streptomyces* hormone controlling antibiotic production in *Streptomyces avermitilis* // Proc. Natl. Acad. Sci. – 2011. – **108**, N39. – P. 16410–16415.
12. *Korynevska A., Heffeter P., Matselyukh B., Elbing L., Micksche M., Stoika R. et al.* Mechanisms underlying the anticancer activities of the angucycline landomycin E // Biochem. Pharmacol. – 2007. – **74**. – P. 1713–1725.
13. *Li W-R., Peng S-Z.* Rational design and synthesis of unsaturated 2,5- dioxopiperazine derivatives as potential protein tyrosine kinase inhibitors // Tetrahedron Lett. – 1998. – **39**. – P. 7373–7376.
14. *Liu J., Brahimi F., Saragovi H.U., Burgess K.* Bivalent diketopiperazine-based tropomysin receptor kinase C (TrkC) antagonists //J. Med. Chem. – 2010. – **53**. – P. 5044–5048.
15. *Martins M., Carvalho I.* Diketopiperazines: Biological activity and synthesis // Tetrahedron. – 2007. – **63**. – P. 9923–9932.
16. *Matselyukh B., MohammadipanahF., Laatsch H., Rohr J., Efremenkova O., Khilya V.* N-methylphenylalanyl-dehydrobutyrine diretopiperazine – a new regulator of antibiotics biosynthesis and morphogenesis in streptomycetes //Microbiology (in press).
17. *Matselyukh B.P., Tymoshenko S.G., Bambura O.I., Kopejko O.P.* Screening and characterization of regulators of landomycin E biosynthesis // Mikrobiol. Zhurn. – 2011. – **73**, N5. – P. 16–20.
18. *Ohnishi Y., Yamazai 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. – 2005. – **69**. – P. 431–439.
19. *Ostash B., Rebets Y., Myronovskyy M., Tsypik O., Ostash I., Kulachkovskyy O. et al.* Identification and characterization of the *Streptomyces globisporus* 1912 regulatory gene *indYR* that affects sporulation and antibiotic production // Microbiology. – 2011. – **157**. – P. 1240–1249.
20. *Panchuk R.R., Lehka L.V., Matselyukh B.P., Kril I.Y., Stoika R.S.* Search for and identification of molecular targets of angucycline antibiotic landomycin E in human tumor cells // Studia Biologica. – 2012. – **6**, N1. – P. 5–18.
21. *Stein D., Cohen S.N.* A cloned regulatory gene of *Streptomyces lividans* can supress the pigment deficiency phenotype of different developmental mutants // J. Bacteriol. – 1989. – **171**. – P. 2258–2261.
22. *Takano E.* γ-Butyrolactones: *Streptomyces* signalling molecules regulating antibiotic production and differentiation // Curr. Opin. Microbiol. – 2006. – **9. –** P. 287–294.
23. *Takano E., Nihira T., Hara Y., Jones J., Gershater C., Yamada Y., Bibb M.* Purification and structural determination of SCB1, a γ-butyrolactone that elicits antibiotic production in *Strep[tomyces coelicolor* A3(2) // J. Biol. Chem. – 2000. – **275**, N15. – P.11010–11016.
24. *van Wezel G.P., McKenzie N.L., Nodwell J.R.* Applying the genetics of secondary metabiolism in model *Actinomycetes* to the discovery of new antibiotics // Methods Enzymol. – 2009. – **458**. – P. 117–141.

Отримано 10.06.2011