The Journal of Antibiotics (2017) 70, 395–399
© 2017 Japan Antibiotics Research Association All rights reserved 0021-8820/17
www.nature.com/ja

ORIGINAL ARTICLE

Decatamariic acid, a new mitochondrial respiration inhibitor discovered by pesticidal screening using drug-sensitive *Saccharomyces cerevisiae*

Yoshihiro Watanabe[1], Takuya Suga[2], Satomi Narusawa[2], Masato Iwatsuki[1,2], Kenichi Nonaka[1,2], Takuji Nakashima[1,2], Yasuo Shinohara[3], Takahiro Shiotsuki[4], Naoya Ichimaru[5], Hideto Miyoshi[6], Yukihiro Asami[1,2], Satoshi Ōmura[2] and Kazuro Shiomi[1,2]

A new decalin, decatamariic acid, was isolated from a cultured broth of the fungus *Aspergillus tamarii* FKI-6817. Its absolute configuration was elucidated by NMR and electronic circular dichroism. Decatamariic acid (10 μM) elicited ~50% inhibition of the ATP production in mitochondria isolated from wild-type *Saccharomyces cerevisiae* without affecting the activities of respiratory enzymes. The action manner of this compound may be interesting as a possible seed for new pesticides.
The Journal of Antibiotics (2017) **70,** 395–399; doi:10.1038/ja.2016.164; published online 18 January 2017

INTRODUCTION

ATP, produced in the mitochondrial inner membrane, is the common energy source for living organisms, including arthropods. Several commercial insecticidal and acaricidal compounds inhibit electron transport system complex I, including fenpyroximate,[1] fenazaquin[2] and pyrimidifen,[3] and against complex III, such as strobilurin A[4] originating from a fungus. They have proved to be safe for mammals by their selective activity against arthropods. Recently, we have established a new screening system aimed at finding a mitochondrial inhibitor, using a recombinant *Saccharomyces cerevisiae*, and have found two new mitochondrial inhibitors, ascosteroside C[5] and trichopolyn VI,[6] produced by fungi. In the field of natural products chemistry and chemical biology, *S. cerevisiae* has been widely used for screening and functional study of bioactive compounds,[7] and it is suitable for bioassay-guided screening of various drug candidates. However, there is a problem in that *S. cerevisiae* can exhibit high levels of drug resistance. Therefore, we used a drug-sensitive BY25929 strain of *S. cerevisiae* that has no multidrug-resistance genes (*yrr1*, *yrs1*, *pdr1* and *pdr3*) in this study.[8] Growth inhibition against the drug-sensitive strain was compared with that of wild-type *S. cerevisiae*, and the cultured broths that inhibited only the drug-sensitive strain were selected. The 9009 samples were screened and 476 and 429 samples inhibited drug-sensitive strain and wild-type strain, respectively. Among the broths having such selectivity, we screened those that showed growth inhibition against drug-sensitive BY25929 only in glycerol-containing medium (1% yeast extract, 2% Peptone, 3% glycerol and 1.5% agar) and not in glucose-containing medium (1% yeast extract, 2% Peptone, 3% glucose and 1.5% agar). Thus we could obtain cultured broths that may affect mitochondrial function.[5,6]

As a result, our screens showed that *Aspergillus tamarii* strain FKI-6817 produced a new compound (FKI-6817A; **1**) that inhibited the growth of drug-sensitive BY25929 strain of *S. cerevisiae*. Compound **1** inhibited the growth of drug-sensitive budding yeast only in a glycerol-containing medium, and it elicited ~50% inhibition of the ATP production in isolated mitochondria. We herein report taxonomic studies of the *A. tamarii* strain and the isolation, physicochemical properties, structural elucidation and biological activities of **1**.

RESULTS AND DISCUSSION

Taxonomy of strain FKI-6817

Colonies on Czapek yeast extract agar (CYA) were 46–47 mm in diameter after 7 days at 25 °C (Figure 1a), velutinous, corrugate, colliculose, with white aerial mycelium, covered with olive (3F8) conidia, with irregular margin; clear exudate was produced, soluble pigment was not produced; reverse were yellow (3B8). Colony colors were designated according to the *Methuen Handbook of Colour*.[9] Colonies on malt extract agar (MEA) were 37–39 mm in diameter after 7 days at 25 °C (Figure 1b), velutinous, colliculose, with white aerial mycelium, covered with olive yellow (3D8) conidia, with irregular margin; exudate and soluble pigment were not

[1]Department of Drug Discovery Sciences, Graduate School of Infection Control Sciences, Kitasato University, Tokyo, Japan; [2]Kitasato Institute for Life Sciences, Kitasato University, Tokyo, Japan; [3]Institute for Genome Research, Tokushima University, Tokushima, Japan; [4]Institute of Agrobiological Sciences, National Agriculture and Food Research Organization, Ibaraki, Japan; [5]Faculty of Bioenvironmental Science, Kyoto Gakuen University, Kyoto, Japan and [6]Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto, Japan
Correspondence: Professor S Ōmura or Professor K Shiomi, Kitasato Institute for Life Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan.
E-mail: omuras@insti.kitasato-u.ac.jp or shiomi@lisci.kitasato-u.ac.jp
Dedicated to Professor Julian Davies for his long-standing career in the studies on antibiotics.
Received 20 August 2016; revised 1 December 2016; accepted 14 December 2016; published online 18 January 2017

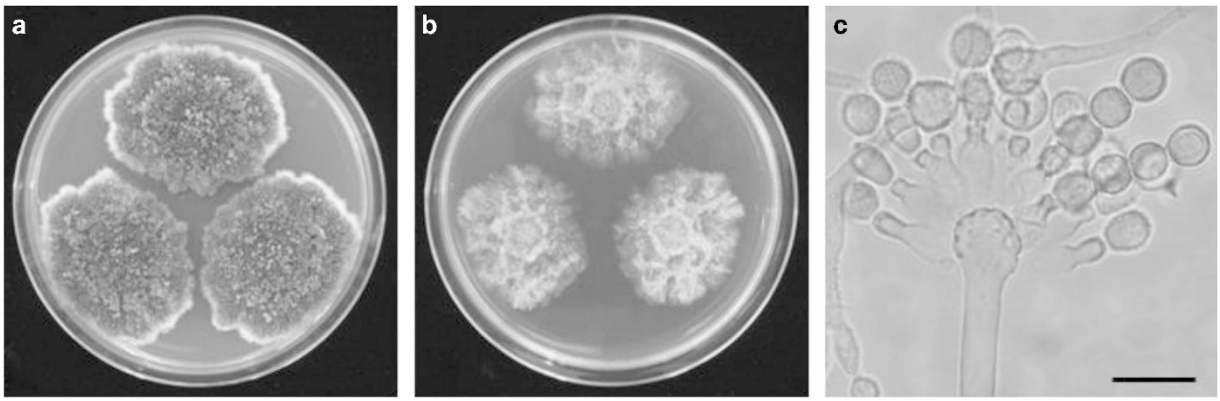

Figure 1 *Aspergillus tamarii* FKI-6817. (**a**) Photograph of colonies grown on Czapek yeast extract agar (CYA) after 7 days. (**b**) Photograph of colonies grown on malt extract agar (MEA) after 7 days. (**c**) Micrograph of conidiophore on MEA. Scale bar: 20 μm

produced; reverse were white (1A1). Colonies on Czapek yeast agar with 20% sucrose (CY20S) were 27–28 mm in diameter after 7 days at 25 °C, velutinous, corrugate, plane, with white aerial mycelium, covered with olive yellow (3C8) conidia, with entire margin; exudate and soluble pigment were not produced; reverse were light yellow (3A5). Colonies on Czapek Dox agar (CZ) were 28–30 mm in diameter after 7 days at 25 °C, velutinous, corrugate, colliculose, with white aerial mycelium, covered with olive (3E8) conidia, with entire margin; exudate and soluble pigment were not produced; reverse were yellowish white (3A2). Colonies on CYA were 48–52 mm in diameter after 7 days at 37 °C, velutinous, corrugate, colliculose, with white (1A1) aerial mycelium, covered with olive (3F8) conidia, with entire margin; exudate and soluble pigment were not produced; reverse were olive (3E4).

Conidiophores on MEA were borne on a basal felt or directly from the agar, stipes were simple, (80–)200–900 × 6–11 μm, rough-walled and uncolored. Vesicles were globose to pyriform, 9.3–17.2 μm wide; uniseriate conidial heads were present (Figure 1c). Phialides were usually covering the entire surface of the vesicle, 7.3–13.0 (–14.7) × (2.3–)3.2–6.0 μm. Conidia borne in chains, were globose, roughened with thick walls, (4.5–)5.5–10(–11.5) μm in diameter. From the above morphological characteristics, strain FKI-6817 was classified into the genus *Aspergillus*.[10]

To determine the most closely related *Aspergillus* species, the DNA sequence of the internal transcribed spacer (ITS), β-tubulin gene (BT2) and calmodulin gene (CMD) regions of FKI-6817 were compared with sequences in the GenBank database by BLASTN 2.3.1 analysis,[11] showing that the strain FKI-6817 had 100% similarity with the ITS sequences of NRRL 20818 (ex-type of *A. tamarii*: GenBank accession number AF004929), 99.3% similarity with the BT2 sequences of NRRL 20818 (GenBank accession number AY017540) and 99.1% similarity with the CMD sequences of NRRL 20818 (GenBank accession number EF661526).

From the results of morphological characteristics and DNA analysis, the strain FKI-6817 was identified as *A. tamarii*.

Physico-chemical properties of 1

Compound **1** was obtained as a colorless oil. It showed optical rotation of $[\alpha]_D^{27}$ +8.8 ($c = 0.1$, CH_3OH), IR absorptions (attenuated total reflectance) of ν_{max} 3324, 2931, 1704, 1450, 1380 and 1172 cm^{-1} and UV λ_{max} (ε) 201 (900) nm in CH_3OH. The molecular formula of **1** was elucidated as $C_{20}H_{32}O_3$ from the $[M+NH_4]^+$ peak at *m/z* 338.2688 (calcd. for $[M+NH_4]^+$, *m/z* 338.2689) in high-resolution electrospray ionization mass spectrometry analysis, accounting for five degrees of unsaturation.

Table 1 NMR data for 1 in CD_3OD

Position	δ_C	δ_H*(int., mult., J in Hz)*
1	37.2	1.25 (1H, m)
		1.73 (1H, m)
2	19.9	1.45 (1H, m)
		1.77 (1H, m)
3	42.8	1.18 (1H, m)
		1.43 (1H, m)
4	34.2	
5	52.7	1.27 (1H, m)
6	20.0	1.70 (1H, m)
		1.72 (1H, m)
7	32.4	2.00 (1H, m)
		2.10 (1H, m)
8	132.5	
9	138.0	
10	40.1	
11	33.6	2.95 (1H, d, 16.8)
		3.10 (1H, d, 16.8)
12	176.8	
13	43.9	2.45 (1H, d, 15.8)
		2.80 (1H, d, 15.8)
14	137.3	
15	124.8	5.30 (1H, dd, 6.4, 12.0)
16	59.4	4.09 (2H, d, 7.2)
17	16.6	1.60 (3H, s)
18	22.1	0.88 (3H, s)
19	33.7	0.91 (3H, s)
20	20.5	1.02 (3H, s)

NMR spectra were recorded at 400 MHz for ^1H and 100 MHz for ^{13}C.

Structure elucidation of 1

The structure of **1** was elucidated by 1D and 2D NMR. The ^1H and ^{13}C NMR spectral data of **1** in CD_3OD are listed in Table 1. The ^1H NMR spectrum of **1** indicated the presence of four methyls, eight sp^3 methylenes, one sp^3 methine and one sp^2 methine. The ^{13}C NMR spectrum of **1** showed the resolved resonances of 20 carbons, including 1 carbonyl carbon (δ_C 176.8). The ^1H-^1H COSY revealed the presence of two partial structures 1-H_2 (δ_H 1.25, 1.73)/2-H_2 (δ_H 1.45, 1.77)/3-H_2 (δ_H 1.18, 1.43) and 6-H_2 (δ_H 1.70, 1.72)/7-H_2

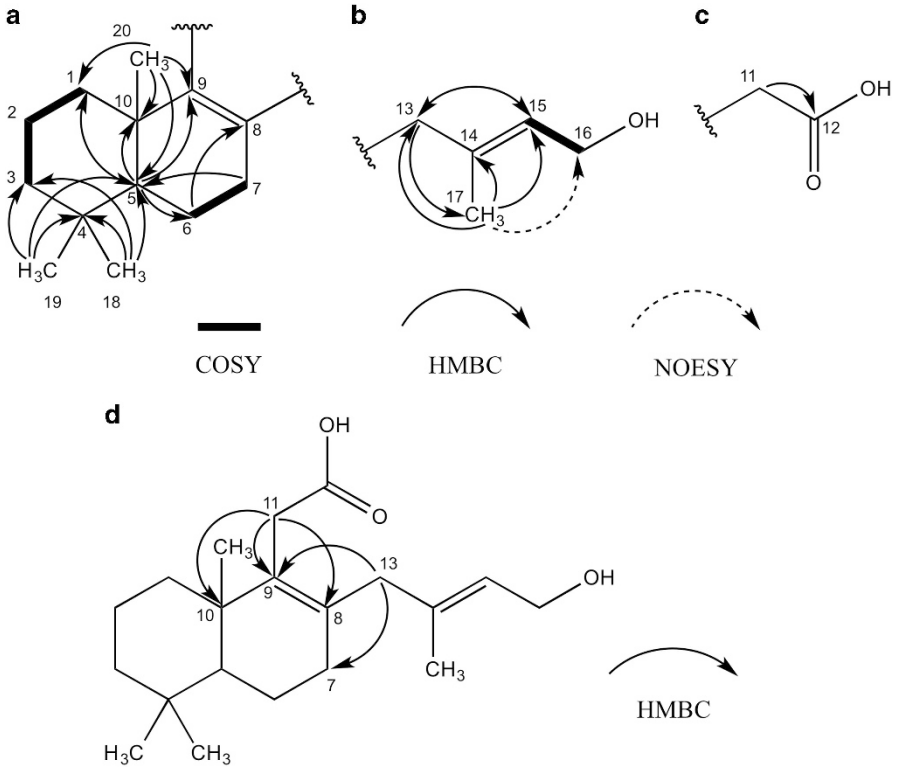

Figure 2 (**a**–**c**) The partial structures and key correlations of gCOSY, HMBC and NOESY in **1**. (**d**) Connection of the partial structures by correlations of HMBC in **1.**

(δ_H 2.00, 2.10). HMBC correlations from 20-H_3 (δ_H 0.88) to C-1 (δ_C 37.2), C-5 (δ_C 52.7), C-9 (δ_C 138.0) and C-10 (δ_C 40.1); from 18-H_3 (δ_H 1.02) and 19-H_3 (δ_H 0.91) to C-3(δ_C 42.8), C-4 (δ_C 34.2) and C-5; from 7-H_2 (δ_H 2.00, 2.10) to C-5; from 6-H_2 (δ_H 1.70, 1.72) to C-8 (δ_C 132.5); and from 5-H (δ_H 1.27) to C-1, C-6 (δ_C 20.0), C-9 and C-10 revealed the partial structure that formed a 5,5,9-trimethyl-$\Delta^{1,2}$-octalin moiety (Figure 2a). The partial structure of 2-methyl-2-buten-4-ol was established by HMBC correlations from 17-H_3 (δ_H 1.60) to C-13 (δ_C 43.9), C-14 (δ_C 137.3) and C-15 (δ_C 124.8); from 13-H_2 (δ_H 2.45, 2.80) to C-15 and C-17 (δ_C 16.6); and from 15-H (δ_H 5.30) to C-13 and the COSY observed between 15-H and 16-H_2 (δ_H 4.09) (Figure 2b). The NOESY between 16-H_2/17-H_3 established the configuration as 14*E*. The C5 unit was connected to the $\Delta^{1,2}$-octalin moiety at the C-8 position, indicated by HMBC correlations from 13-H_2 to C-9 and C-7 (δ_C 32.4) (Figure 2d). Finally, the HMBC correlation from 11-H_2 (δ_H 2.95, 3.10) to C-12 (δ_C 176.8) and the correlations from 11-H_2 to C-8, C-9 and C-10 indicated that the remaining ethanoic acid moiety (Figure 2c) was linked to the $\Delta^{1,2}$-octalin moiety at the C-9 position, and the planar structure of **1** was assigned as shown in Figure 2d. This diterpene was named decatamariic acid. The relative configuration of **1** was elucidated by NOESY analysis. The NOESY correlations of 2-H_{ax}/20-H_3, 3-H_{eq}/18-H_3, 5-H/19-H, 6-H_{ax}/18-H and 6-H_{ax}/20-H_3 suggested that the relative configuration of **1** was 5*R**,10*R** (Figure 3a).

Absolute configuration of 1

Comparing the electronic circular dichroism (ECD) spectra of **1** with the calculated ECD spectra provided information about the absolute configuration of **1**. The lowest energy conformer of **1** was run with the Monte Carlo algorithm implemented in Spartan'14 using the Merck molecular force field. Conformational searches run by semiempirical PM3 calculations in Gaussian 09[12] were further refined by density functional theory at the B3LYP/TZVP level, which yielded additional relevant conformers. The calculated conformer structures were consistent with our ROESY analyses. As the Boltzmann distribution of **1** was 0.331 as the highest value, it was suggested that the partial structures of propionic acid and 3-methylpent-2-en-1-ol are rotating. To conduct accurate calculation, the core structure without propionic acid and 3-methylpent-2-en-1-ol was calculated by the method mentioned above. The calculated conformers for the core structure of **1** are shown in Supplementary Information S1, together with their atomic coordinates and energies. The ECD spectra of **1** were simulated by time-dependent density functional theory at the same level of theory for the relevant conformers. Comparing the experimental and calculated ECD spectra showed that the absolute configuration of **1** was 5*R*,10*R* (Figure 3b).

Biological activity of 1

The biological activities of **1**, together with electron transport inhibitors strobilurin B (complex III inhibitor) and carboxin (complex II inhibitor), were evaluated against a drug-sensitive *S. cerevisiae* strain BY25929 and two endogenous AAC-disrupted *S. cerevisiae* strains (one *Tribolium castaneum* ADP/ATP carrier (AAC) and one *Acyrthosiphon pisum* AAC) in glycerol-containing medium. An AAC-disrupted *S. cerevisiae* strain with empty vector was evaluated in glucose-containing medium[13,14] (Table 2). The BY25929 strain was also evaluated in glucose-containing medium. Yeast *S. cerevisiae* can produce ATP predominantly in its mitochondria in glycerol-containing medium; thus if the compound can inhibit *S. cerevisiae* growth in this system, it may inhibit the mitochondrial functions. In

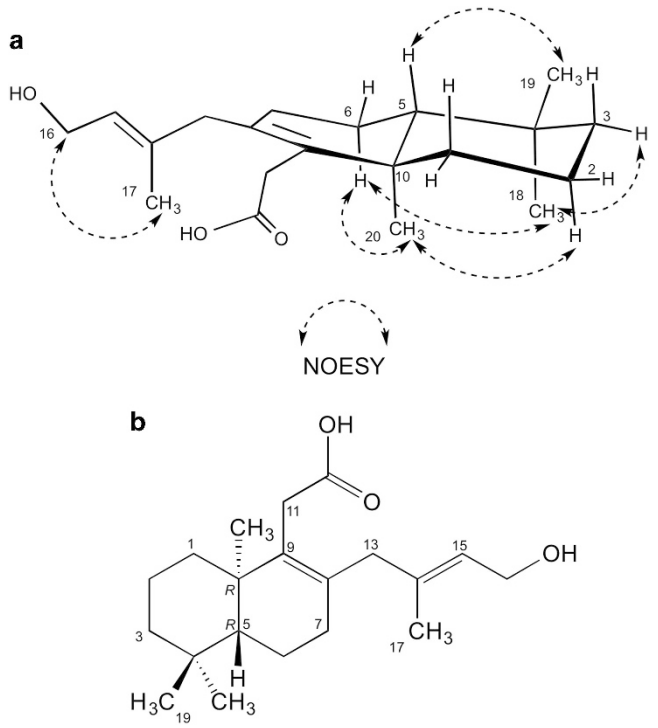

Figure 3 Structure of **1**. (**a**) The relative configuration of **1** by NOESY. (**b**) The absolute configuration of **1**.

Table 2 Growth inhibition activity of 1 against various recombinant *S. cerevisiae*

		Drug-sensitive S. cerevisiae		*ΔAAC* S. cerevisiae		
Compound	*μg per disc*	*Glycerol medium*	*Glucose medium*	*Empty vector strain*	Tribolium castaneum *strain*	Acyrthosiphon pisum *strain*
1	10.0	13.5	–	–	–	–
	3.0	8.5	–	–	–	–
	1.0	–	–	–	–	–
	0.3	–	–	–	–	–
Strobilurin B	3.0	45.4	–	–	47.0	16.5
	1.0	40.0	–	–	46.6	15.3
	0.3	39.2	–	–	45.6	–
	0.1	37.7	–	–	41.6	–
Carboxin	3.0	20.9	–	–	19.1	24.1
	1.0	13.4	–	–	13.5	17.0
	0.3	7.3	–	–	8.7	10.0
	0.1	–	–	–	–	–

Results of paper disc assays of various *S. cerevisiae* strains. The drug-sensitive BY25929 strain was cultured in glycerol- or glucose-containing medium. Three endogenous AAC-disrupted and single-copy-type yeast shuttle vector pRS314YA2P-expressing strain were used. An 'Empty vector' strain was cultured in glucose-containing medium, and *Tribolium castaneum* AAC-expressing and *Acyrthosiphon pisum* AAC-expressing organisms were cultured in glycerol-containing medium. Test compounds were dissolved in MeOH at appropriate concentrations. Inhibition zone diameters are shown in millimeters. –; No inhibition.

contrast, *S. cerevisiae* produces ATP mainly in the cytosol in glucose-containing medium. If a compound inhibits *S. cerevisiae* growth in glucose-containing medium, its target may be other than mitochondria.[5] Compound **1** (3 and 10 μg per disc) inhibited the growth of drug-sensitive *S. cerevisiae* in glycerol-containing media but did not inhibit growth in glucose-containing media.

Thus **1** inhibited some mitochondrial functions in drug-sensitive *S. cerevisiae*. Strobilurin B and carboxin inhibited the drug-sensitive *S. cerevisiae* and insect (*T. castaneum* or *A. pisum*) AAC-expressing *S. cerevisiae* in glycerol-containing medium. However, **1** did not inhibit AAC-expressing *S. cerevisiae* in glycerol-containing medium. This suggests that **1** was basically ineffective in insect (*T. castaneum* or *A. pisum*) AAC-expressing *S. cerevisiae* strains because they are not drug-sensitive, and thus **1** may have a mechanism of action different from the other drugs. Therefore, we studied the inhibitory activity of **1** using isolated mitochondria from *S. cerevisiae*.[15] ATP production was reduced to 50% by 10 μM of **1** without affecting the activities of respiratory enzymes in mitochondria isolated from wild-type *S. cerevisiae*. These results strongly suggest that **1** inhibited ATP production in mitochondria. In addition, cell growth inhibition assays were performed using several tumor cell lines. The IC_{50} values of **1** were 250, 118, 95, 162 and 88 μM against A549, Panc1, HT29, HT1299 and HeLa S3 cells, respectively. Compounds that have a decalin skeleton, such as forskoditerpene A[16] and havardic acid B,[17] have similar structures to **1**, but there are no reports that these compound inhibit mitochondrial function.

In conclusion, **1** was isolated from a culture broth of the fungus *A. tamarii* (strain FKI-6817). The structure of **1** was elucidated by NMR study, and the absolute configuration of **1** was revealed by ECD study. Compound **1** was identified as a new decalin. We showed that **1** inhibited mitochondrial ATP production in *S. cerevisiae*. Further comprehensive chemical and biological studies of **1** may lead to the development of much-needed potent new insecticides with novel modes of action.

MATERIALS AND METHODS

General experiments

Reverse-phase column chromatography was conducted on YMC-gel ODS-A (150 μm). A Pegasil ODS SP 100 column was purchased from Senshu Scientific Co. (Tokyo, Japan). Liquid chromatography-electrospray ionization-tandem mass spectrometry (LC/MS/MS) was performed on a QSTAR Hybrid LC/MS/MS using MeOH solvent system containing 2 mM ammonium acetate (AB Sciex, Framingham, MA, USA). NMR spectra were measured with a Varian XL-400 spectrometer (Agilent Technologies, CA, USA; ^{1}H NMR at 400 MHz, ^{13}C NMR at 100 MHz) in CD_3OD. The chemical shifts are expressed in parts per million and are referenced to MeOH-d_4 (3.31 p.p.m.) in the ^{1}H NMR spectra and to MeOH-d_4 (49.0 p.p.m.) in the ^{13}C NMR spectra. IR spectra (attenuated total reflectance) were obtained on a Fourier transform IR spectrometer (FT-210, Horiba Ltd., Kyoto, Japan). UV spectra were measured with a spectrophotometer (U-2801, Hitachi Ltd., Tokyo, Japan). Optical rotation was measured with a polarimeter (P-2200, JASCO Corp., Tokyo, Japan). ECD spectra were measured with a J-720 (JASCO Corp.).

Taxonomic studies of the strain FKI-6817

The strain FKI-6817 was isolated from soil collected on Haha-jima, the Bonin Islands, Tokyo, Japan. For determination of the morphological characteristics of FKI-6817 using the methodology of Klich,[10] the isolate was inoculated as three-point cultures on CYA, MEA, Czapek yeast agar with 20% sucrose (CY20S) and Czapek Dox agar (CZ) and grown at 25 °C (also at 37 °C on CYA) for 7 days in the dark. Colony colors were designated according to the *Methuen Handbook of Colour*.[9] For determination of the micro-morphological characteristics of FKI-6817, microscopic slides were prepared using colonies from MEA. The slides were examined with a Vanox-S AH-2 microscope (Olympus, Tokyo,

Japan), and digital photomicrographs were taken with a DP25 digital camera (Olympus). DNA extraction and PCR amplification of the ITS region, including the 5.8S rRNA gene, BT2 and CMD regions, were conducted under the conditions detailed by Nonaka *et al.*[18] Sequencing products were purified using the BigDye XTerminator Purification Kit (Applied Biosystems, Foster City, CA, USA), and samples were analyzed on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). Contigs were assembled using the forward and reverse sequences with the SeqMan Pro program from the Lasergene 10 package (DNASTAR Inc., Madison, WI, USA). The sequences determined in this study were deposited at the GenBank with accession numbers LC002782 (ITS), LC002782 (BT2) and LC002782 (CMD).

Yeast growth inhibition assay

The growth inhibitory effects of **1**, strobilurin B and carboxin were evaluated using the paper disc method on agar plates inoculated with recombinant *S. cerevisiae* strains. *S. cerevisiae* strain BY25929, whose multidrug-resistance genes (Δ*yrr1*, Δ*yrs1*, Δ*pdr1*, Δ*pdr3*) are deleted, was provided by the National Bio-Resource Project, Japan. This yeast was grown on both glycerol-containing medium (1% yeast extract, 2% Peptone, 3% glycerol and 1.5% agar) and glucose-containing medium (1% yeast extract, 2% Peptone, 2% glucose and 1.5% agar). *S. cerevisiae* WB-12 pRS314YA2P/y2NtcAAC, an endogenous AAC-disrupted and *T. castaneum* AAC-expressing strain (accession number XP_973257), and *S. cerevisiae* WB-12 pRS314YA2P/y2NapAAC, an endogenous AAC-disrupted and *A. pisum* AAC-expressing strain (accession number NM_001162030), were grown on a glycerol-containing medium. *S. cerevisiae* WB-12 pRS314YA2P, an endogenous AAC-disrupted and single-copy yeast shuttle vector pRS314YA2P-expressing strain (empty vector strain), was grown on a glucose-containing medium as the references.[5,6] Sterile filter discs impregnated with each compound solution (10 μl) were placed on the agar plate, and the plates were incubated at 30 °C for 48 h. After incubation, the inhibition zones were measured.

Fermentation

A. tamarii strain FKI-6817 was grown and maintained on an agar 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 loop of spores was inoculated into the seed medium (100 ml) consisting of 2.0% glucose, 0.5% Polypepton (Nihon Pharmaceutical Co., Japan), 0.2% yeast extract, 0.1% KH_2PO_4, 0.05% $MgSO_4{\cdot}7H_2O$ and 0.1% agar (adjusted to pH 6.0 before sterilization) in each of five 500 ml Erlenmeyer flasks. The flasks were incubated on a rotary shaker (210 r.p.m.) at 27 °C for 2 days. The seed culture (25 ml) was inoculated into each of the 20 culture bags (Ulpack 47, Hokken Co. Ltd., Tochigi, Japan) containing a production medium (500 g of wet rice). Static fermentation was continued at 27 °C for 14 days.

Isolation

The stationary culture (10 kg) was extracted with 50% ethanol (15 liters). After filtration, the filtrate was concentrated *in vacuo* to remove the ethanol. The obtained aqueous solution was applied to an ODS chromatography column (600 ml resin, YMC Co., Kyoto, Japan). After washing with H_2O (5 liters), the column was eluted stepwise with 20% methanol (2.0 liters), 40% methanol (2.0 liters), 50% methanol (2.0 liters), 60% methanol (2.0 liters) and 100% methanol with 0.1% trifluoroacetic acid (TFA). The active compound was eluted with 100% methanol with 0.1% TFA, the active fractions being concentrated *in vacuo* before being applied to an ODS chromatography column (300 ml resin). After washing with H_2O (1.0 liters), the column was eluted stepwise with 70% methanol (1.0 liters), 80% methanol (1.0 liters), 90% methanol (1.0 liters), 100% methanol (1.0 liters) and 100% methanol with 0.1% TFA. The active compound was eluted with 80% methanol, and the solution was concentrated *in vacuo*. The remaining aqueous solution (600 ml) was extracted with ethyl acetate (3 × 600 ml), and the organic layer was concentrated *in vacuo* to dryness. The ethyl acetate extract (400 mg) was purified by HPLC (Pegasil ODS, 10 i.d. × 250 mm, Senshu Scientific Co.) with an isocratic solvent system of 40% acetonitrile–water at a flow rate of 4.0 ml min^{-1}. The peak with the retention time of 47–55 min was collected and freeze-dried to afford **1** (11.2 mg).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The study was supported by the Programme for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry from the Bio-oriented Technology Research Advancement Institution of Japan and by the Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry, Japan. We are grateful to Dr Kenichiro Nagai and Ms Noriko Sato, School of Pharmacy, Kitasato University for help in obtaining NMR and MS data and are grateful to Dr Rokuro Masuma and Dr Hidetaka Yuge, Kitasato University for suggestions concerning the experiments.

1 Friedrich, T. *et al.* Two binding sites of inhibitors in NADH: ubiquinone oxidoreductase (complex I). Relationship of one site with the ubiquinone-binding site of bacterial glucose: ubiquinone oxidoreductase. *Eur. J. Biochem.* **15**, 691–698 (1994).
2 Balba, H. Review of strobilurin fungicide chemicals. *J. Environ. Sci. Health B* **42**, 441–451 (2007).
3 Eisinger, M. *et al.* Pyrimidifen intoxication. *Ann. Emerg. Med.* **42**, 289–291 (2003).
4 Ago, P. Mucidin, the first Czechoslovakian antibiotic. *Minerva Med.* **25**, 37–38 (1970).
5 Suga, T. *et al.* Ascosteroside C, a new mitochondrial respiration inhibitor discovered by pesticidal screening using recombinant *Saccharomyces cerevisiae*. *J. Antibiot. (Tokyo)* **68**, 649–652 (2015).
6 Suga, T. *et al.* Trichopolyn VI: a new peptaibol insecticidal compound discovered using a recombinant *Saccharomyces cerevisiae* screening system. *J. Gen. Appl. Microbiol.* **61**, 82–87 (2015).
7 Chinen, T., Nagumo, Y. & Usui, T. Construction of a genetic analysis-available multidrug sensitive yeast strain by disruption of the drug efflux system and conditional repression of the membrane barrier system. *J. Gen. Appl. Microbiol.* **60**, 160–162 (2014).
8 Miyamoto, Y. *et al.* Identification of *Saccharomyces cerevisiae* isoleucyl-tRNA synthetase as a target of the G1-specific inhibitor reveromycin A. *J Biol. Chem.* **277**, 28810–28814 (2002).
9 Kornerup, A. & Wanscher, J. H. *Methuen Handbook of Colour* 3rd edn. Eyre Methuen, London, UK, (1978).
10 Klich, M. A. *Identification of Common Aspergillus Species*, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, (2002).
11 Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**, 3389–3402 (1997).
12 Frisch, M. J. *et al. Gaussian 09, Revision D.01*, Gaussian, Wallingford, CT, USA, (2009).
13 Hashimoto, M. *et al.* Expression of the bovine heart mitochondrial ADP/ATP carrier in yeast mitochondria: significantly enhanced expression by replacement of the N-terminal region of the bovine carrier by the corresponding regions of the yeast carriers. *Biochim. Biophys. Acta* **1409**, 113–124 (1999).
14 Asami, Y. *et al.* RK-805, an endothelial cells growth inhibitor, produced by *Neosartorya* sp. *Tetrahedron* **60**, 7085–7091 (2004).
15 Yamada, A. *et al.* Ca^{2+}-induced permeability transition can be observed even in yeast mitochondria under optimized experimental conditions. *Biochim. Biophys. Acta* **1787**, 1486–1491 (2009).
16 Shan, Y. *et al.* Diterpenes from *Coleus forskohlii* (WILLD.) BRIQ. (Labiatae). *Chem. Pharm. Bull.* **56**, 52–56 (2008).
17 Jolad, S. D. *et al.* Havardic acids A-F and havardiol, labdane diterpenoids from *Grindelia havardii*. *Phytochemistry* **26**, 483–489 (1987).
18 Nonaka, K. *et al. Penicillium viticola*, a new species isolated from a grape in Japan. *Mycoscience* **52**, 338–343 (2011).

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