J. Gen. Appl. Microbiol., **61**, 57–62 (2015)
doi 10.2323/jgam.61.57

jGAM

Full Paper

Ukulactone C, a new NADH-fumarate reductase inhibitor produced by *Talaromyces* sp. FKI-6713

(Received January 6, 2015; Accepted January 23, 2015)

Satoru Kaifuchi,[1] Mihoko Mori,[1,2] Kenichi Nonaka,[1,2] Rokuro Masuma,[2] Satoshi Ōmura,[2] and Kazuro Shiomi[1,2,*]

[1] *Graduate School of Infection Control Sciences, Kitasato University, Tokyo 108-8641, Japan*
[2] *Kitasato Institute for Life Sciences, Kitasato University, Tokyo 108-8641, Japan*

Screening for NADH-fumarate reductase inhibitors led to the isolation of a new ukulactone analog, ukulactone C, as a major polyene compound produced by *Talaromyces* sp. FKI-6713. The structure of the compound was elucidated as a reduced analog of ukulactone A by 1D- and 2D-NMR experiments. Ukulactone C possessed a potent inhibitory activity (IC_{50} = 62 nM) against NADH-fumarate reductase of the roundworm *Ascaris suum in vitro*.

Key Words: electron transport enzyme inhibitor; NADH-fumarate reductase; *Talaromyces*; ukulactone

Introduction

Our long-term research program is focused on the discovery of new antiparasite antibiotics produced by naturally occurring microorganisms. Several parasite diseases have been significantly curtailed or overcome in developed and developing countries, but re-emerging diseases caused by parasites remain a continual problem worldwide. In developing countries, where resources and medicines are scarce or misused, parasitic diseases can quickly become a major threat to public health. Helmintic parasitic diseases are also important in animal health as they represent a major threat to human food supplies, thereby creating a comprehensive obstacle to improvements in both human health and socioeconomic development.

We selected anaerobic energy metabolism as a target for a specific screening project (Kita et al., 2007). Many multicellular helminths living in their hosts cannot get enough oxygen, they consequently use an anaerobic metabolism mechanism to meet their energy needs. Electrons supplied from NADH, produced through glycolysis and the TCA cycle, pass through complex I, ubiquinone, complex III and complex IV (from low to high redox potential). In mammals, the final step involves oxygen accepting the electrons to produce water. The proton density gradient caused by this electron transport is used to generate ATP at F_OF_1-ATPase (complex V). Electrons are also passed from succinate to ubiquinone by complex II and move to complex III and complex IV. In the anaerobic mechanism, succinate is produced from fumarate by the reverse enzymatic reaction of complex II, which is called fumarate reductase, and succinate is subsequently changed to volatile acids. Rhodoquinone is used for this reverse reaction instead of ubiquinone, and rhodoquinone produced by fumarate reductase is reduced at complex I. This system is not as effective as mammalian oxidative phosphorylation because glucose cannot be degraded to carbon dioxide and water. However, it does allow the production of ATP in the absence of oxygen.

Many inhibitors of the electron transport system and oxidative phosphorylation have been reported from microbial origins, such as piericidin (complex I inhibitor), antimycin (complex III inhibitor), and oligomycin (complex V inhibitor) (Ueki et al., 2000). A complex II inhibitor, siccanin, is used to treat dermatomycosis, and some analogs of a complex III inhibitor, strobilurin, are used in agriculture for protection against phytopathogenic fungi. However, inhibitors having specificity to the anaerobic metabolism have not been reported. Thus, we screened inhibitors of NADH-fumarate reductase composed of complex I and the reverse reaction of complex II of helminth

*Corresponding author: Kazuro Shiomi, Graduate School of Infection Control Sciences, Kitasato University, 5-9-1, Shirokane, Minato-ku, Tokyo 108-8641, Japan.
TEL and FAX: +81-3-5791-6131 E-mail: shiomi@lisci.kitasato-u.ac.jp

None of the authors of this manuscript has any financial or personal relationship with other people or organizations that could inappropriately influence their work.

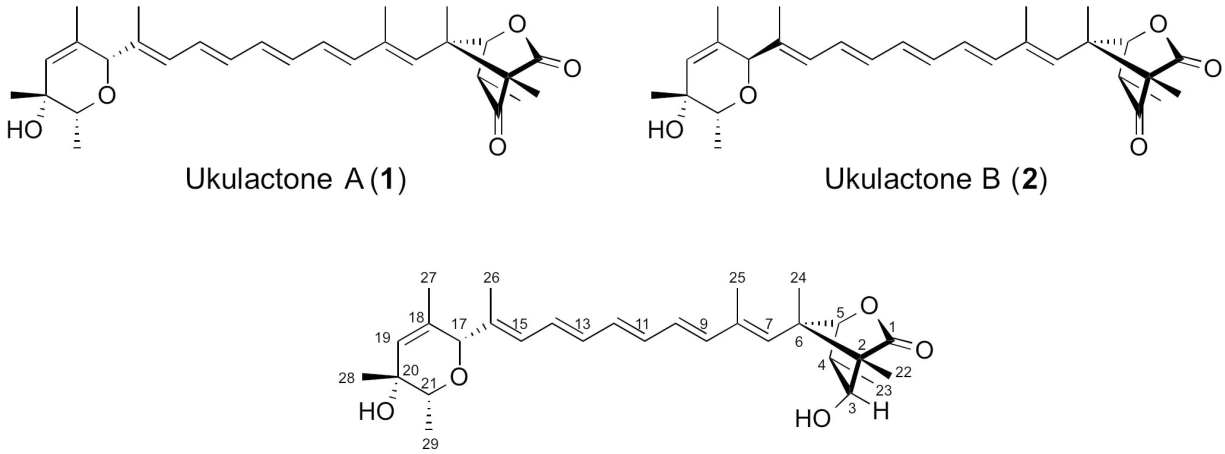

Fig. 1. Structures of ukulactones A–C (**1**–**3**).

Table 1. Physicochemical data of **3**.

Appearance	Pale yellow syrup
Molecular weight	468
Molecular formula	$C_{29}H_{40}O_5$
HR-ESI-MS	
Found	491.2767 $[M+Na]^+$
Calcd.	491.2773 $[M+Na]^+$
$[\alpha]_D^{24}$ (c = 0.1, MeOH)	+3.6
UV λ_{max} nm (log ε) in MeOH	241 (3.6), 293 (sh, 4.2), 308 (sh, 4.5), 322 (4.8), 338 (5.0), 356 (5.0)
IR ν_{max} cm^{-1} (KBr)	3733, 3394, 2973, 2938, 1774
Solubility	
Soluble	MeOH, $CHCl_3$, EtOAc
Insoluble	H_2O

anaerobic energy metabolism using the mitochondrion fraction of the roundworm *Ascaris suum*. Several new compounds have been isolated in this screening, such as nafuredin (Ōmura et al., 2001; Ui et al., 2001), paecilaminol (Ui et al., 2006a) and verticipyrone (Ui et al., 2006b).

Recently, we isolated novel compounds which we named ukulactones A (**1**) and B (**2**) (Fig. 1) from the culture broth of *Penicillium* sp. FKI-3389 (Mori et al., 2011). They have a common skeleton composed of two rings, 2-oxabicyclo[2.2.1]heptane-3,5-dione and 5,6-dihydro-2*H*-pyran, connected with pentaene, and **2** is an epimer of **1** at the junction of the pyran ring and pentaene. Compound **1** was a potent NADH-fumarate reductase inhibitor and inhibited complex I of *Ascaris suum* at the IC_{50} value of 0.055 μM, whereas its inhibition against bovine complex I was 28 μM (IC_{50}). Compound **2**, the epimer of **1**, was 200-fold weaker than **1** in inhibiting NADH-fumarate reductase (IC_{50}; 2.4 nM for **1**, 470 nM for **2**).

We have now isolated a new ukulactone analog named ukulactone C (**3**), from the culture broth of a fungus, *Talaromyces* sp. FKI-6713, which showed a potent inhibitory activity against NADH-fumarate reductase (Fig. 1). Here, we report the taxonomy of the producing strain and the fermentation, isolation, structure elucidation, and some bioactivity of **3**.

Materials and Methods

Microorganisms. The fungal strain FKI-6713 was isolated from a soil sample collected in Haha-jima (Bonin Islands), Tokyo, Japan. The ITS region of FKI-6713 was compared to sequences in the GenBank database by BLASTN 2.2.30 analysis (Altschul et al., 1997). This strain had a 96.3% similarity to CBM-FA-0948 (holotype of *Talaromyces hachijoensis*, GenBank accession number AB176620). This strain resembled the genus *Penicillium* in morphology. From the results of morphological and sequencing analyses, the producing strain FKI-6713 was classified as an unidentified species of genus *Talaromyces*. The ITS sequence of the strain FKI-6713 was deposited at the DNA Data Bank of Japan with the accession number LC016747.

Culture of the fungal strain. The strain *Talaromyces* sp. FKI-6713 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 steriliza-

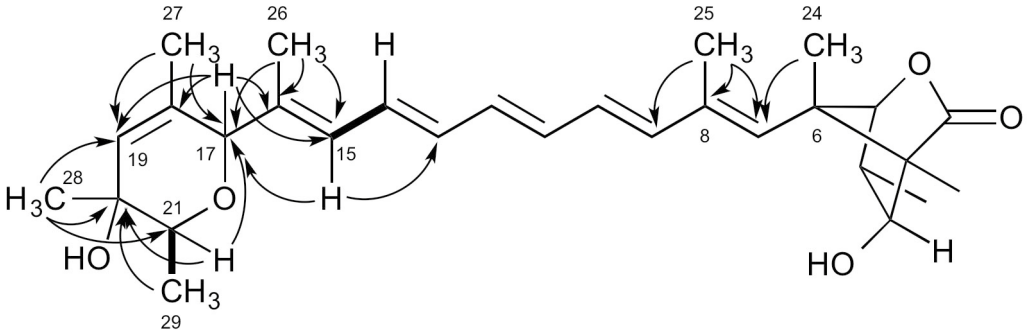

Fig. 2. COSY (bold line) and HMBC (arrow) correlations of **3**.

tion). A loopful of spores of this strain was inoculated into two 500-ml Erlenmeyer flasks, each containing 100 ml of a seed medium consisting of 2% 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) and incubated on a rotary shaker at 27°C for 5 days. Ten milliliters of the seed culture was inoculated into each of 20 culture bags (Ulpack 47, Hokken Co. Ltd., Tochigi, Japan) containing a production medium (500 g of water sodden rice), and the production culture was kept in a static condition at 25°C for 14 days.

Isolation of ukulactone C. Moldy rice (10 kg) was extracted with 10 l of MeOH. After the rice was separated by filtration, the extract was evaporated *in vacuo* to remove MeOH. The aqueous residue (2.5 L) was extracted three times with the same volume of EtOAc and the organic layer was concentrated *in vacuo* to yield a brown oil (3.65 g). The oil was applied to a silica gel open column (60ø × 100 mm, particle size: 0.063–0.200 mm, Merck, Darmstadt, Germany), and eluted with an *n*-hexane-EtOAc-MeOH system to give 5 fractions (100:0:0, 90:10:0, 80:20:0, 50:50:0 and 0:0:100, each 1 l). The 50:50:0 fraction (260 mg) was purified by preparative HPLC (column, Pegasil ODS, 20ø × 250 mm, Senshu Scientific Co.; solvent, 55% CH_3CN aq.; flow rare, 7.0 ml/min; detection, UV at 210 nm). The peak at the retention time of 48.3 min was collected and concentrated *in vacuo* to dryness to yield ukulactone C (**3**, 18.4 mg).

Physicochemical analysis. UV spectra were measured using a Hitachi UV/Vis spectrometer U-2810. Optical rotations were recorded by means of a JASCO DIP-1000 polarimeter. FT-IR spectra were conducted on Horiba FT-170. HR-ESIMS spectra were measured on a JEOL JMS-T100LP. NMR spectra were recorded on a 400 MHz NMR spectrometer (400-MR, Agilent Technologies). For calibration of ^1H and ^{13}C chemical shifts, the carbon signal and residual proton signal of $CDCl_3$ were used (δ_C 77.0 and δ_H 7.26).

Measurement of enzyme-inhibitory activity. Inhibitory activities against NADH-fumarate reductase and NADH oxidase were assayed using sub-mitochondrial particles of *Ascaris suum* and bovine heart, respectively, by a procedure previously described (Mori et al., 2011).

Measurement of cytotoxicity. MRC-5 cells were plated on 96-well flat bottom plates at a density of 1.5×10^4 cells/well with 200 μl of culture medium and incubated at 37°C with 5% CO_2 for 2 days. Test compounds in 50% dimethylsulfoxide were added to each well. After 2 days cultivation at 37°C with 5% CO_2, cell density and morphological changes in the cells were observed under a microscope. After observation, 10 μl of WST-8 solution (Dojindo Co.) was added to the cells and the plate was incubated at 37°C with 5% CO_2 for 2 h. Then, absorbance at 450 nm was measured.

Results and Discussion

Isolation and structure elucidation of ukulactone C (3)

A solid culture of *Talaromyces* sp. FKI-6713 was extracted with MeOH. The extract was subsequently extracted with EtOAc, and then the organic layer was purified by silica gel column chromatography and HPLC. Ukulactone C (**3**, 18.4 mg) was isolated and subsequently proved to be a major NADH-fumarate reductase inhibitor of this culture. We also isolated a small amount of the structurally-related analog. The analog has the same molecular weight as that of **3**, and seemed to be a stereoisomer of **3**. However, we could not clarify its structure due to its instability.

The physicochemical data of **3** is shown in Table 1. The appearance of **3** was a pale yellow syrup. The characteristic absorption maxima at 322, 338 and 356 nm in the UV spectrum, suggested that **3** has a pentaene substructure, similar to other known ukulactones. The molecular formula was established to be $C_{29}H_{40}O_5$ by HR-ESIMS (observed $[M+Na]^+$ at 491.2767, calcd $[M+Na]^+$ 491.2773). This indicated **3** has two more hydrogen atoms compared to ukulactones A (**1**) and B (**2**). The IR spectrum indicated the presence of hydroxyl (3733 and 3394 cm^{-1}) and carbonyl (1774 cm^{-1}) groups. From these analyses, **3** seemed to be a reduced analog of **1** and **2**.

The ^1H and ^{13}C NMR data of **3** are shown in Table 2. Analysis of the ^1H and ^{13}C NMR, DEPT and HSQC spectra revealed the presence of eight methyl groups, one sp^3 methine carbon, three quaternary carbons, four oxymethine carbons, nine sp^2 methine carbons, three sp^2 quaternary carbons, and one carbonyl group. Compared to **1**, there was an extra oxymethine carbon (δ 79.9) whereas one ketone carbon was lost in the structure of **3**.

The ^1H NMR spectrum of **3** was similar to that of **1** except for a new signal at 3.39 ppm. The ^1H and ^{13}C chemical shifts of the pyran ring unit of **3** were in good accordance with those of **1** in $CDCl_3$ (Mori et al., 2011). COSY

Table 2. ^{1}H and ^{13}C NMR data of **1** (in $CDCl_3$, ^{1}H: 400 MHz, ^{13}C: 100 MHz, 22°C).

Position	δ_H (Int., mult., *J* in Hz)	δ_C (mult.)	
1		177.9	s
2		60.0	s
3	3.39 (1H, d, 3.5)	79.9	d
4	2.26 (1H, qdd, 7.0, 3.5, 2.0)	45.0	d
5	4.74 (1H, d, 2.0)	88.2	d
6		56.2	s
7	5.74 (1H, s)	131.4	d
8		135.2	s
9	6.26 (1H, overlapped)	137.3	d
10	6.25 (1H, overlapped)	128.3	d
11	6.26 (1H, overlapped)	133.2	d
12	6.29 (1H, overlapped)	133.4	d
13	6.32 (1H, overlapped)	133.7	d
14	6.45 (1H, dd, 14.0, 11.0)	128.5	d
15	6.16 (1H, br.d, 11.0)	130.6	d
16		135.9	s
17	4.37 (1H, s)	85.3	d
18		136.4	s
19	5.68 (1H, br.s)	130.8	d
20		67.1	s
21	3.48 (1H, q, 6.0)	77.2	d
22	1.23 (3H, s)	6.6	q
23	1.18 (3H, d, 7.0)	14.3	q
24	1.19 (3H, s)	16.9	q
25	1.84 (3H, br.s)	14.1	q
26	1.70 (3H, br.s)	12.0	q
27	1.48 (3H, s)	18.5	q
28	1.15 (3H, s)	23.5	q
29	1.22 (3H, d, 6.0)	14.2	q

and HMBC correlations clarified that **3** had the same 3,6-dihydro-3-hydroxy-2,3,5-trimethyl-2*H*-pyran ring unit and pentaene substructure as **1** and **2** (Fig. 2; Mori et al., 2011). Therefore, it was suggested that the structural alteration occurred at the oxabicyclo[2.2.1]heptane unit in **3**.

Structure elucidation of the oxabicyclo ring substructure in **3** was carried out by comparing to the NMR data of that of **1**. The direct connections between hydrogens and carbons were assigned by an HSQC spectrum. The spin system from the oxymethine proton H-3 (δ 3.39) to another oxymethine proton H-5 (δ 4.74) through H-4 (δ 2.26) shown by COSY revealed the connection of C-3–C-4–C-5 (Fig. 3). The HMBC correlations from singlet methyl protons H_3-22 to carbonyl carbon C-1, quaternary carbons C-2 and C-6, and oxymethine carbon C-3, from doublet methyl protons H_3-23 to C-3, methine carbon C-4 and oxymethine carbon C-5, from oxymethine proton H-5 to C-1, C-2, C-3 and C-4, from singlet methyl protons H_3-24 to C-2, C-5, quaternary carbon C-6 and sp^2 methine carbon C-7 were observed. These NMR data revealed a hydroxyl moiety attached to position **3** of the oxabicyclo ring in **3**. Thus, the oxabicyclo ring substructure of 3 was assigned as shown in Fig. 3.

Because of signal overlapping at δ 6.2–6.3 ppm in the ^{1}H NMR spectrum, it was difficult to confirm the presence of the spin system and the coupling constants of seven olefin protons (from H-9 to H-15) in a pentaene moiety. However, the similarity of ^{13}C chemical shifts of the olefin methyl carbons C-25 (δ 14.1) and C-26 (δ 12.0) with those of **1** suggested the configuration of the pentaene

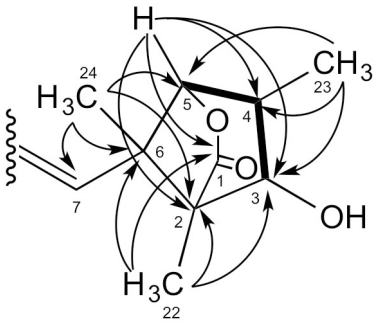

Fig. 3. COSY (bold line) and HMBC (arrow) correlations of the oxabicyclo ring substructure of **3**.

moiety in **3** was also all-*trans* (Mori et al., 2011).

The HMBC correlations from the methyl protons H_3-24 to C-7, and H_3-25 to C-7, C-8 and C-9 revealed that this oxabicyclo unit connected to C-7 of the pentaene, and the correlations from H_3-26 to C-15, C-16 and C-17 clarified that the pyran ring was connected to the other end of pentaene at position 17 (Fig. 2). Thus, the planar structure of **3** was elucidated.

We conducted NOESY and differential NOE experiments to analyze relative configurations of the pyran ring and oxabicyclo unit. The observed NOE correlations are shown in Fig. 4. By NOE correlations from H-17 to H-21, H_3-26 and H_3-27, and those from H_3-28 to H-19 and H-21, the relative conformation of the dihydropyran ring was indicated to be 17*S*,20*R*,21*R*, which is the same configuration of **1**. The values of ^{1}H and ^{13}C chemical shifts at position 17 were different among **1** (17*S* epimer; ^{1}H δ4.36, ^{13}C δ 85.2) and **2** (17*R* epimer; ^{1}H δ 4.30, ^{13}C δ 80.7) (Mori et al., 2011). The values of **3** (^{1}H δ4.37, ^{13}C δ85.3) are also in good agreement with those of **1**. In the oxabicyclo unit, the NOE correlations from H-3 to H_3-22 and H_3-23, from H-4 to H-5 and from H-5 to H_3-24 and H_3-25 were observed (Fig. 4). The relative configuration of positions 3 and 4 in reduced shimalactones A and B was studied by Wei et al. (2006). In **3**, the values of ^{1}H chemical shifts and the coupling constant of H-3 (3.4 Hz) were almost the same as those of the 3*S*,4*R*-type of the reduced compound. The differential NOE experiments also supported this finding. Therefore, the relative configuration of the oxabicyclo unit was suggested to be 2*S*,3*S*,4*R*,5*S*,6*S*. We concluded **3** is a new ukulactone analog which has a hydroxyl moiety at position 3 instead of a ketone group in the oxabicyclo ring. This is the first report of a reduced-type ukulactone analog originating as a natural compound.

Although we reported the producing strain of ukulactone A (**1**) and B (**2**) to be *Penicillium* sp. FKI-3389, we recently reclassified the producing strain as *Talaromyces allahabadensis* (unpublished). The ukulactone C-producing strain FKI-6713 is also a *Talaromyces* species, although different from the ukulactone A-producing *Talaromyces* species. HPLC analysis revealed that the strain FKI-6713 produced **3** as a major polyene compound with small amounts of minor analogs, however, **1** and **2** were not contained in the metabolites of this strain, even though the culture condition was suitable for the production of **1** and

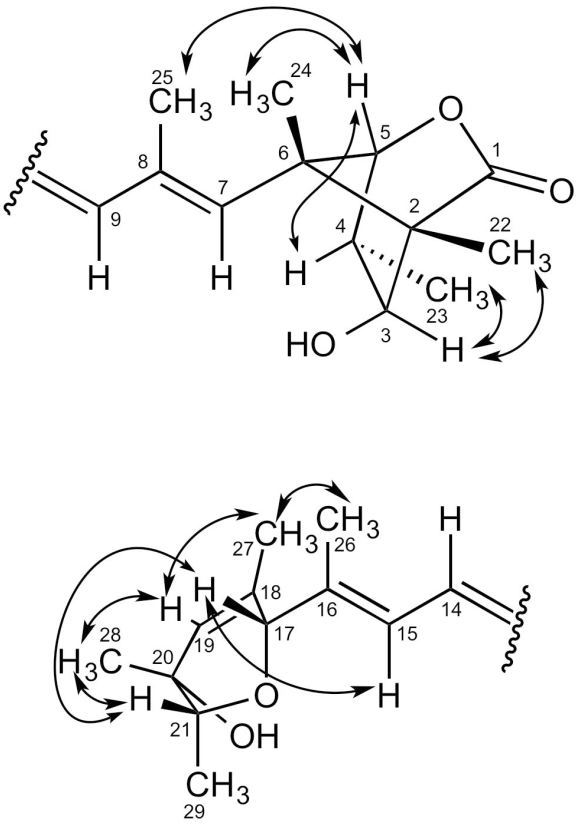

Fig. 4. NOE correlations of the pyran ring (left) and the oxabicyclo unit (right) of **3**.

2. All of the other natural ukulactone-related compounds, shimalactones A and B (Wei et al., 2005, 2006), prugosenes A1–A3 (Lang et al., 2007), coccidiostatin A (Jayasuriya et al., 2007) and wartmannilactones E, F and H (Dong et al., 2009), have a 3-ketone-type of oxabicyclo ring in their structures. The ukulactone C-producing strain may therefore have a unique 3-ketone reducing enzyme.

Biological activities

We reported that ukulactone-type polyene compounds show potent NADH-fumarate reductase inhibitory activities, especially the 17*S*-epimer ukulactone A (**1**) which was significantly more potent than the 17*R*-epimer ukulactone B (**2**), because the conformation of the dihydropyran ring changes due to the alteration of the configuration at position 17 (Mori et al., 2011). As ukulactone C (**3**) has an *S* configuration at position 17, the same as **1**, inhibitory activity against NADH-fumarate reductase should be retained.

Inhibitory activities of **3** against electron transport system enzymes were evaluated using sub-mitochondrial particles of *Ascaris suum* and bovine heart (Table 3). The IC_{50} value of **3** against NADH-fumarate reductase was 62 nM, about 26 times weaker than that of **1**, and about 5 times weaker than that of prugosene A1 (IC_{50} 13 nM), whose structure is equal to 8-demethylated **1**. However **3** is more active compared to the 17*R*-epimer, **2**. Compound **3** has an *S* configuration at position 17, the same as **1**, and thus the inhibitory activity against NADH-fumarate reductase should be retained. With respect to the inhibitory activity against mammalian enzyme, NADH oxidase (complexes

Table 3. Inhibitory activity against electron transport enzymes.

	IC_{50} (nM)	
	NADH-fumarate reductase	NADH oxidase
Ukulactone A (**1**)*	2.4	9,000
Ukulactone B (**2**)*	470	16,000
Ukulactone C (**3**)	62	10,000

*The inhibitory activities were reported by Mori et al. (2011).

I+III+IV), **3** showed a similar IC_{50} value (10,000 nM) to the 17*S*-epimers (**1** and prugosene A1). These data suggested that the conformation of the dihydropyran ring may be more important than the 2-oxabicyclo[2.2.1]heptane unit for bestowing *Ascaris* enzyme-specific inhibition.

The cytotoxicity of **3** was evaluated using human fibroblast cells (MRC-5). Morphological change and growth inhibition against human T-lymphocyte Jurkat cells were observed using **1** at IC_{50} of 21 μM (Mori et al., 2011). However, **3** showed no cytotoxicity against MRC-5 cells even at 50 μM. Consequently, the 3-hydroxyl-type ukulactone analog **3** may become a good candidate for anthelmintic development.

Acknowledgments

We thank Dr. Kenichiro Nagai and Ms. Noriko Sato, School of Pharmacy, Kitasato University for the measurement of mass and NMR spectra. This work was supported by The Ministry of Education, Culture, Sports, Science and Technology (MEXT) Grant-in-Aid for Young Scientists (B) 21780115 (to MM) and Kitasato University Research Grant for Young Researchers (to MM).

References

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z. et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.*, **25**, 3389–3402.

Dong, Y., Lin, Y., Lu, X., Zheng, Z., Ren, Z. et al. (2009) Cathepsin B inhibitory tetraene lactones from the fungus *Talaromyces wortmannii*. *Helv. Chim. Acta*, **92**, 567–574.

Jayasuriya, H., Guan, Z., Dombrowski, A. W., Bills, G. F., Polishook, J. D. et al. (2007) Isolation, structure, and coccidiostat activity of coccidiostatin A. *J. Nat. Prod.*, **70**, 1364–1367.

Kita, K., Shiomi, K., and Ōmura, S. (2007) Parasitology in Japan: Advances in drug discovery and biochemical studies. *Trends Parasitol.*, **23**, 223–229.

Lang, G., Wiese, J., Schmaljohann, R., and Imhoff, J. F. (2007) New pentaenes from the sponge-derived marine fungus *Penicillium rugulosum*: structure determination and biosynthetic studies. *Tetrahedron*, **63**, 11844–11849.

Mori, M., Morimoto, H., Kim, Y., Ui, H., Nonaka, K. et al. (2011) Ukulactones A and B, new NADH-fumarate reductase inhibitors produced by *Penicillium* sp. FKI-3389. *Tetrahedron*, **67**, 6582–6586.

Ōmura, S., Miyadera, H., Ui, H., Shiomi, K., Yamaguchi, Y. et al. (2001) An anthelmintic compound, nafuredin, shows selective inhibition of complex I in helminth mitochondria. *PNAS*, **98**, 60–62.

Ueki, M., Machida, K., and Taniguchi, M. (2000) Antifungal inhibitors of mitochondrial respiration: discovery and prospects for development. *Curr. Opin. Anti-infect. Investig. Drugs*, **2**, 387–398.

Ui, H., Shiomi, K., Yamaguchi, Y., Masuma, R., Nagamitsu, T. et al. (2001) Nafuredin, a novel inhibitor of NADH-fumarate reductase, produced by *Aspergillus niger* FT-0554. *J. Antibiot.*, **54**, 234–238.

Ui, H., Shiomi, K., Suzuki, H., Hatano, H., Morimoto, H. et al. (2006a) Paecilaminol, a new NADH-fumarate reductase inhibitor, produced by *Paecilomyces* sp. FKI-0550. *J. Antibiot.*, **59**, 591–596.

Ui, H., Shiomi, K., Suzuki, H., Hatano, H., Morimoto, H. et al. (2006b) Verticipyrone, a new NADH-fumarate reductase inhibitor, produced by *Verticillium* sp. FKI-1083. *J. Antibiot*., **59**, 785–790.

Wei, H., Itoh, T., Kinoshita, M., Kotoku, N., Aoki, S. et al. (2005) Shimalactone A, a novel polyketide, from marine-derived fungus *Emericella variecolor* GF10. *Tetrahedron*, **61**, 8054–8058.

Wei, H., Itoh, T., Kotoku, N., and Kobayashi, M. (2006) Shimalactones, neuritogenic polyketides from a marine-derived fungus *Emericella variecolor* GF10. *Heterocycles*, **68**, 111–123.