Chemistry of Natural Compounds, Vol. 40, No. 3, 2004

METABOLITES OF THE SEA ISOLATE OF BACTERIA *Streptomyces* SP. 6167

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UDC 577.181.3+615.332:579.873.71

Sea isolate of Streptomyces *sp. 6167 was found to produce four macrolide antibiotics feigrisolides A, B, and D, and dinactin. The chemical structures of the compounds were determined using 1D and 2D NMR spectrometry and electrospray mass spectrometry (ESMS). It was shown that the feigrisolides are cytotoxic to Ehrlich carcinoma tumor cells and to egg-cells of the sea urchin* Strongylocentrotus intermedius *and antimicrobial to the bacteria* Bacillus cereus *and* Escherichia coli.

Key words: marine actinobacteria *Streptomyces* sp., macrolide antibiotics, feigrisolides, biological activity.

Actinobacteria isolated from marine sources are interesting as biologically active compounds [1-5]. Many of them are various types of macrolides [6-8]. We investigated metabolites of marine bacteria and first showed that *Streptomyces* sp. 6167 cultivated in liquid medium synthesizes a series of macrolides. Herein we describe the isolation, structure determination, and biological activity of the macrolides.

The total ethylacetate extract of the cultivation medium and bacterial mass was separated over silica gel using $CHCl_3$:CH_3OH systems of increasing polarity. Tests for antibacterial activity showed that fractions eluted from silica gel by $CHCl_3$:CH_3OH (95:5) (fraction **1**) and (90:10) (fraction **2**) contained active compounds. Gel-filtration chromatography of fraction **1** over Sephadex LH-20 ($CHCl_3$:CH_3OH, 60:40) produced three chromatographically pure compounds **1**, **2**, and **3**.

The molecular weight of **1** is 216 Da according to quasimolecular ions produced by electrospray mass spectrometry (ESMS) performed in two regimes: $[M + Na]^+$ = 239 and $[M - H]^-$ = 215. The ^1H and ^{13}C NMR spectra and the HSQC spectrum (in DMSO-d_6, δ) of **1** showed two methyls (^1H 0.86 → ^{13}C 10.0, ^1H 0.92 → ^{13}C 13.1), three methine protons bound to C bearing an O (^1H 4.0 → ^{13}C 80.7, H-3; ^1H 3.88 → ^{13}C 75.6, H-6; ^1H 3.5 → ^{13}C 68.6, H-8). The spectra also have signals for four methyl groups (^1H 1.55, 1.45 → ^{13}C 43.1, 2H-7; ^1H 1.88, 1.40 → ^{13}C 31.6, 2H-5; ^1H 1.76, 1.55 → ^{13}C 27.2, 2H-4; ^1H, 1.30-1.40 → ^{13}C 30.6, 2H-9) and one methine group (^1H 2.36 → ^{13}C 39.6, H-2). These data and the ^1H—^1H COSY spectrum of **1** (Fig. 1) indicate that the compound is feigrisolide B (**1**), which was isolated previously from the soil isolate of *Streptomyces griseus* [9].

The molecular weight of **2** is 400 Da according to quasimolecular ions from ESMS in two regimes: $[M + Na]^+$ = 423 and $[M - H]^-$ = 399. ^1H and ^{13}C NMR spectra (CD_3OD, δ) and the HSQC spectrum of **2** showed four methyls (^1H 0.93 → ^{13}C 10.85, 3H-22; ^1H 1.08 → ^{13}C 14.43, H_3-18; ^1H 1.09 → ^{13}C 14.47, 3H-23; ^1H 1.24 → ^{13}C 21.45, 3H-24); six protons bound to C bearing an O (^1H 3.95-4.14 → ^{13}C 82.56, H-1; 82.74, H-8; 78.24, H-5; 78.73, H-14; ^1H 5.0 → ^{13}C 71.37, H-12; ^1H 3.74 → ^{13}C 71.99, H-20). The spectra also contain signals for seven methylene protons (^1H 2.0-1.6 → ^{13}C 32.93, H-7/15; 29.92, H-6/16; 32.2, 2H-21; 33.04, H-7/15; 30.20, H-6/16; 44.4, H-13/19; 44.65, H-13/19), and two methine groups (^1H 2.45 → ^{13}C 47.66, H-9; 47.92, H-2). The data for **2** show that they are identical to those for feigrisolide C, which was isolated previously from the soil isolate of *Streptomyces griseus* [9].

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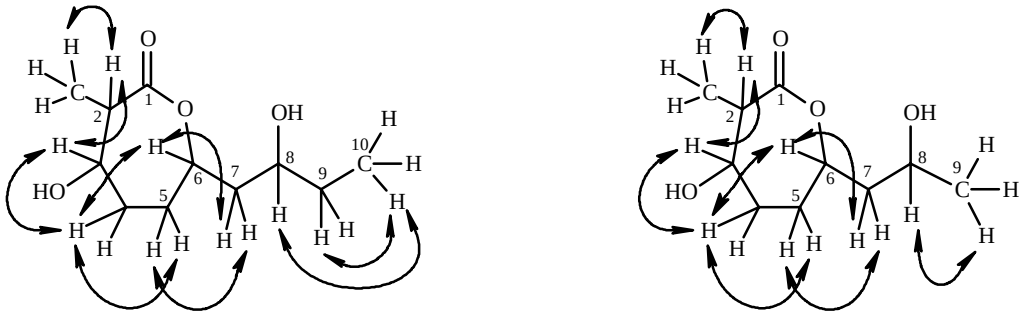
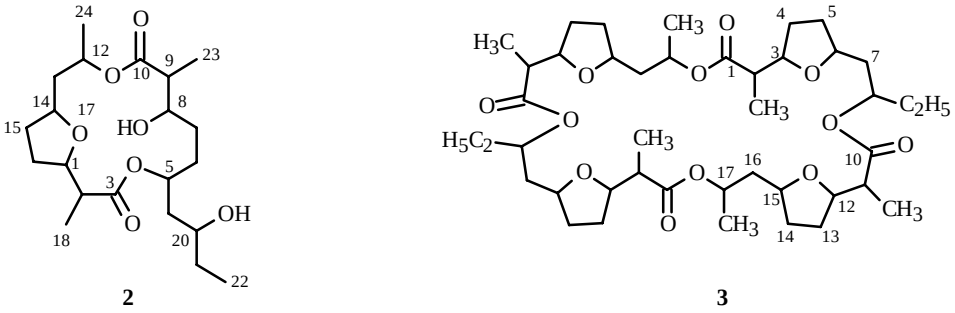
 0009-3130/04/4003-0282 ©2004 Plenum Publishing Corporation



1 **4**

Fig. 1. $^{1}H—^{1}H$ COSY couplings in feigrisolides B (**1**) and A (**4**).

A molecular weight of 765 Da for **3** was found from quasimolecular ions for ESMS in two regimes: $[M + Na]^+ = 788$ and $[M + NH_4]^+ = 783$. The electron-impact mass spectrum (EIMS) confirmed the molecular ion of 765. 2D NMR spectroscopy (COSY-45, HMBC, HSQC) and ^{1}H and ^{13}C NMR spectra indicate that **3** is identical to the macrolide antibiotic dinactin, which was isolated from *Streptomyces aureus* [10, 11]. According to ESMS, we determined that **3** is synthesized together with homologs of molecular weight 779 and 793 Da.



2 **3**

Gel filtration chromatography over Sephadex LH-20 in CH_3OH of fraction **2** produced pure **4** of molecular weight 202 Da, which was established for the quasimolecular ions produced by ESMS in two regimes: $[M + Na]^+ = 225$ and $[M - H]^- = 201$. ^{1}H and ^{13}C NMR spectra and 2D NMR spectroscopy ($^{1}H—^{1}H$ COSY, HMQC, HMBC) (Fig. 1) determined that **4** is a homolog of **1** and is identical to feigrisolide A, which was isolated previously from the soil isolate of *Streptomyces griseus* [9].

We showed that the total feigrisolides exhibit weak antimicrobial activity (IC = 4 mg/mL) toward *Bacillus cereus* and *Escherichia coli*. Compounds **1**, **2**, and **4** showed cytotoxic activity. Feigrisolide B was the most active toward tumor cells (IC_{50} 14.0 μM) and fertilized egg cells of sea urchin (IC_{50} 4.7 μM). The cytotoxic activity of the less hydrophobic feigrisolide A is 7 and 30, respectively, times lower than that of feigrisolide B. At concentrations higher than the cytotoxic values, the studied compounds did not lyse erythrocytes (Table 1). Cytotoxic and antimicrobial activities have been studied for certain macrolides [6-8, 12]. However, the mechanism of action of these compounds has not been studied. The lack of hemolytic activity for **1**, **2**, and **4** indicates that their mechanism of action is not due to destruction of biological membranes, which is characteristic of membranotropic compounds [13].

Thus, the actinomycete *Streptomyces* sp. 6167 is a good source of biologically active macrolides. The study of their activities and mechanisms of action is undoubtedly interesting.

TABLE 1. Biological Activity of Feigrisolides (μM)

Compound	IC_{50}*	IC_{50}**
Feigrisolide B (**1**)	14.0	4.7
Feigrisolide A (**4**)	99.0	124.0
Feigrisolide C (**2**)	125.0	125.0

Concentration: *inhibiting development of tumor cells; **blocking initial division of fertilized egg cells of sea urchin; LC_{100} > 200 at which 100% of erythrocytes lyse. The suspension of murine erythrocytes was incubated at 37°C for 2 h.

EXPERIMENTAL

^1H and ^{13}C NMR spectra of compounds in DMSO-d_6 and CD_3OD were measured on Bruker DRX-500 and DPX-300 spectrometers with TMS internal standard. Mass spectra were recorded on Varian MAT 371 (70 eV) and Varian 311 A (70 eV) spectrometers. Chromatographic monitoring was performed on Polygram SIL g/UV 254 plates (Macherey-Nagel & Co) using $CHCl_3$:CH_3OH (95:5 and 90:10). Compounds on chromatography plates were developed using anisaldehyde prepared as before [14]. Cytotoxic and hemolytic activities were studied by the published methods [15]. Antibacterial activity was tested by the literature method [16].

Cultivation of Bacteria and Isolation of 1, 2, 3, and 4. Bacteria were cultivated in 250-mL Ehrlenmeyer flasks in media (g/L): malt extract 10, glucose 4, yeast extract 4, pH 7.8, distilled water 50%, and artificially prepared seawater 50%.

Fermentation was performed with continuous rocking for 6 d at 29°C. The total volume of culture medium was 24 L.

Biomass was separated from culture medium by filtration. The bacterial cell wall was destroyed by ultrasound. The culture medium and biomass were extracted with ethylacetate. The weight of the total ethylacetate extract was 3.8 g. The extract was chromatographed over silica gel using $CHCl_3$:CH_3OH mixtures of increasing polarity. Fractions **1** and **2** were obtained. Chromatography over Sephadex LH-20 of fraction **1** using $CHCl_3$:CH_3OH (60:40) and subsequent rechromatography produced feigrisolides B and C and dinactin; of fraction **2** using CH_3OH, feigrisolide A.

Yields of feigrisolides A, B, and C, and dinactin per weight of total extract were 0.78, 0.13, 1.3, and 0.18%, respectively.

Feigrisolide B (1) is a colorless oil, very soluble in $CHCl_3$ and CH_3OH. It acquires a brown color on reaction with anisaldehyde. IR spectrum (KBr, ν, cm^{-1}): 3402 (OH), 1712 (C=O). Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 198 (10) $[M - H_2O]^+$, 169 (100) $[M - H_2O - C_2H_5]^+$, 125 (60) $[M - H_2O - C_4H_9O]^+$. ESMS: $[M + Na]^+ = 239$, $[M - H]^- = 215$.

Feigrisolide C (2) is a colorless oil, very soluble in CH_3OH. Reaction with anisaldehyde imparted a dark brown color. IR spectrum (KBr, ν, cm^{-1}): 3444 (OH), 1729 (C=O). ESMS: $[M + Na]^+ = 423$, $[M - H]^- = 399$.

Dinactin (3) was isolated as a brown oil, very soluble in $CHCl_3$. Reaction with anisaldehyde imparts a dark brown color. IR spectrum (KBr, ν, cm^{-1}): 1730, 1712 (OC=O). ESMS: $[M + Na]^+ = 788$, $[M + NH_4]^+ = 783$.

Feigrisolide A (4) is a colorless oil, very soluble in CH_3OH. It acquires a brown color on reaction with anisaldehyde. IR spectrum (KBr, ν, cm^{-1}): 3410 (OH), 1724 (C=O). Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 184 (18) $[M - H_2O]^+$, 169 (25) $[M - H_2O - CH_3]^+$, 125 (60) $[M - H_2O - C_3H_7O]^+$, ESMS: $[M + Na]^+ = 225$, $[M - H]^- = 201$.

ACKNOWLEDGMENT

The work was supported by grants RFBR No. 03-04-49528, Presidium of the RAS Program Molecular and Cellular Biology No. 04-1-05-005, and FED RAS No. 04-3-A-05-059.

REFERENCES

1. K. Gebhardt, J. Schimana, P. Krastel, K. Dettner, J. Rheinheimer, A. Zeeck, and H. Fiedler, *J. Antibiot.*, **66**, 794 (2002).
2. T. Furumai, K. Eto, T. Sasaki, H. Higuchi, H. Onaka, N. Saito, T. Fujita, H. Naoki, and Y. Igarashi, *J. Antibiot.*, **55**, 873 (2002).
3. R. Asolkar, R. Maskey, E. Helmke, and H. Laatsch, *J. Antibiot.*, **55**, 893 (2002).
4. G. K. Oleinikova, T. A. Kuznetsova, F. Huth, H. Laatsch, V. V. Isakov, L. S. Shevchenko, and G. B. Elyakov, *Russ. Chem. Bull.*, **50**, 2231 (2001).
5. T. Kuznetsova, A. Dmitrinok, M. Sobolevskaya, L. Shevchenko, and V. Mikhailov, *Russ. Chem. Bull.*, **10**, 3353 (2002).
6. K. Gustafson, M. Roman, and W. Fenical, *J. Am. Chem. Soc.*, **111**, 7519 (1989).
7. W. Fenical, *Chem. Rev.*, **93**, 1673 (1993).
8. C. Pathirana, P. Jensen, and W. Fenical, *Tetrahedron Lett.*, **33**, 7663 (1992).
9. Y. Tang, I. Sattler, R. Thiericke, and S. Grabley, *J. Antibiot.*, **53**, 934 (2000).
10. M. Haneda, Y. Nawata, T. Hayashi, and K. Ando, *J. Antibiot.*, **27**, 555 (1974).
11. E. Meyers, F. Pansy, D. Perlman, D. Smith, and F. Weisenborn, *J. Antibiot.*, **18**, 128 (1965).
12. D. Tapiolas, M. Roman, and W. Fenical, *J. Am. Chem. Soc.*, **113**, 4682 (1991).
13. N. G. Prokof′eva, N. I. Kalinovskaya, P. A. Luk′yanov, and T. A. Kuznetsova, *Biol. Morya (Vladivostok)*, **22**, No. 3, 179 (1996).
14. E. Stahl, ed., *Thin-Layer Chromatography: A Laboratory Handbook* (translated from the German), Academic, New York (1965).
15. N. Prokof′eva, E. Chaikina, A. Kicha, and N. Ivanchina, *Comp. Biochem. Physiol., Part B*, **134**, 695 (2003).
16. N. G. Prokof′eva, M. M. Anisimov, N. M. Rebachuk, M. M. Kisileva, and N. D. Pokhilo, *Izv. Akad. Nauk, Ser. Biol.*, **6**, 645 (2002).