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Andrastins A~C, New Protein Farnesyltransferase Inhibitors, Produced by *Penicillium* sp. FO-3929

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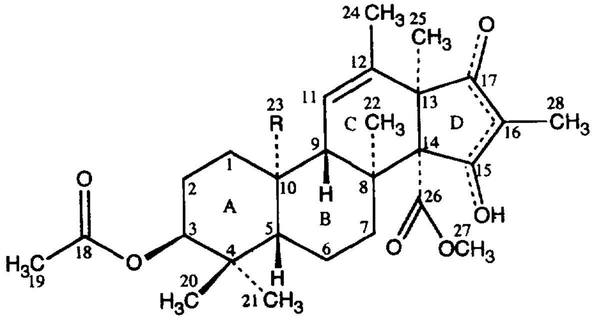
Abstract: New protein farnesyltransferase inhibitors, andrastins A~C (**1**~**3**) have been isolated from the cultured broth of *Penicillium* sp. FO-3929. The structure of **1** enriched by [1,2-^{13}C]acetate was elucidated by INADEQUATE and ^{13}C-^{13}C spin decoupling experiments. The structure of **2** and **3** were also elucidated by NMR experiments. Andrastins had an *ent*-5α-14β-androstane skeleton with keto-enol tautomerism.

Ras proteins are subjected to posttranslational farnesylation at a cysteine the fourth residue from the C-terminus, in which protein farnesyltransferase (PFTase) is concerned. Inhibition of PFTase would alter membrane localization and activation of Ras proteins$^{1)}$. In the course of screening for PFTase inhibitors of microbial origin, we have found gliotoxins and pepticinnamins previously$^{2\sim4)}$. Additional new PFTase inhibitors, andrastins A, B, and C (**1**~**3**), were found from the cultured broth of *Penicillium* sp. FO-3929$^{5,6)}$. In this paper, the structure elucidation of **1**~**3** is described.



	R
Andrastin A (**1**)	CHO
Andrastin B (**2**)	CH_2OH
Andrastin C (**3**)	CH_3

HR FAB-MS of **1** revealed its molecular formula, $C_{28}H_{38}O_7$$^{5)}$. First, the structure of **1** was examined by ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, and HMBC experiments (Fig. 1)$^{6)}$. Three partial structures, $-CH_2-CH_2-CH-O-$, $-CH-CH_2-CH_2-$, and $-CH-CH=$, were deduced by the ^{1}H-^{1}H COSY. They were connected to form rings A, B, and C by the HMBC experiment. The HMBC experiment revealed the arrangements of one acetyl (C-18 and 19), five methyls (C-20, 21, 22, 24, and 25), one aldehyde (C-23), and one quaternary carbon (C-17). The remaining atoms, $C_5H_7O_4$, could not be assigned by the HMBC.

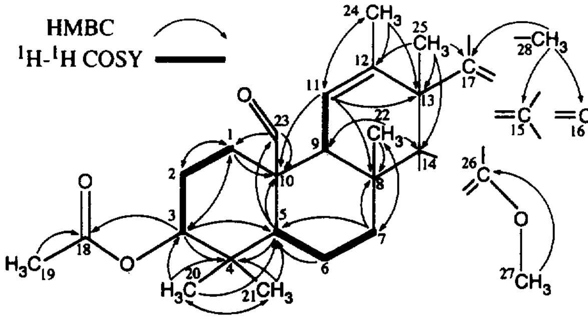


Fig. 1. Partial structure of 1 elucidated by ^1H-^1H COSY and HMBC.

Because 1 was assumed to be synthesized via mevalonate pathway from its partial structure, we carried out the incorporation experiment with [1,2-^{13}C]acetate to enrich ^{13}C signals. Sodium [1,2-^{13}C]acetate (1 mg/ml) was added to the 40-hours cultured broth and the broth was incubated for a further 60 hours. From 1 liter of the cultured broth, 80 mg of labeled 1 was obtained. The 2D-INADEQUATE of the labeled 1 confirmed the bonds of rings A, B, and C except the bonds of C-8 (δ 42.8)/C-14 (δ 68.6)/C-13 (δ 57.8). Moreover, the cross peaks of C-15 (δ 187.4)/C-16 (δ 114.5), C-14/C-26 (δ 171.8), and C-16/C-28 (δ 6.1) were observed. Then the ^{13}C-^{13}C spin decoupling experiments were conducted for the unidentified carbons (Fig. 2). Irradiation of C-15 and C-17 (δ 200.4) simplified the C-16 signal and irradiation of C-14 simplified the C-15 signal. Therefore a cyclopentane ring (ring D) was deduced and the long-range coupling between H_3-27 (δ 3.58)/C-26 in the HMBC revealed the methoxy residue attached to C-26. At the ring D, two carbonyl like carbons, C-15 (δ 187.4) and C-17 (δ 200.4), were neighbors of olefinic C-16 (δ 114.5). So C-15 should be an oxy-olefinic carbon. The ^{13}C signals of C-15 and C-17 were very broad suggesting that they might be in an equilibrium between keto-enol tautomers. Thus the planer structure of 1 was elucidated.

The relative configuration of 1 was examined by the differential NOE experiments. The NOEs between

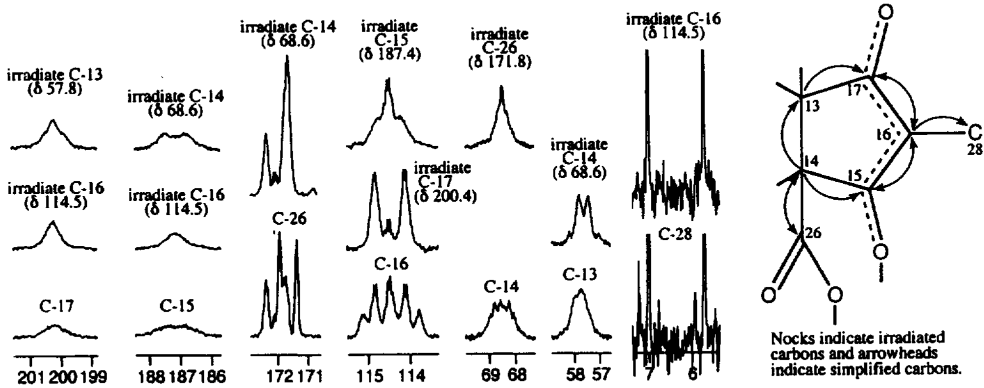


Fig. 2. Structure of 1 elucidated by ^{13}C-^{13}C spin decoupling experiments.

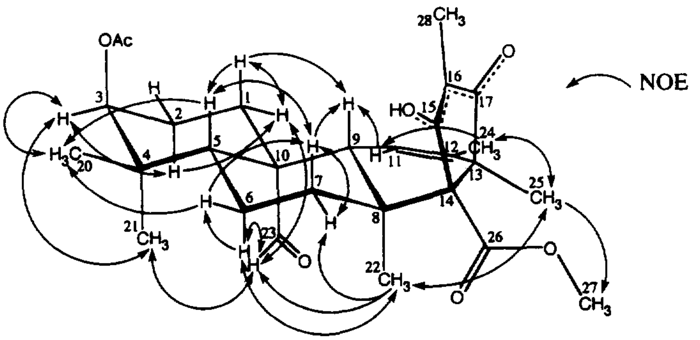


Fig. 3. NOE experiments of **1**.

H-3 (δ 4.62)/H_3-21 (δ 0.88), H-5 (δ 1.84)/H_3-20 (δ 0.95), H_α-6 (δ 2.08)/H_3-22 (δ 1.24), H_α-6/H-23 (δ 10.18), H_3-21/H-23, H_3-22/H-23, H_3-22/H_3-25 (δ 1.16), and H_3-25/H_3-27 (δ 3.58) suggested the relative configuration of **1** (Fig. 3). Absolute configuration of the skeleton of **1** was elucidated as an enantiomer of 5α,14β-androstane by the X-ray analysis of 15-(*p*-bromobenzoyl)-andrastin A[8].

The molecular formula of **2** and **3** were elucidated by HR FAB-MS as $C_{28}H_{40}O_7$ and $C_{28}H_{40}O_6$, respectively. The ^1H and ^{13}C NMR spectra of them resembled those of **1** except the proton and carbon signals of C-23. According to the HMBC experiment of **2**, the cross peaks from oxymethylene protons (δ 3.77 & 3.92, H_2-23) to C-1 (δ 30.2), C-5 (δ 50.4), C-9 (δ55.1), and C-10 (δ 43.1) indicated that **2** had a hydroxymethyl residue instead of aldehyde of **1**. Similarly, **3** was shown to have a methyl residue at C-10 by the long-range couplings from methyl protons (δ 0.95, H_3-23) to C-1 (δ 34.4), C-5 (δ 50.3), C-9 (δ 54.4), and C-10 (δ43.3). Thus the structures of **2** and **3** were elucidated.

The IC_{50} values of **1**, **2**, and **3** against PFTase (from human monocyte cells THP-1) were 24.9, 47.1, and 13.3 μM, respectively.

Recently KOSEMURA *et al.* reported citreohybridonol[9,10] that was isolated from the mycelium of a hybrid fungi and had antifeedant and insecticidal activities. Citreohybridonol had a γ-lactone ring bridged from C-6 to C-23 of andrastins. Citreohybridonol group was not sesterterpene and biosynthesized from a sesquiterpene and a tetraketide[9,10].

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6. The seed culture (600 ml) of *Penicillium* sp. FO-3929 was inoculated into a 50-liter jar fermenter containing 30 liters of a production medium, and fermented at 27°C for 112 hours. The broth supernatant was extracted with EtOAc and concentrated to give a brown oil (8.5 g). The oil was chromatographed over silica gel (430 g) using $CHCl_3$-MeOH. Andrastins were eluted with $CHCl_3$-MeOH (99:1 and 96:4). The fractions containing andrastins (4.5 g) were chromatographed over ODS silica gel (450 ml) using CH_3CN-0.05% H_3PO_4 (3:2) for **1** and **2** and CH_3CN-0.05% H_3PO_4 (4:1) for **3** to give yellow powders of crude **1** (1.71 g), **2** (340 mg), and **3** (189 mg). They were further purified by HPLC (Senshu pak Pegasil ODS, ϕ20 x 250 mm, CH_3CN-0.05% H_3PO_4 (3:2) for **1**, CH_3CN-0.05% H_3PO_4 (1:1) for **2**, and CH_3CN-0.05% H_3PO_4 (4:1) for **3**) to give white powders of **1** (1.48 g), **2** (330 mg), and **3** (36.6 mg).
7. NMR experiments were performed on JEOL JNM-EX270 or Valian Unity 400 spectrometers using the solvent signal (CD_3OD, 3.31 ppm for ^1H and 49.0 ppm for ^{13}C) as references. *Andrastin A (1)* — ^1H NMR (270 MHz) δ 10.18 (1H, s, 23-H), 5.39 (1H, br. s, 11-H), 4.62 (1H, dd, J=2.4, 2.4, 3-H), 3.58 (3H, s, 27-H_3), 3.00 (1H, ddd, J=4.0, 12.9, 13.0, 7-H), 2.30 (1H, ddd, J=3.3, 3.3, 12.4, 1-H), 2.25 (1H, ddd, J=3.1, 3.1, 12.9, 7-H), 2.13 (1H, br. s, 9-H), 2.08 (1H, m, 6-H), 2.05 (3H, s, 19-H_3), 2.05 (1H, m, 2-H), 1.84 (1H, dd, J=2.4, 15.7, 5-H), 1.75 (3H, br. s, 24-H_3), 1.70 (1H, m, 6-H), 1.59 (3H, s, 28-H_3), 1.59 (1H, m, 2-H), 1.24 (3H, s, 22-H_3), 1.16 (3H, s, 25-H_3), 0.98 (1H, ddd, J=5.0, 12.4, 13.0, 1-H), 0.95 (3H, s, 20-H_3), 0.88 (3H, s, 21-H_3); ^{13}C NMR (67.8 MHz) δ 206.8 (d, C-23), 200.4 (s, C-17), 187.4 (s, C-15), 172.2 (s, C-18), 171.8 (s, C-26), 137.0 (s, C-12), 123.6 (d, C-11), 114.5 (s, C-16), 79.0 (d, C-3), 68.6 (s, C-14), 57.8 (s, C-13), 54.8 (d, C-9), 53.4 (s, C-10), 52.2 (q, C-27), 49.6 (d, C-5), 42.8 (s, C-8), 38.0 (s, C-4), 33.5 (t, C-7), 29.0 (t, C-1), 27.1 (q, C-20), 24.3 (t, C-2), 21.5 (q, C-21), 21.1 (q, C-19), 19.84 (q, C-24), 19.76 (q, C-22), 17.9 (t, C-6), 16.0 (q, C-25), 6.4 (q, C-28). *Andrastin B (2)* — ^1H NMR (270 MHz) δ 5.71 (1H, br. s), 4.67 (1H, dd, J=2.6, 2.6), 3.92 (1H, d, J=12.2), 3.77 (1H, d, J=12.2), 3.56 (3H, s), 2.81 (1H, m), 2.26 (1H, m), 2.10 (3H, m), 2.04 (3H, s), 1.90 (1H, br. s), 1.75 (3H, br. s), 1.60 (3H, s), 1.57 (1H, m), 1.53 (1H, m), 1.50 (1H, m), 1.34 (3H, s), 1.17 (3H, s), 1.01 (1H, m), 0.99 (3H, s), 0.90 (3H, s); ^{13}C NMR (67.8 MHz) δ 202.1, 186.8, 172.5, 172.0, 134.2, 127.1, 114.4, 79.9, 68.9, 62.5, 58.1, 55.1, 52.0, 50.4, 43.1, 42.8, 37.4, 34.2, 30.2, 28.4, 25.5, 21.5, 21.2, 19.8, 18.4, 18.0, 16.0, 6.4. *Andrastin C (3)* — ^1H NMR (270 MHz) δ 5.40 (1H, br. s), 4.63 (1H, dd, J=2.4, 2.4), 3.57 (3H, s), 2.77 (1H, m), 2.10 (1H, m), 2.04 (1H, m), 2.03 (3H, s), 1.96 (1H, m), 1.81 (1H, m), 1.75 (3H, br. s), 1.60 (3H, s), 1.59 (1H, m), 1.57 (1H, m), 1.51 (1H, m), 1.43 (1H, m), 1.31 (3H, s), 1.18 (3H, s), 1.12 (1H, m), 0.95 (3H, s), 0.93 (3H, s), 0.88 (3H, s); ^{13}C NMR (67.8 MHz) δ 201.7, 188.0, 172.5, 172.0, 136.4, 126.1, 114.4, 79.6, 68.8, 58.1, 54.4, 52.0, 50.3, 43.4, 38.1, 37.7, 34.4, 34.0, 28.2, 23.5, 22.0, 21.1, 19.8, 18.8, 18.1, 17.3, 16.1, 6.3.
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