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COMMUNICATION TO THE EDITOR

A cryptic antibiotic triggered by monensin

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Recently, we reported the identification of promomycin, a new polyether closely related to lonomycin, based on its activity inducing antibiotic production in some *Streptomyces* spp.[1] Originally, we discovered that promomycin produced by *Streptomyces scabrisporus* strain 153 promoted antibiotic production in *Streptomyces griseorubiginosus* srain 574, but we also revealed that other polyethers including monensin exert similar stimulatory effects on antibiotic production. Promomycin and monensin exhibit antibacterial activity by themselves due to the function as ionophores,[1,2] but we observed that they stimulated antibiotic production in some *Streptomyces* spp. at subinhibitory concentrations.[1] Although the mechanism of this stimulation is not yet known, we expect that cryptic antibiotics, production of which is triggered by such a specific stimulatory event include those of unknown structure. Hence, we isolated and studied the chemical structure of the monensin-dependent antibiotic produced by *S. griseorubiginosus*.

S. griseorubiginosus strain 574 was inoculated into 80 ml MSS medium (containing (%): starch, 3; sucrose, 2; soybean flour (Sigma-Aldrich, Tokyo, Japan), 1; polypepton, (Difco, Detroit, MI, USA) 0.5; NaCl, 0.3; $CaCO_3$, 0.5; and soybean oil, 0.1 (pH 7.0)) (chemicals were purchased from Wako Pure Chemicals (Osaka, Japan) if not indicated otherwise) added with 5 μg ml^{-1} monensin prepared in 500 ml baffled Erlenmeyer's flask, and grown for 6 days at 28 °C with rotary shaking at 135 r.p.m. A volume of ~1.6 l of culture supernatant thus obtained was added to 80 ml of activated charcoal to adsorb the metabolite. The charcoal was first washed with water, and the antibiotic was eluted with 400 ml of water containing 50% acetone. This antibiotic activity was not detected in the culture without the supply of monensin. The eluate was evaporated to dryness, dissolved in 10 ml water and applied to reverse-phase columns using a Purif-compact chromatography system (Moritex, Tokyo, Japan) (flow rate, 5 ml per min). First, the water solution was applied to a Purif-pack ODS 100 μm, size 60 (Moritex). The column was washed first with 300 ml of water and then with 300 ml of 10 % methanol in water, and the activity principle was eluted with 300 ml of 20% methanol in water into a single batch. The eluate was evaporated, dissolved in 10 ml water and applied to a Purif-pack ODS 30 μm, size 60 (Moritex, Tokyo). The elution was carried out similarly as in the previous step, except that the eluate was collected into 10 ml aliquots. The active fractions were combined, lyophilized, dissolved in 2 ml of water and applied to gel-filtration column chromatography using Sephadex G-15 (ϕ 25 mm×400 mm) (GE Healthcare, Tokyo, Japan). Column was developed with water at flow rate 0.8 ml per min. Thereafter, the active fractions were combined, lyophilized, dissolved in 2 ml water and applied to a reverse phase column (Capsel Pak Inertsil ODS; ϕ 10 mm×250 mm) (Shiseido, Tokyo, Japan) by using HPLC system (class VP, Shimadzu, Kyoto, Japan). The column was isocratically developed with 30% methanol in water, and the activity fractions corresponding to a single UV absorption peak were manually collected. The eluates were combined and lyophilized to be the final purified preparation. By this process, ca. 1 mg of the purified substance was obtained from 30 l of culture broth of strain 574.

On the basis of the Mass and NMR spectral data (supplied as Supplementary text), the purified substance was identified to be a known isonitrile antibiotic (SF2768) (Figure 1).[3] SF2768 was originally isolated based on its fungicidal activity, but was also known for its bactericidal activity.[3] *Streptomyces* spp. used in the original discovery produced this substance in a conventional culture condition; hence, the addition of monensin is not essential for the production of SF2768 by the original producer. However, the evidence obtained in this study indicates that monensin stimulates the synthesis of structurally unrelated substance. This makes us assume that monensin affects the genetic control rather than biosynthesis of certain secondary metabolites, and expect that the addition of the group of polyether yet harbors potential to awaken the production of cryptic secondary metabolites in *Streptomyces*.

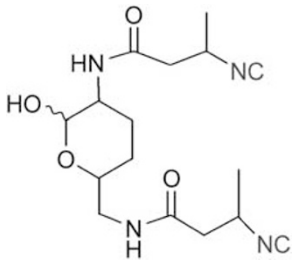

Figure 1 Structure of SF2768.

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1 Amano, S. *et al.* Promomycin, a polyether promoting antibiotic production in *Streptomyces* spp. *J Antibiot.* **63,** 486–491 (2010).
2 Butaye, P., Devriese, L. A. & Haesebrouck, F. Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on gram-positive bacteria. *Clin. Microbiol. Rev.* **16,** 175–188 (2003).
3 Tabata, Y., Hatsu, M., Amano, S., Shimizu, A. & Imai, S. SF2768, a new isonitrile antibiotic obtained from *Streptomyces. Sci. Rep. Meiji Seika Kaisha* **34,** 1–9 (1995).

Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)