Andrastin D, Novel Protein Farnesyltransferase Inhibitor Produced by *Penicillium* sp. FO-3929

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Ras proteins have been shown to be post-translationally farnesylated on a specific carboxy-terminal cysteine by protein farnesyltransferase (PFTase). Inhibition of PFTase is expected to alter membrane localization and activation of Ras proteins[1]. In the course of screening for PFTase inhibitors of microbial origin, we have previously discovered gliotoxins[2], pepticinnamins[3,4], kurasoins[5,6], and andrastins[7~9]. Andrastins A~C (**1**~**3**, Fig. 1), were isolated from the cultured broth of *Penicillium* sp. FO-3929 and showed moderate inhibition against PFTase. They are biosynthesized from a sesquiterpene and a tetraketide and have an *ent*-5α,14β-androstane skeleton with a keto-enol tautomerism at the cyclopentane ring. We carried out further isolation for PFTase inhibitors from the cultured broth of strain FO-3929 and found andrastin D (**4**, Fig. 1). In this paper, fermentation, isolation, structure elucidation, and biological properties of **4** are described.

A stock culture of strain FO-3929 was inoculated into six 500-ml Erlenmeyer flasks containing 100 ml of a seed medium consisting of glucose 2.0%, yeast extract (Oriental Yeast Co.) 0.2%, $MgSO_4 \cdot 7H_2O$ 0.05%, Polypepton (Daigo Nutritive Chemicals) 0.5%, KH_2PO_4 0.1%, and agar 0.1% (pH 6.5) and incubated on a rotary shaker at 27°C for 2 days. Then 600 ml of the seed culture was transferred into a 50-liter jar fermenter containing 30 liters of a production medium consisting of soluble starch (Wako Pure Chemical Ind.) 1.5%, glycerol 0.5%, soybean meals 1.0%, fermipan (Gist-brocades) 0.3%, KCl 0.3%, $CaCO_3$ 0.2%, $MgSO_4 \cdot 7H_2O$ 0.05%, and KH_2PO_4 0.05% (pH 6.5). The fermentation was carried out at 27°C for 112 hours with agitation of 250 rpm and aeration of 15 liters/minute.

The cultured broth (30 liters) was centrifuged and the supernatant was adjusted to pH 3 with 6N HCl. Andrastins were extracted with EtOAc. The EtOAc layer was concentrated under reduced pressure to give a brown oil, which was applied onto a silica gel column with $CHCl_3$. Fractions were eluted with $CHCl_3$ and $CHCl_3$-MeOH. The fractions eluted with $CHCl_3$ - MeOH (99:1 and 96:4) were concentrated under reduced pressure to give a yellow powder (4.5 g). The powder was dissolved in a small volume of MeOH, applied on a ODS silica gel column (450 ml, YMC*GEL ODS-AQ-120-S50, YMC Co., Ltd.) prepared with CH_3CN-0.05% H_3PO_4 (3:2) and eluted with the same solution. Compound **4** was separated from the other andrastins by this chromatography, and the fractions containing **4** were concentrated under reduced pressure to give a yellow powder (150 mg). It was further purified by HPLC under the following conditions: column, Senshu pak Pegasil ODS (i.d. 20×250 mm, Senshu Scientific Co., Ltd.); mobile phase, CH_3CN-0.05% H_3PO_4 (3:2); flow rate, 8 ml/minute; detection, UV 285 nm. The fraction of **4** eluted at 35 minutes was concentrated to remove CH_3CN, extracted with EtOAc at pH 3, and concentrated to dryness to give a white powder of **4** (58.5 mg).

The molecular formula of **4** was deduced to be $C_{26}H_{36}O_5$ (*m/z* 429.2642 $(M+H)^+$; calcd for $C_{26}H_{37}O_5$, 429.2641) by HR-FAB-MS. The other physico-chemical properties of **4** were as follows. MP 114~120°C. $[\alpha]_D$ −69.6° (*c* 1.0, MeOH). UV λ_{max} (MeOH) nm (ε) 211 (11,130), 235 (sh, 3,600), 255 (2,960), 290 (sh, 2,440). IR ν_{max} (KBr) cm^{-1} 3440, 2950, 2880, 1741, 1704, 1618, 1457, 1432, 1382, 1319, 1209, 1135, 1029, 1002. The UV and IR spectra of **4** were similar to other andrastins.

The structure of **4** was examined by ^1H and ^{13}C NMR, DEPT, ^1H-^1H COSY, HMQC, and HMBC experiments. Chemical shifts in the ^1H and ^{13}C NMR of **4** are shown in Tables 1 and 2 in comparison with those of **3**. The ^{13}C NMR spectra of **4** resembled those of **3** except carbon signals of ring A. Therefore the structure of rings B, C, and D were supposed to be the same as that of other andrastins. The ^1H-^1H COSY and the HMBC experiments confirmed the structure of rings B, C, and D (Fig. 2). As for ring A of **4**, an acetyl (δ 172.5 and 21.1) and C-3 methine (δ 79.6) carbon signals of **3** were not observed and a ketone signal (δ 219.5) appeared. The cross peaks from H_2-1 (δ 1.30, 2.07), H_2-2 (δ 2.35, 2.64), H_3-20 (δ 1.09), and H_3-21 (δ 1.06) to the ketone carbon were observed in the HMBC experiment, which indicated that C-3 of the ring A is a carbonyl group. Therefore the structure of **4** was elucidated as 3-deacetoxy-3-oxo derivative of **3**.

The relative configuration of **4** was examined by the differential NOE experiments. As shown in Fig. 3, 5α,14β-androstane skeleton was elucidated by NOEs

Fig. 1. Structures of andrastins A, B, C, and D (**1**~**4**).

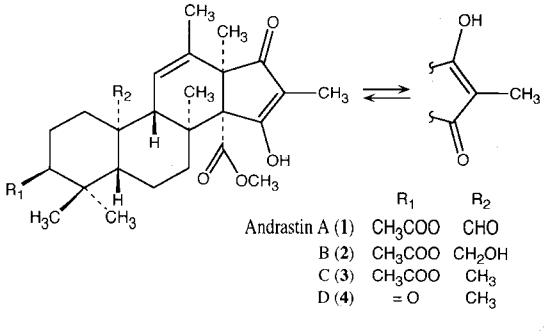

Table 1. ^{1}H NMR data of **3** and **4** in CD_3OD.

Position	**3** (270 MHz)	**4** (400 MHz)
1	1.12 m, 1.57 m	1.30 ddd (6.4, 11.7, 13.0), 2.07 ddd (3.6, 7.1, 13.0)
2	1.59 m, 1.96 m	2.35 ddd (3.6, 6.4, 16.0), 2.64 ddd (7.1, 11.7, 16.0)
3	4.63 dd (2.4, 2.4)	
5	1.43 m	1.45 dd (2.3, 12.3)
6	1.51 m, 2.04 m	1.54 dddd (2.3, 3.2, 4.0, 13.6), 1.72 dddd (3.4, 12.3, 13.1, 13.6)
7	2.10 m, 2.77 m	2.15 ddd (3.2, 3.4, 13.3), 2.78 ddd (4.0, 13.1, 13.3)
9	1.81 m	1.80 br s
11	5.40 br s	5.43 br s
19	2.03 s	
20 (eq)	0.88 s	1.09 s
21 (ax)	0.93 s	1.06 s
22	1.31 s	1.34 s
23	0.95 s	1.08 s
24	1.75 br s	1.80 br s
25	1.18 s	1.20 s
27	3.57 s	3.57 s
28	1.60 s	1.58 s

The CD_3OD signal (3.31 ppm) was used as a reference. The coupling constants (Hz) are in parentheses.

Table 2. ^{13}C NMR data of **3** and **4** in CD_3OD.

Position	**3** (67.8 MHz)	**4** (100 MHz)
1	34.4 t	39.9 t
2	23.5 t	34.8 t
3	79.6 d	219.5 s
4	37.7 s	48.6 s
5	50.3 d	55.6 d
6	18.8 t	20.2 t
7	34.0 t	33.6 t
8	38.1 s	37.7 s
9	54.4 d	54.0 d
10	43.4 s	43.1 s
11	126.1 d	125.7 d
12	136.4 s	136.9 s
13	58.1 s	58.1 s
14	68.8 s	68.8 s
15	188.0 s	186.6 s
16	114.4 s	114.6 s
17	201.7 s	201.1 s
18	172.5 s	
19	21.1 q	
20 (eq)	28.2 q	26.6 q
21 (ax)	22.0 q	21.6 q
22	18.1 q	18.0 q
23	17.3 q	16.8 q
24	19.8 q	19.9 q
25	16.1 q	16.1 q
26	172.0 s	171.9 s
27	52.0 q	52.1 q
28	6.3 q	6.3 q

The CD_3OD signal (49.0 ppm) was used as a reference.

Fig. 2. Structure of **4** elucidated by ^{1}H-^{1}H COSY and HMBC.

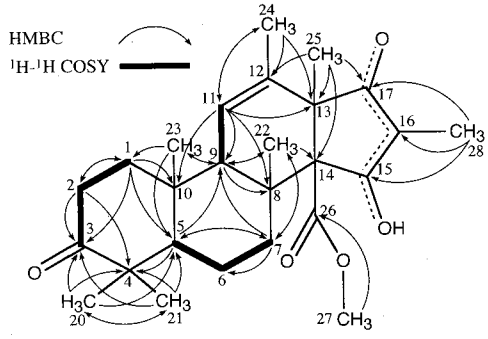

Fig. 3. NOE experiments of **4**.

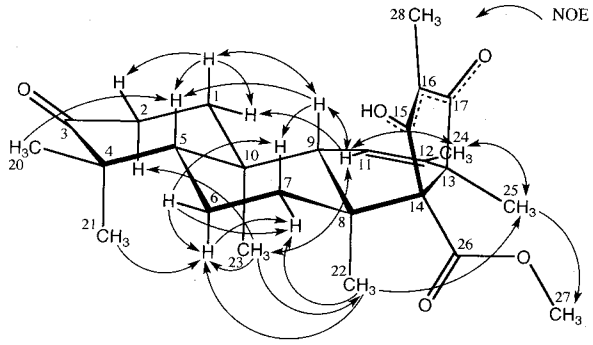

Table 3. Inhibitory activity of andrastins against PFTase.

	IC_{50} (μM)
Andrastin A (**1**)	24.9
Andrastin B (**2**)	47.1
Andrastin C (**3**)	13.3
Andrastin D (**4**)	25.7

between H_β-1 (δ 1.30)/H-5 (δ 1.45), H_β-1/H-9 (δ 1.80), H-5/H-9, H_α-6 (δ 1.72)/H_3-22 (δ 1.34), H_α-6/H-23 (δ 1.08), H_3-22/H_3-23, H_3-22/H_3-25 (δ 1.20), and H_3-25/H_3-27 (δ 3.57). Because **4** was coproduced with **1**, the absolute configuration of **4** was suggested to be the same as that of **1**. Thus the structure of **4** was deduced to be *ent*-15-hydroxy-14β-methoxycarbonyl-4,4,8,12,16-pentamethyl-3,17-dioxo-5α-androst-11,15-diene and its tautomer (Fig. 1).

The PFTase inhibitory activity of **4** was measured according to the method described previously[3]. The IC_{50} value of **4** against PFTase are shown in Table 3. The inhibitory activity of **4** was similar to that of **1** and weaker than that of **3**. Compound **4** showed no antimicrobial activities against bacteria, yeast, and fungi tested.

Recently some fungal metabolites were classified as meroterpenoid (mixed polyketide-terpenoid) group[10]. The 25 carbons were not originated solely from iso-

prenoid units, but were originated from a tetraketide and a sesquiterpene. Among meroterpenoids, andrastins and citreohybridones have *ent*-5α,14β-androstane skeleton[9,11]. All of their C-3 positions are attached by acetoxy moiety. Compound **4** is the first one having 3-keto moiety among *ent*-5α,14β-androstane meroterpenoids. Its C-3 hydroxyl seems to be oxidized instead being acetylated. Such 3-keto moiety was observed in the other meroterpenoid group of compound, terretonin[12,13], and their biosynthetic relationship is interested.

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