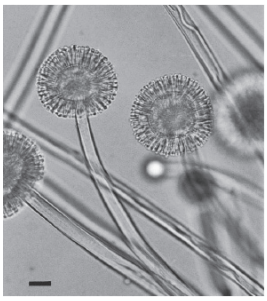
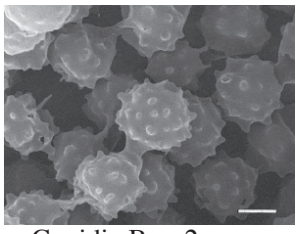
Nafuredin

1. Discovery, producing organism and structure[1-7)]

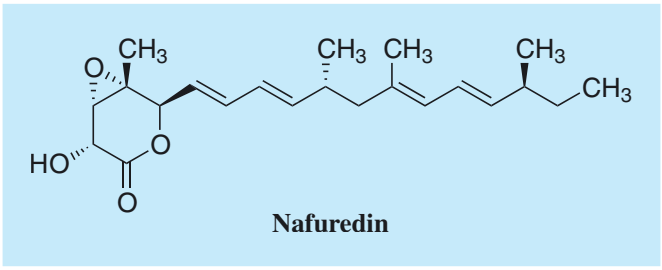
Nafuredin was isolated from the culture broth of *Aspergillus niger* strain FT-0554 and recognized as an inhibitor of helminth NADH-fumarate reductase. Its target was revealed to be complex I (NADH-quinone oxidoreductase), and it was identified as a selective inhibitor of helminth complex I. Nafuredin showed anthelmintic activity against *Haemonchus contortus* in *in vivo* studies. The structure of nafuredin was elucidated as β,γ-epoxy-δ-lactone with a branched side chain, and its absolute configuration was revealed synthetically.



Conidiophore and conidiogenous cells
Bar: 20 μm



Conidia Bar: 2 μm
Aspergillus niger FT-0554



Nafuredin

2. Physical data

White powder. $C_{22}H_{32}O_4$; mol wt 360.50. Sol. in DMSO, EtOH, EtOAc, $CHCl_3$. Insol. in H_2O, hexane.

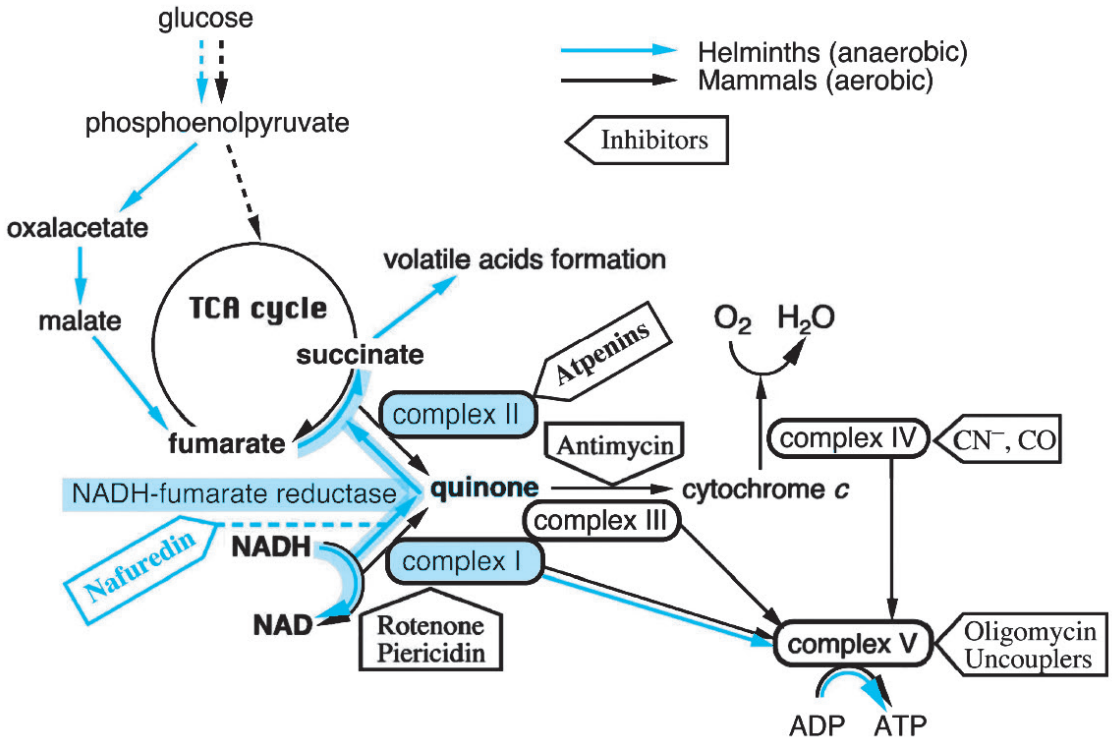
3. Biological activity[1,4)]

1) Effects on electron transport enzymes

		IC_{50} (nM)			
	Complex	*Ascaris suum* (adult)	*Ascaris suum* (L2)	*Haemonchus contortus* (adult)	Rat liver
NADH-fumarate reductase	I+II	12	NT	NT	1,000
NADH-ubiquinone reductase	I	8	8.9	86	10,000
NADH-rhodoquinone reductase	I	24	9.0	195	>100,000
Rhodoquinol-fumarate reductase	II	80,000	NT	NT	NT
Succinate-ubiquinone reductase	II	>100,000	NT	NT	>100,000

NT : not tested

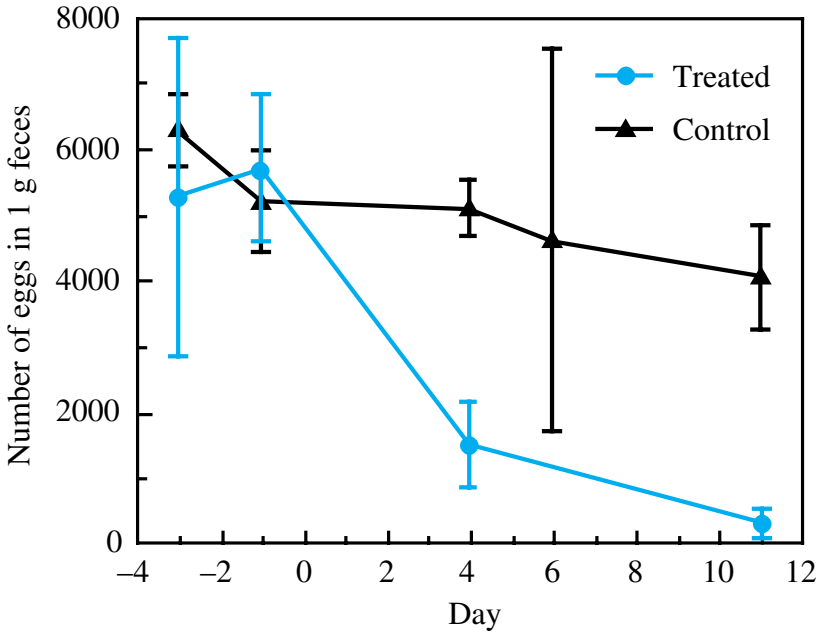
Many adult parasites live in low oxygen environments. Such organisms generate ATP differently from aerobic mammals. Anaerobic parasites fix CO_2 to phosphoenolpyruvate producing oxaloacetate, which is converted into malate and transported to the mitochondria. Malate is then converted to fumarate, and reduced to succinate by complex II which uses NADH as a reducing agent. This system, composed of complexes I and II, is called NADH-fumarate reductase. The quinone used in this system is not ubiquinone but rhodoquinone. In a screening of NADH-fumarate reductase inhibitors for new anthelmintics, we found nafuredin and atpenin (See p. 92).



2) Kinetic analysis of nafuredin inhibition of *A. suum* complex I

The inhibition is noncompetitive with NADH (K*i* = 8.1 nM) and competitive with rhodoquinone (K*i* = 8.3 nM).

3) Effect on *Haemonchus contortus* infected sheep



Eggs per gram of feces from *H. contortus* infected sheep on different days before and after oral treatment with 2 mg/kg nafuredin compared to untreated infected sheep are shown. Values are the mean of two trials (control) or three trials (treated animals) ± S.D.

4) Toxicity

There were no signs of side effects or reduction of body weight during the testing period in either sheep (2 mg/kg p.o.) or mice (50 mg/kg p.o. and i.p.).

5) Antimicrobial activity (10 μg/6 mm disc, paper disc method)

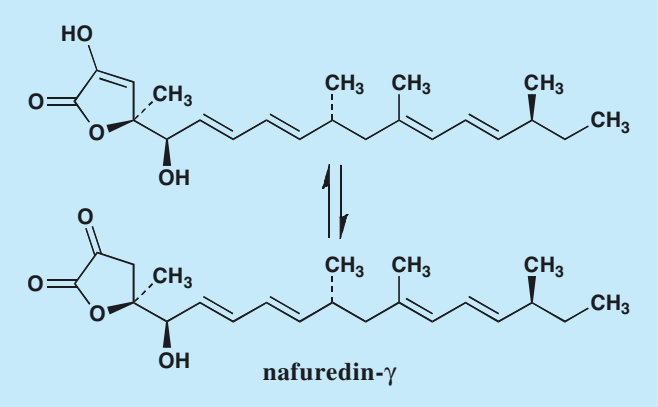
Test organism	Inhibitory zone (mm)	Test organism	Inhibitory zone (mm)
Bacillus subtilis ATCC6633	—	*Bacteroides fragilis* ATCC23745	—
Staphylococcus aureus ATCC6538p	—	*Acholeplasma laidlawii* PG8	—
Micrococcus luteus ATCC9341	—	*Pyricularia oryzae* KF180	±
Mycobacterium smegmatis ATCC607	—	*Candida albicans* KF1	12
Escherichia coli NIHJ	—	*Saccharomyces cerevisiae* KF26	—
Pseudomonas aeruginosa IFO3080	+	*Aspergillus niger* ATCC6275	—
Xanthomonas campestris pv. *oryzae* KB88	—	*Mucor racemosus* IFO4581	10

4. Biosynthesis[2)]

Nafuredin is biosynthesized from a nonaketide and four methionines (branched methyl carbons).

5. Nafuredin-γ[8)]

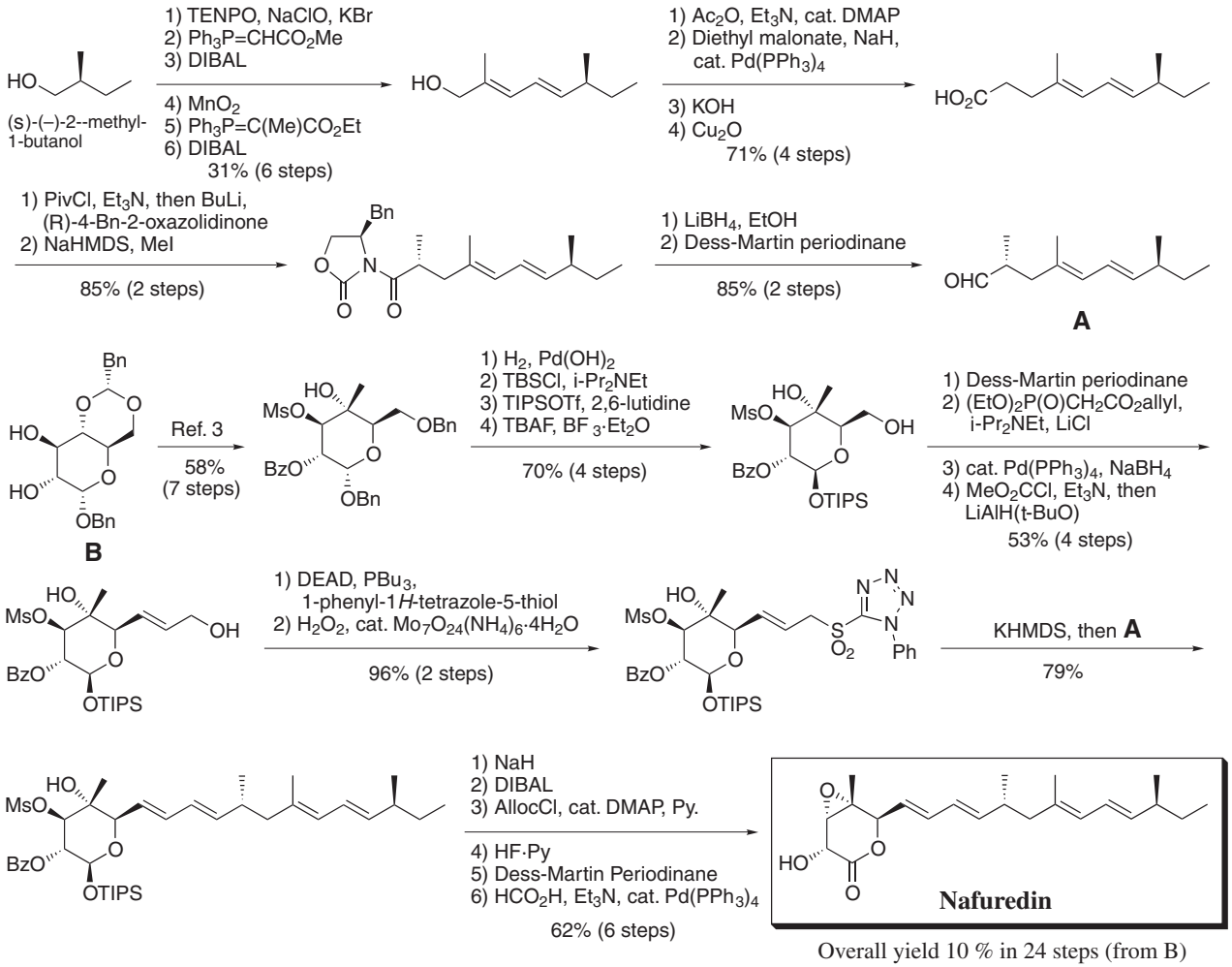
Nafuredin is easily converted to a compound named nafuredin-γ by weak alkaline treatment. The structure of nafuredin-γ was elucidated as a γ-lactone form of nafuredin with keto-enol tautomerism. Nafuredin-γ shows similar complex I inhibitory activity as nafuredin, and it also possesses anthelmintic activity *in vivo*.



nafuredin-γ

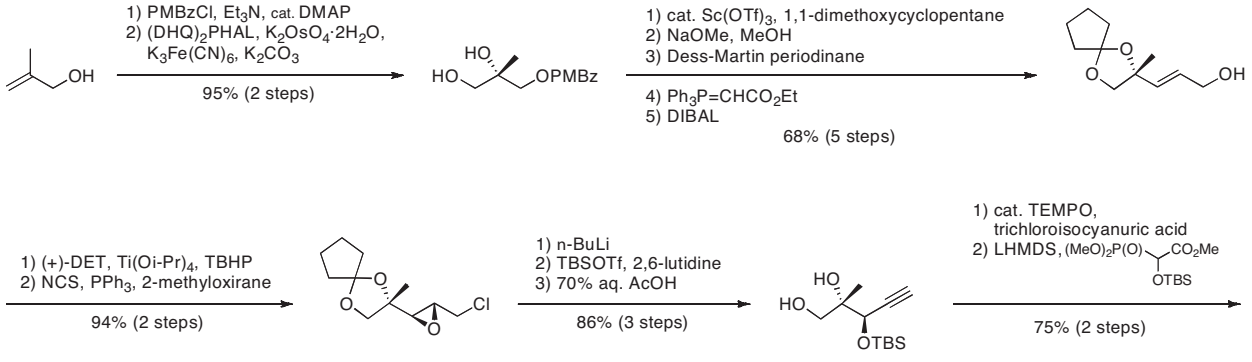
6. Total synthesis[9,10] (See Appendix-I)

1) Total synthesis of nafuredin



Overall yield 10 % in 24 steps (from B)

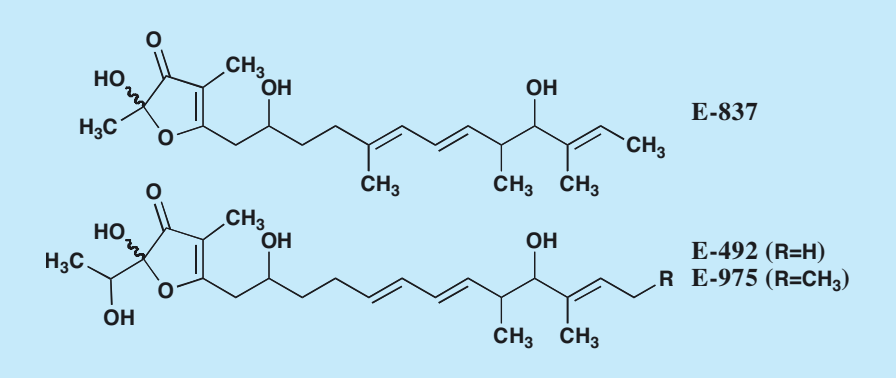
2) Total synthesis of nafuredin-γ



Overall yield 20% in 18 steps

7. Close structural relatives[11)]

Analyses of type I polyketide synthase gene clusters of *Streptomyces aculeolatus* NRRL 18422 and *Streptomyces* sp. Eco86 predicted three 5-alkenyl-3-furanones, one (E-837) from the former and two (E-492, E-975) from the latter strain. They were isolated and their inhibitory activity against NADH-fumarate reductase were evaluated, because they were structurally similar to nafuredin- γ. However their showed only moderate inhibitory activity (IC_{50} = 1-4 μg/ml).



8. References

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