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Isolation of New Thiopeptide Berninamycin E from *Streptomyces atroolivaceus*

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Thiopeptide antibiotic, berninamycins A, was originally isolated from *Streptomyces bernensis*. In this report, new berninamycin analogue named berninamycin E was isolated using several procedures using column chromatography from acetone extract of *S. atroolivaceus* NBRC 12741. High resolution ESI-MS spectrum data determined the molecular fomula of berninamycin E as $C_{51}H_{53}N_{15}O_{15}S$. The structure of berninamycin E was determined using NMR and MS spectra.

Key Words: Berninamycin, *Streptomyces atroolivaceus*, NMR spectrum, Antibiotic.

INTRODUCTION

Thiopeptide antibiotics are a class of sulfur containing highly modified cyclic peptides with interesting biological properties[1]. So far, structurally wide variety of thiopeptide antibiotics including important substance such as thiostrepton have been isolated mainly from actinomycetes[2-5]. Structure of antibiotics in this class mostly contains heterocyclic rings such as thiazole, oxazole and pyridine and dehydrated amino acids including dehydroalanine and dehydrobutyric acid[6]. The mode of action of this antibiotic family is potent inhibition of protein biosynthesis in broad range of gram-positive bacteria by binding to their ribosomal subunits[7,8]. On the other hand, several thiopeptides were reported to induce the tipA gene, which produces the proteins TipAL and TipsAS that belong to the MerR family of transcription regulators[9]. This class of thiopeptides has very importance not only as antibacterial agents but also as biochemical reagents.

Berninamycins A-D were originally isolated from *Streptomyces bernensis*[10-13] and recently we reported identification of berninamycin A (Fig. 1) from different species, *S. atroolivaceus*[14]. As a result of further chemical investigation, we found new berninamycin analogue named berninamycin E (Fig. 1). Here we describe the isolation and structure determination of berninamycin E from *S. atroolivaceus*.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were obtained with a JEOL ECA-600 in DMSO-d_6 at 27.0 ºC. The resonance of residual DMSO-d_6 at δH 2.49 was used as internal reference for ^{1}H NMR spectrum. ESI-TOF MS spectra were recorded by a JEOL JMS-T100LP mass spectrometer. For in-source collision-induced dissociation (CID) experiment using ESI-TOF MS spectrometer, orifice 1, orifice 2 and ring lens voltage were set at 100, 10 and 12 V, respectively.

Bacterial strain: The bacterium *Streptomyces atroolivaceus* (type strain, NBRC12741) was obtained from the NBRC culture collection (NITE Biological Resource Center, Japan). Cultivation was performed using ISP2 agar medium[15] with incubation at 30 ºC for 6 days.

Isolation of berninamycin E: ISP2 agar medium (500 mL) which *S. atroolivaceus* grew on was extracted with 500 mL of acetone. The acetone extract were concentrated to an aqueous suspension and was subjected to open column chromatography (CHP20P, 5 × 10 cm) eluted with 20 % MeOH, 60 % MeOH and MeOH. The MeOH fraction was subjected to reversed-phase HPLC using ODS column (Nacalai Tesque, Cosmosil MSII 4.6 × 250 mm). Sequential two step elution was performed for HPLC separation; step A: isocratic elution for 5 min with the solvent system consisted of MeCN/H_2O/TFA (30:70:0.1) with 1 mL/min flow rate; Step B: gradient elution for 25 min from MeCN/H_2O/TFA (30:70:0.1) to MeCN/H_2O/TFA (70:30:0.1) with 1 mL/min flow rate. The UV detector of HPLC was set at the absorbance of 220 nm to yield berninamycin E as a colourless amorphous powder: HR ESI-MS *m/z* 1148.3671 $[M+Na]^+$ calcd. for $C_{51}H_{54}N_{15}O_{15}S$ (Δ -2.67 mmu).

RESULTS AND DISCUSSION

The culture media of *S. atroolivaceus* was extracted by acetone and the acetone extract was filtered and concentrated

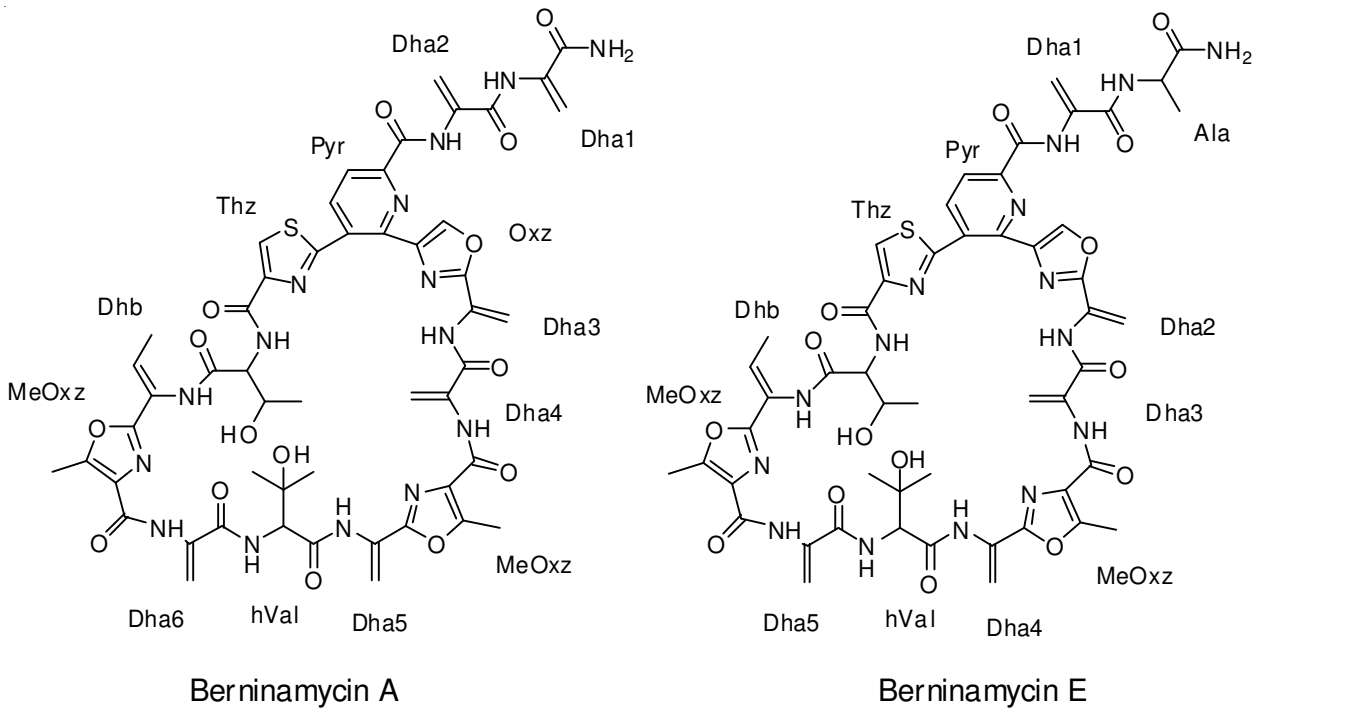

Fig. 1. Chemical structures of berninamycins A and E; abbreviations: thiazole (Thz), oxazole (Oxz), methyl oxazoline (MeOxz), pyridine (Pyr), dehydroalanine (Dha), dehydrobutyric acid (Dhb), hydroxyl valine (hVal), alanine (Ala).

to aqueous residue by the rotary evaporator. The acetone extract was subjected to open column chromatography using hydrophobic resin eluted with 20 % MeOH, 60 % MeOH and MeOH. The new minor component named berninamycin E was isolated along with berninamycin A by repeatedly subjecting MeOH fraction to preparative HPLC. The content of berninamycin E in the extract was very low compared to berninamycin A.

Structure determination of berninamycin E was accomplished by combination of ESI-MS and NMR spectrum data. High resolution ESI-MS spectrum data determined the molecular formula as $C_{51}H_{53}N_{15}O_{15}S$. Considering that molecular formula of berninamycin A is $C_{51}H_{51}N_{15}O_{15}S$, berninamycin E was thought to have the structure of berninamycin A with hydrogenation of one double bond. To obtain further information about the chemical structure, ^{1}H NMR spectrum of berninamycin E was measured using DMSO-d_6 as a solvent. The comparison of ^{1}H NMR spectrum with that of berninamycin A indicated banishment of methylene peaks of dehydroalanine1 (Dha1, 5.67 and 5.99 ppm with asterisks in Fig. 2a) and the emergence of methyl residue (1.28 ppm with arrow in Fig. 2b) in the spectrum of berninamycin E. Because of the low yield of berninamycin E, measurement of 2D NMR spectra did not give enough quality of spectra to analyze. To obtain further information about the structure, in-source collision-induced dissociation (CID) experiment was accomplished. As a result, major fragmentation ion peaks at 1084.3 and 1015.5 were observed, which were corresponding to fragments as shown in Fig. 3. Based on the data that (1) the similar chemical shifts of berninamycin E compared to berninamycin A except for banishment of methylene peaks of Dha1 and emergence of methyl residue and (2) MS-fragmentation analysis indicating the sequence of NH_2-Ala-Dha1, we concluded that berninamycin E had the structure as shown in Fig. 1.

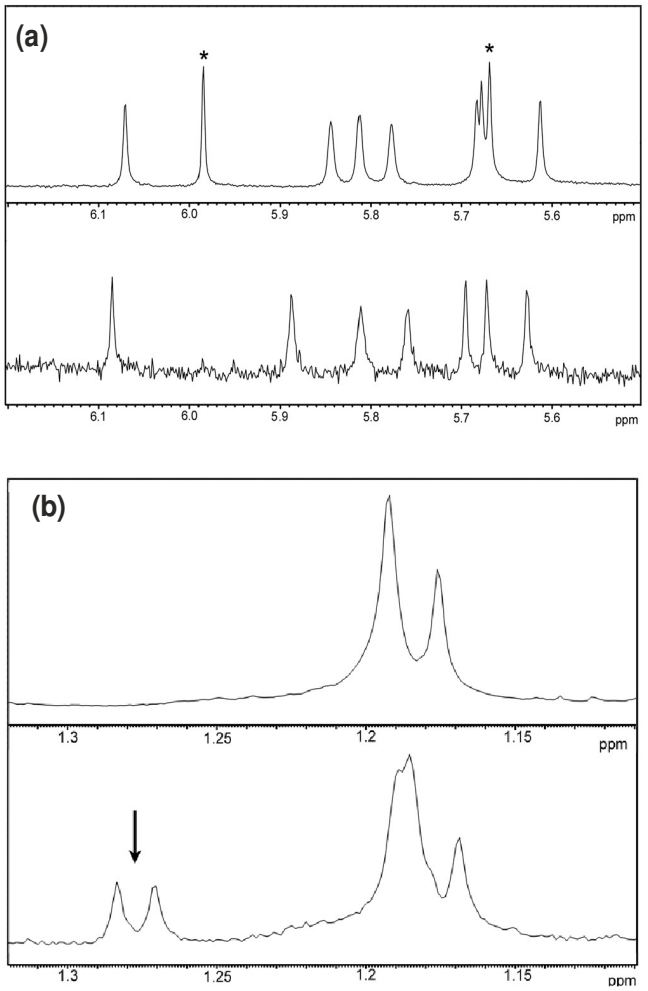

Fig. 2. Comparison of ^{1}H NMR spectra between berninamycin A (upper) and berninamycin E (bottom); a) enlarged spectrum from 5.5 to 6.2 ppm, b) enlarged spectrum from 1.1 to 1.3 ppm

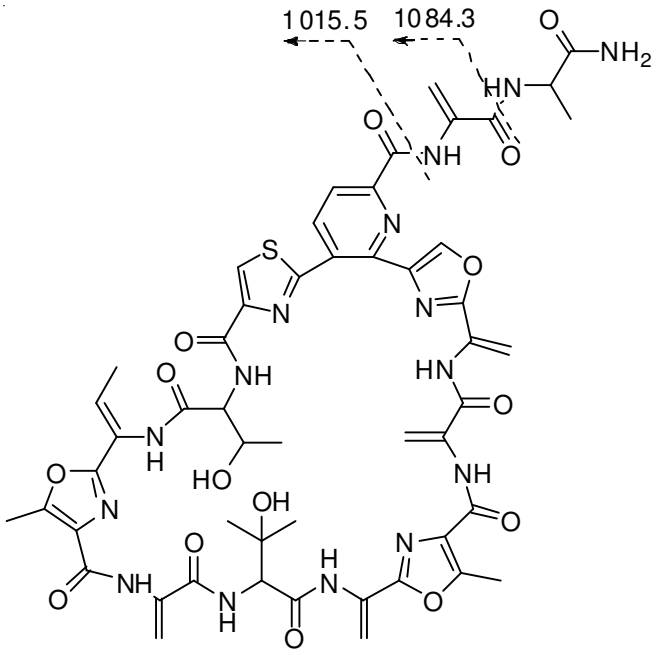

Fig. 3. ESI-MS fragments of berninamycin E by in-source CID experiment

Conclusion

New berninamycin analogue named berninamycin E was isolated from Streptomyces atroolivaceus. The structure was determined by comparison with ^{1}H NMR spectrum of berninamycin A and ESI-MS spectra.

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REFERENCES

1. M.C. Bagley, J.W. Dale, E.A. Merritt and X. Xiong, *Chem. Rev*., **105**, 685 (2005).
2. W.P. Jambor, B.A. Steinberg and L.O. Suydam, *Antibiot. Ann*., **3**, 562 (1955).
3. J.D. Dutcher and J. Vandeputte, *Antibiot. Ann*., **3**, 560 (1955).
4. R. Donovick, J.F. Pagano, H.A. Stout and M.J. Weinstein, *Antibiot. Ann*., **3**, 554 (1955).
5. B. Anderson, D.C. Hodgkin and M.A. Viswamitra, *Nature*, **225**, 233 (1970).
6. R.A. Hughes and C.J. Moody, *Angew. Chem. Int. Ed. Engl*., **46**, 7930 (2007).
7. F. Reusser, *Biochemistry,* **8**, 3303 (1969).
8. N. Naaktgeboren, K. Roobol, J. Gubbens and H.O. Voorma, *Eur. J. Biochem.,* **70**, 39 (1976).
9. J. Thompson, E. Cundliffe and M.J. Stark, *J. Gen. Microbiol*., **128**, 875 (1982).
10. R.C. Lau and K.L. Rinehart, *J. Antibiot. (Tokyo)*, **47**, 1466 (1994).
11. J.M. Liesch, J.A. McMillan, R.C. Pandey, I.C. Paul, K.L. Rinehart and F. Reusser, *J. Am. Chem. Soc*., **98**, 299 (1976).
12. J.M. Liesch, D.S. Millington, R.C. Pandey and K.L. Rinehart, *J. Am. Chem. Soc*., **98**, 8237 (1976).
13. J.M. Liesch and K.L. Rinehart, *J. Am. Chem. Soc*., **99**, 1645 (1977).
14. A. Ninomiya and S. Kodani, *Natural Products: An Indian J.,* **7**, 163 (2011).
15. E.B. Shirling and D. Gottlieb, *Int. J. Syst. Bacteriol*., **16**, 3130 (1966).