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NOTE

Sartorypyrone D: a new NADH-fumarate reductase inhibitor produced by *Neosartorya fischeri* FO-5897

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NADH-fumarate reductase (NFRD) is an enzyme composed of complex I (NADH-quinone reductase) and complex II (quinol-fumarate reductase) in an anaerobic electron transport chain. The NFRD system uses fumarate as a terminal electron acceptor in the mitochondrial electron transport chain and can generate ATP in the absence of oxygen.[1] This system allows helminths to live in anaerobic circumstances inside host mammals. Mammals do not have NFRD in their mitochondria, therefore a selective inhibitor of NFRD is expected to be a good anthelmintic medicine.

We have screened for inhibitors of NFRD using *Ascaris suum* (roundworm) mitochondria and previously reported the discovery of nafuredin,[2] atpenins,[3] paecilaminol,[4] verticipyrone[5] and ukulactones[6] from fungal secondary metabolites. In the course of this screening, we obtained a new NFRD inhibitor, sartorypyrone D (**1**). In this paper, we report the structural elucidation of **1,** and the NFRD inhibitory activities of **1** and its derivatives, sartorypyrone A (**2**)[7] and aszonapyrones A (**3**)[8] and B (**4**),[9] produced by a fungal strain *Neosartorya fischeri* FO-5897 (Figure 1).

Strain FO-5897 was isolated from a soil sample collected in Funabashi city, Chiba, Japan. Morphologically, this strain was classified in the genus *Aspergillus*. The ITS sequence of strain FO-5897 was elucidated and deposited at the DNA Data Bank of Japan, with the accession number AB921976. The ITS sequence of FO-5897 was compared with sequences in the MycoBank database by MycoID, pairwise sequence alignments[10] and it had a 99.3% similarity with that of CBS 544.65 (neotype of *A. fischeri*). From this information and morphological characteristics, FO-5897 was identified with *N. fischeri* (anamorph: *A. fischeri*).[11]

The strain *N. fischeri* FO-5897 was maintained on an LcA slant consisting of 0.1% glycerol, 0.08% KH_2PO_4, 0.02% K_2HPO_4, 0.02% $MgSO_4{\cdot}7H_2O$, 0.02% KCl, 0.2% $NaNO_3$, 0.02% yeast extract and 1.5% agar (adjusted to pH 6.0 before sterilization). A loopful of spores of the strain was inoculated into 100 ml of seed culture medium consisting of 2.0% glucose, 0.5% Polypepton (Nihon Pharmaceutical, Tokyo, Japan), 0.2% yeast extract, 0.2% KH_2PO_4, 0.05% $MgSO_4{\cdot}7H_2O$ and 0.1% agar (adjusted to pH 6.0 before sterilization) in a 500-ml Erlenmeyer flask, followed by incubation on a rotary shaker at 27 °C for 3 days. One milliliter of the seed culture was inoculated into each of 76 500-ml Erlenmeyer flasks containing a production medium (50 g of water sodden rice) and the culture was maintained in static condition at 25 °C for 14 days.

Moldy rice (3.8 kg) was extracted with 7.6 l of MeOH. After the rice was separated by filtration, 1 l of water was added to the extract and MeOH was then removed from the extract *in vacuo*. The aqueous solution was extracted with the same volume of EtOAc three times and the organic layer was concentrated *in vacuo* to afford a brown oil (3.17 g). The oil was applied to a silica gel open column (50ϕ × 120 mm, 0.063–0.200 mm, Merck, Darmstadt, Germany) and eluted with *n*-hexane-$CHCl_3$-MeOH system to give eight fractions (100:0:0, 80:20:0, 50:50:0, 0:100:0, 0:99:1, 0:98:2, 0:90:10 and 0:0:100, each 1 l). The sixth fraction (0:98:2 fr., 534 mg) was applied to an ODS column (30ϕ × 40 mm, Senshu Scientific Co., Tokyo, Japan) and eluted with each 100 ml of 50, 60, 65, 70, 75, 80, 85, 90 and 100% MeOH. The 75% MeOH eluate (17.1 mg) was applied to preparative HPLC (Pegasil ODS, 20ϕ × 250 mm, Senshu Scientific Co.) with 65% aqueous CH_3CN containing 0.1% TFA (flow rate, 7.0 ml min^{-1}; detection, UV 300 nm). The peaks with retention times of 24 and 37 min were collected and concentrated *in vacuo* to dryness to afford aszonapyrone B (**4**, 3.4 mg) and sartorypyrone D (**1**, 6.8 mg), respectively. The fifth fraction of silica gel column chromatography (0:99:1 fraction, 715 mg) was chromatographed by an ODS column (30ϕ × 35 mm, Senshu Scientific Co., eluted with aqueous MeOH system). The 80% MeOH fraction (237 mg) was purified by preparative HPLC (Pegasil ODS, 20ϕ × 250 mm, Senshu Scientific Co.) with 65% aqueous CH_3CN containing 0.1% TFA (flow rate, 7.0 ml min^{-1}; detection, UV 300 nm) to afford aszonapyrone A (**3**, Rt 70 min, 37 mg) and sartorypyrone A (**2**, Rt 78 min, 103 mg).

Compound **1** was yellowish white amorphous, $[\alpha]_D^{23} = -14.3$ ($c = 0.1$, $CHCl_3$), UV (MeOH) λ_{max} nm (log ε) 203 (4.6), 230 (sh, 3.5) and 290 (4.0). The molecular formula was established as $C_{26}H_{38}O_4$ with eight degrees of unsaturation by HR-ESI-MS

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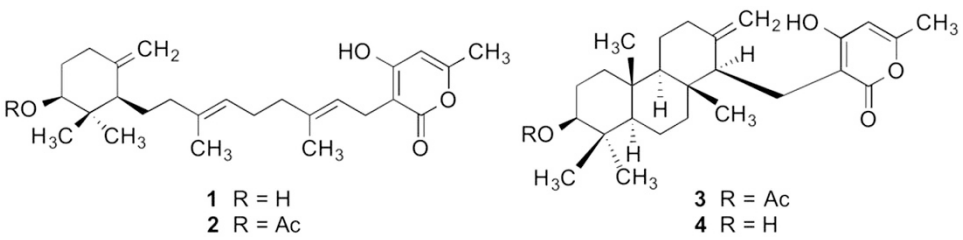

Figure 1 Structures of sartorypyrone D (**1**) and sartorypyrone A (**2**) and aszonapyrone A (**3**) and aszonapyrone B (**4**).

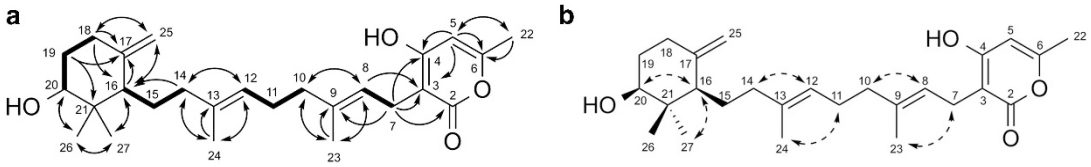

Figure 2 (**a**) ^{1}H-^{1}H COSY (bold lines) and ^{1}H-^{13}C HMBC (arrows) correlations of sartorypyrone D (**1**). (**b**) Selected NOESY correlations of **1**.

Table 1 ^{1}H and ^{13}C NMR spectral data of sartorypyrone D in $CDCl_3$ (^{1}H, 400 MHz, ^{13}C, 100 MHz)

Position	*δ_C (p.p.m.)*	*δ_H (p.p.m.), int., mult. (J in Hz)*
2	165.9	—
3	100.7	—
4	165.8	—
5	100.7	5.82, 1H, s
6	160.3	—
7	22.9	3.23, 2H, br.d (7.0)
8	120.4	5.32, 1H, br.t (7.0)
9	140.6	—
10	39.6	2.09, 2H, br.t (7.0)
11	26.0	2.10, 2H, br.t (7.0)
12	123.2	5.03, 1H, br.t (7.0)
13	136.3	—
14	38.6	1.75, 1H, m
		2.05, 1H, m
15	23.9	1.55, 1H, m
		1.60, 1H, m
16	51.2	1.63, 1H, br.d (11.0)
17	147.3	—
18	32.4	1.98, 1H, ddd (13.0, 12.0, 5.0)
		2.33, 1H, ddd (13.0, 5.0, 5.0)
19	32.0	1.52, 1H, m
		1.86, 1H, dddd (13.0, 5.0, 5.0, 5.0)
20	77.3	3.43, 1H, dd (10.0, 5.0)
21	40.4	—
22	19.7	2.18, 3H, s
23	16.3	1.78, 3H, s
24	16.2	1.59, 3H, s
25	108.5	4.60, 1H, s
		4.86, 1H, s
26	16.2	0.74, 3H, s
27	26.1	1.02, 3H, s

Table 2 NFRD and NADH oxidase inhibitory activities and antimicrobial activities of compounds 1–4

Enzyme	*Complex*	*IC_{50} (μM)*			
		1	***2***	***3***	***4***
NFRD and NADH oxidase inhibitory activities					
NADH-fumarate reductase	I+II	1.7	0.6	8.7	72.5
NADH oxidase	I+III+IV	3.0	1.3	87.0	241.2
		Inhibition zone (mm)			
		1	***2***	***3***	***4***
Antimicrobial activities (10 μg per 6 mm paper disk)					
Gram (+)					
Bacillus subtilis		8.0	8.0	7.0	—
Kocuria rhizophila		9.0	11.0	14.0	—
Mycobacterium smegmatis		10.0	10.0	10.0	14.0
Gram (−)					
Escherichia coli		—	—	—	—
Xanthomonas oryzae		—	—	—	—
Yeast					
Candida albicans		—	—	—	—
Saccharomyces cerevisiae		—	—	—	—
Fungi					
Aspergillus niger		—	—	—	—
Mucor racemosus		—	—	—	—

Abbreviation: IC_{50}, half-maximal inhibitory constant.

(*m/z* 437.2669 ([M+Na]$^{+}$, calculated for $C_{26}H_{38}O_4Na$, 437.2667). The IR spectrum (KBr) showed characteristic absorptions at 3428, 2935, 1681, 1585, 1450, 1265 and 1025 cm^{-1}, suggesting the presence of hydroxyl and carbonyl groups.

The similarities of UV and ^{1}H NMR spectra suggested **1** was an analog of **2**. The MW of **1** was 42 mass units smaller than that of **2**, which suggested **1** to be a deacetylated derivative of **2**. By analysis of the 1D and 2D NMR spectra (Figure 2a and Table 1), **1** was assigned as 20-*O*-deacetylated sartorypyrone A.

The relative configuration of **1** was elucidated by NOESY. The coupling constant of H-20 (J = 9.5 Hz) suggested that it is axial, and the NOESY correlation between H-16 and H-20 suggested H-16 was also axial. Therefore, the relative configurations of positions 16 and 20 are deemed to be 16*S**, 20*S**. Furthermore, the configurations of the

two double bonds in the geranyl moiety were determined to be 8*E*, 12*E*, as the NOESY correlations were observed between H-7 and H_3–23, between H-8 and H-10, between H-11 and H_3-24 and between H-12 and H-14. Thus, the relative configuration of **1** was elucidated as the same as that of **2** (Figure 2b). Compound **1** is a new analog of sartorypyrone A (**2**), and it was named sartorpyrone D. Although the structure of sartorypyrone D has been proposed as a hypothetical biosynthetic intermediate of sartorypyrone A,[7] we could show its existence as a metabolite of the producing fungus strain, *N. fischeri*, in this study.

The inhibitory activities of compounds **1–4** against mitochondrial respiratory enzymes were measured using submitochondrial particles of *A. suum* and bovine heart, as previously described.[6] As shown in Table 2, sartorypyrones D (**1**) and A (**2**) inhibited NFRD potently, but also showed comparable inhibitory activity against mammalian NADH oxidase, which was composed of complex I, III and IV. In contrast to **1** and **2**, cyclized compounds, aszonapyrones A (**3**) and B (**4**), exhibited moderate NFRD inhibitions. However, the selectivity against mammalian NADH oxidase was higher than **1** and **2**. These results suggest that the geranyl moiety connecting two rings in sartorypyrones might have an important role in NFRD inhibition similar to other NFRD inhibitors.[2,3,5,6] In addition, acetylated compounds, sartorypyrone A (**2**) and aszonapyrone A (**3**), showed more potent inhibitory activities against both enzymes comparing with that of each nonacetylated analogs (Table 2). Therefore the acetyl moiety in both the compounds may contribute to enhance these inhibitory activities.

The antimicrobial activities of **1–4**, against nine species of microorganisms were measured by a paper disk method[12] (Table 2). A paper disk (6 mmϕ, Advantec, Toyo Roshi Kaisha, Ltd., Tokyo, Japan) containing 10 μg of each sample was placed on the agar plate. Compounds **2** and **3** was reported to show anti-Gram-positive bacterial activities.[13] In our study, **1, 2** and **3** showed antibacterial activities against all tested Gram-positive bacteria, *B. subtilis*, *K. rhizophila* and *M. smegmatis*, whereas **4** showed antibacterial activity only against *M. smegmatis*.

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