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Absolute configuration of nafuredin, a new specific NADH-fumarate reductase inhibitor

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Abstract—Nafuredin, a new specific NADH-fumarate reductase inhibitor, was isolated from the culture broth of a fungal strain *Aspergillus niger* FT-0554. The stereoselective synthesis of three degradation products obtained by ozonolysis of nafuredin allowed elucidation of the absolute configuration of nafuredin.

NADH-fumarate reductase (NFRD) is a terminal electron transport system of anaerobic energy metabolism. Some kinds of parasites use this metabolism to generate ATP instead of the classical glycolysis, TCA cycle and electron transport systems. So an inhibitor of NFRD is potentially a selective antiparasitic agent. In the course of our screening for NFRD inhibitors, nafuredin (**1**) was obtained from the fermentation broth of a fungal strain *Aspergillus niger* FT-0554 isolated from a marine sponge.[1,2] Nafuredin (**1**) inhibited NFRD of *A. suum* with an IC_{50} value of 12 nM without cytotoxicity and showed good specificity to NFRD of helminths. Although the planar structure of **1** was elucidated by spectroscopic analyses and an additional NOE experiment showed the relative configuration of the β,γ-epoxy-δ-lactone moiety in **1**,[2] the stereochemistry at C(10) and C(16) was unknown. Herein we report the elucidation of the absolute configuration of **1** by degradation and synthetic studies.

Our strategy for the elucidation of the absolute configuration of **1** is shown in Fig. 1. Ozonolysis of **1** would afford three degradation products, which would be compared with the analytical data of the optically

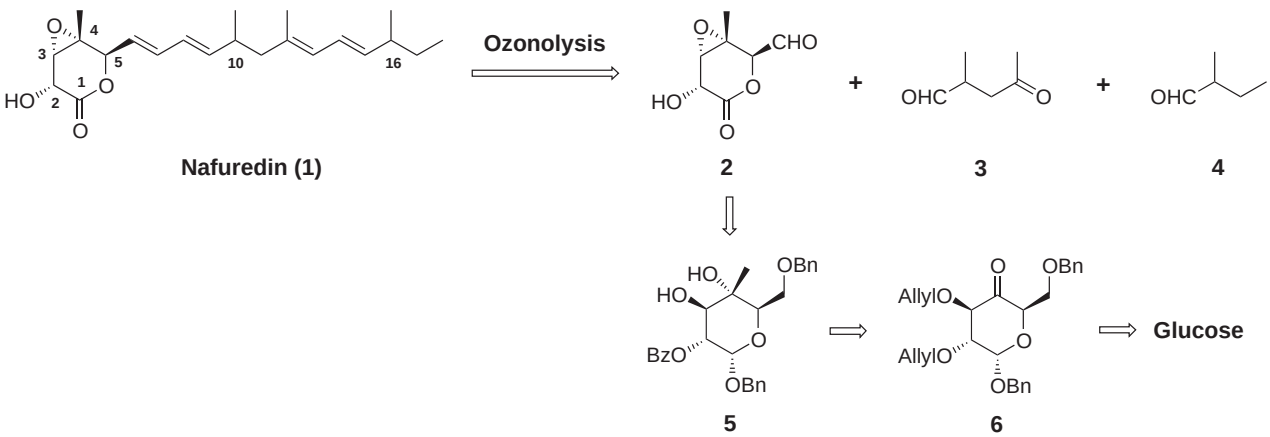

Figure 1.

Keywords: NADH-fumarate reductase (NFRD); nafuredin; absolute configuration.
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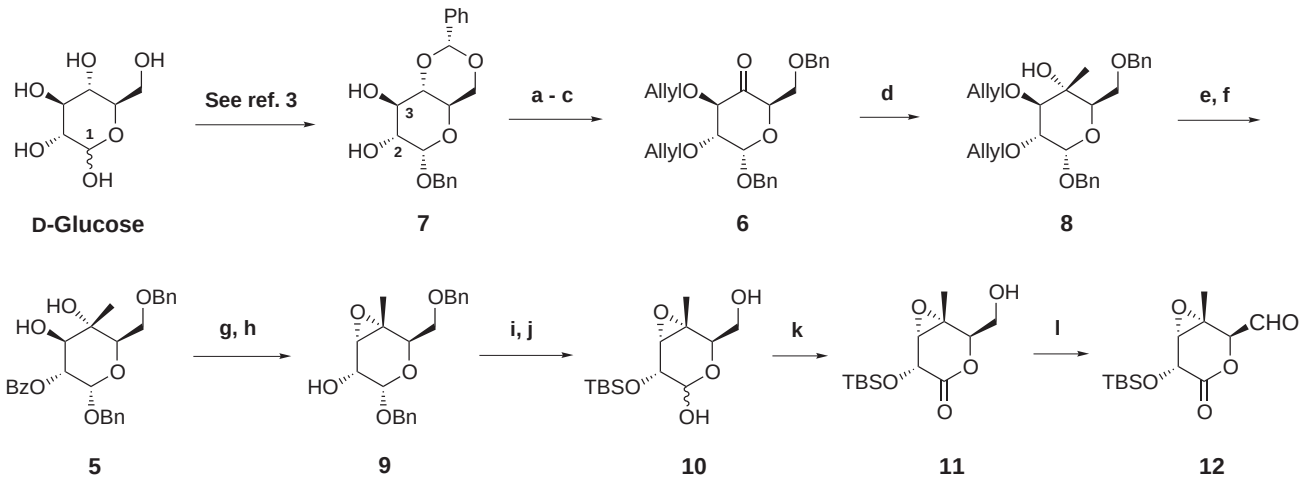
active synthetic compounds (**2**–**4**). The optically active lactone **2** would be derived from glucose via selective protection and deprotection of the hydroxyl groups, stereoselective 1,2-addition at C(4), and epoxidation.

D-Glucose as the starting material was used to synthesize the optically active **2**, and converted to the known diol **7**[3] (Scheme 1). Regioselective and reductive opening of benzylidene acetal[4] after protection of diol **7** as allyl ether followed by Dess–Martin oxidation[5] afforded ketone **6** without complication. Treatment of ketone **6** with MeLi furnished the desired *tert*-alcohol **8** (66% yield) together with its epimer (19% yield).[6] Deprotection of the allyl group and subsequent regioselective benzoylation at C(2)[7] gave diol **5**. Mesylation of **5** followed by epoxidation by treating with sodium hydride yielded epoxy alcohol **9** after quenching with methanol. Compound **9** was protected with *t*-butyldimethylsilyl (TBS) chloride and the corresponding TBS ether was converted to lactol **10** by hydrogenolysis.[8] Fetizon oxidation[9] of **10** led to lactone **11**,[10] which was subjected to Dess–Martin oxidation to afford aldehyde **12**. Though the structure of aldehyde **12** was confirmed by ^{1}H NMR of the resulting mixture, it was very unstable and did not allow purification with silica gel as it possessed a β,γ-epoxy aldehyde unit. So, we realized it was difficult to isolate **12** and **2** for comparison of their analytical data. Next we expected that lactone **11** would be also derived from **1** via ozonolysis and reduction.

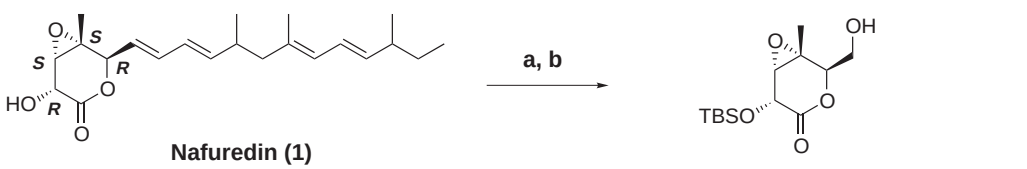
Compound **1** protected as TBS ether was subjected to ozonolysis and quenched with dimethyl sulfide; the resulting aldehyde was treated with sodium borohydride in one pot to produce lactone **11** in 75% overall yield from **1**.

Fortunately, lactone **11** synthesized from D-glucose was identical with that derived from natural nafuredin (**1**) in all respects ($[\alpha]_D$, ^{1}H and ^{13}C NMR, IR, and FABMS). This result shows that the absolute configuration of the lactone moiety in **1** is (2*R*,3*S*,4*S*,5*R*) as depicted in Scheme 2.

We next synthesized the optically active and racemic degradation products **3** and **4** as shown in Scheme 3. In order to synthesize the optically active (*R*)-**3**, (*S*)-(+)-3-hydroxyisobutyric acid methyl ester **13**[11] was converted to nitrile **14** in four steps: (1) protection of the primary alcohol with *t*-butyldiphenylsilyl (TBDPS) chloride; (2) reduction of the ester with DIBAL; (3) mesylation of the resulting primary alcohol; and (4) nucleophilic displacement with NaCN. Compound **14** was treated with MeLi to furnish the corresponding ketone after acidic work-up, which was subjected to deprotection of the TBDPS ether and subsequent nitroxyl radical oxidation[12] to yield the optically active (*R*)-**3**. Next, 2-benzyloxyethanol **15**[11] was subjected to Dess–Martin oxidation and the resulting aldehyde was treated with 1-triphenylphosphoranylidene-2-propanone to yield

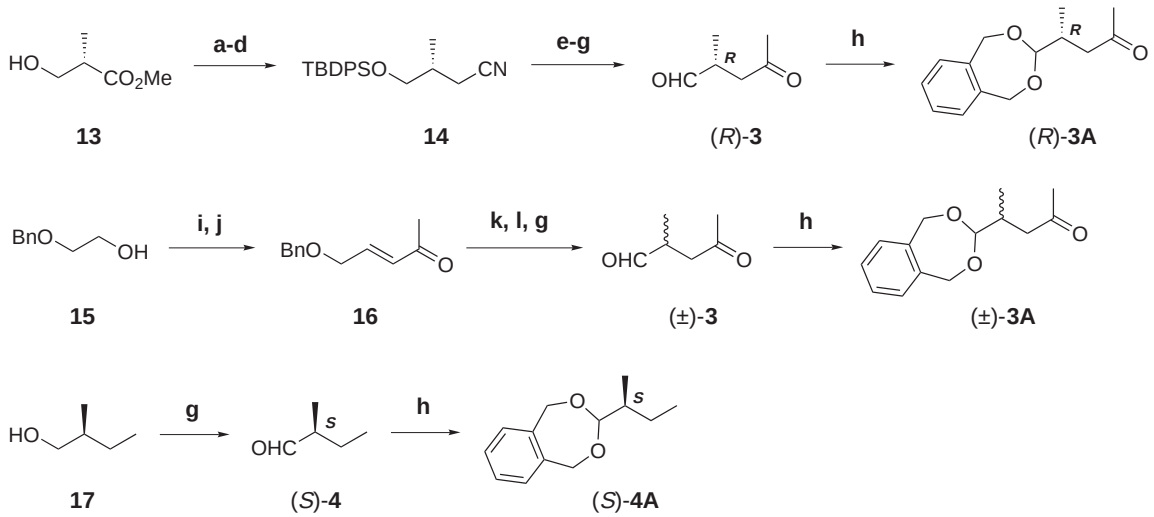


Scheme 1. (a) AllylBr, NaH, DMF, quant.; (b) $NaBH_3CN$, HCl (in Et_2O), THF, 86%; (c) Dess–Martin periodinane, CH_2Cl_2, 98%; (d) MeLi, Et_2O, 66%; (e) Pd/C, cat. *p*-TsOH, MeOH–H_2O, quant.; (f) Bu_2SnO, benzene, then BzCl, Et_3N, dioxane, 91%; (g) MsCl, *i*-Pr_2NEt, CH_2Cl_2, 94%; (h) NaH, THF, then MeOH, 95%; (i) TBSCl, imidazole, DMF, 90%; (j) H_2, $Pd(OH)_2$, EtOH, quant.; (k) Ag_2CO_3, Celite, benzene, 80%; (l) Dess–Martin periodinane, CH_2Cl_2, quant.



11 (Synthetic) $[\alpha]_D^{22} = -34.4^\circ$ (c = 0.18, $CHCl_3$)
11 (Natural) $[\alpha]_D^{22} = -32.9^\circ$ (c = 0.14, $CHCl_3$)

Scheme 2. (a) TBSCl, imidazole, THF; (b) O_3, MeOH, Me_2S, then $NaBH_4$, 75% (two steps).



Scheme 3. (a) TBDPSCl, *i*-Pr_2NEt, CH_2Cl_2, quant.; (b) DIBAL, CH_2Cl_2, 99%; (c) MsCl, *i*-Pr_2NEt, CH_2Cl_2, 93%; (d) NaCN, DMSO, 80%; (e) MeLi, Et_2O, then aq. H_2SO_4, quant.; (f) TBAF, THF, quant.; (g) TEMPO, NaClO, KBr, $NaHCO_3$, CH_2Cl_2, 50–60%; (h) 1,2-ditrimethylsiloxymethylbenzene, TMSOTf, CH_2Cl_2, 80–85%; (i) Dess–Martin periodinane, CH_2Cl_2, 70%; (j) 1-triphenylphosphoranylidene-2-propanone, benzene, 72%; (k) $Me_2Cu(CN)Li$, Et_2O, 85%; (l) H_2, Pd/C, EtOH, 80%.

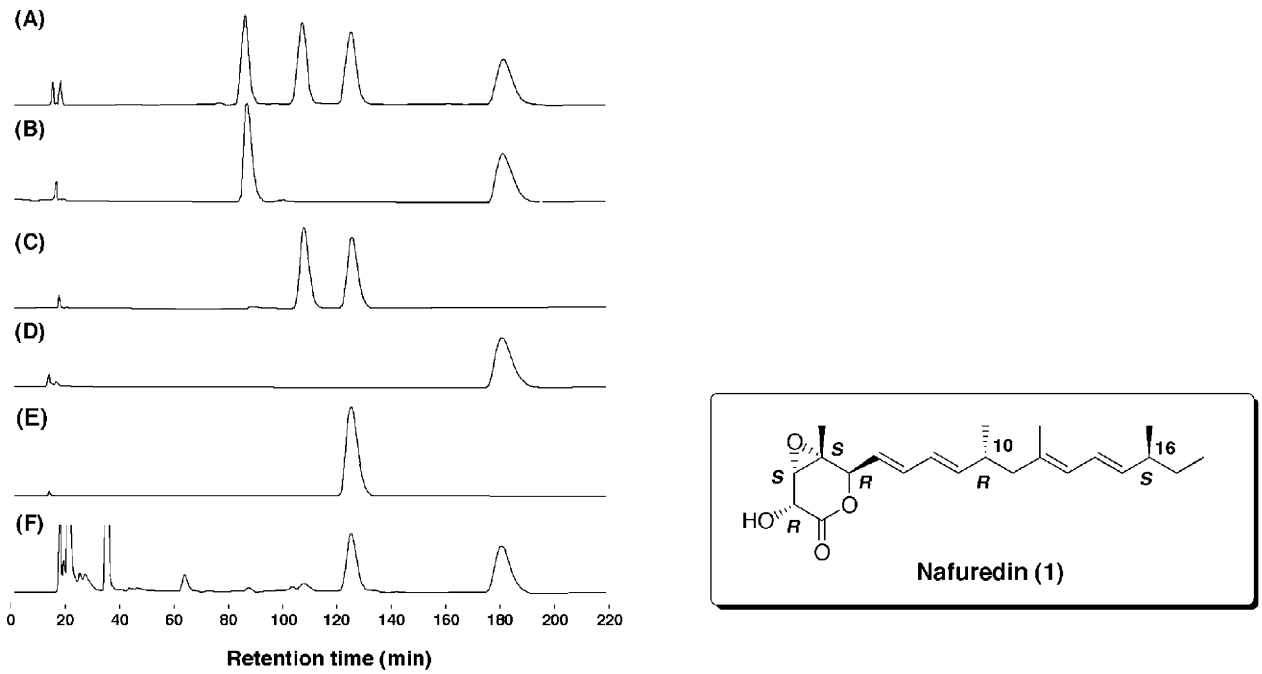


Figure 2.

α,β-unsaturated ketone **16**. Treatment of **16** with $Me_2Cu(CN)Li$ followed by hydrogenolysis and nitroxyl radical oxidation led to racemic **3**. The optically active (*S*)-**4** was derived from (*S*)-(−)-2-methyl-1-butanol **17**[11] by nitroxyl radical oxidation.

We next analyzed the degradation products (±)-**3** and (±)-**4**[11] with various chiral HPLC columns; however, we could not find the conditions to separate each enantiomer. Therefore, **3** and **4** were converted to acetals **3A** and **4A**, respectively. When the 1:1 mixture of acetals (±)-**3A** and (±)-**4A** was injected to a Daicel-OJ column (0.46 cm ϕ×25 cm), and eluted with hexane–isopropanol (3:7) (Senshu HPLC system; 10 μl injection; UV, 207 nm; flow rate, 0.3 ml/min), each enantiomer proved to be separated completely as shown in Fig. 2(A). Fig. 2(B) and (C) show HPLC analytical data of (±)-**3A** and (±)-**4A**, respectively. Next, (*R*)-**3A** and (*S*)-**4A** were also subjected to HPLC analysis to show that their retention times are 127 and 183 min, respectively, as shown in Fig. 2(D) and (E).

Nafuredin (**1**) was subjected to ozonolysis and quenched with dimethyl sulfide; the resulting aldehydes **3** and **4** were converted to acetals **3A** and **4A** in one pot, whose TLC behaviors in several solvent systems

were identical with those of synthetic acetals **3A** and **4A**. After standard work-up, HPLC analysis of the resulting mixture showed clearly that the absolute configuration of the side chain in **1** was (10*R*) and (16*S*) as shown in Fig. 2(F).

In summary, the absolute configuration of nafuredin (**1**) has been elucidated as (2*R*,3*S*,4*S*,5*R*,6*E*,8*E*,10*R*, 12*E*,14*E*,16*S*)-3,4-epoxy-2-hydroxy-4,10,12,16-tetramethyl-6,8,12,14-octadecatetraeno-5-lactone by stereoselective synthesis and HPLC analysis of the degradation products obtained by ozonolysis of **1**. Further studies for the total synthesis of nafuredin are in progress.

Acknowledgements

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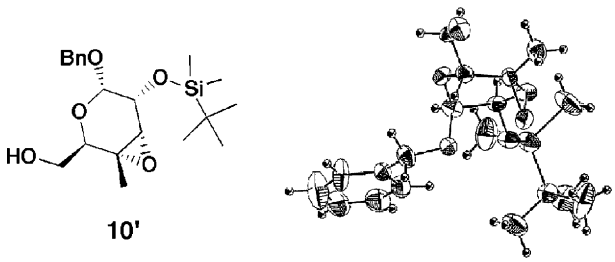
8. The stereochemistry of monobenzyl compound **10′** obtained by hydrogenolysis (30 min) of TBS protected **9** was confirmed by X-ray crystallographic analysis as follows. This X-ray data has been deposited with the CCDC (CCDC 157565).



10′

9. Fetizon, M.; Golfier, M.; Mourgues, P.; Louis, J.-M. *Organic Syntheses by Oxidation with Metal Compounds*; Plenum Press: New York, 1986; pp. 503–567.
10. **11**: mp = 135–137°C; $[\alpha]_D^{25} = -34.4$ ($c = 0.18$, $CHCl_3$); IR (KBr) cm^{-1} = 3466, 3460, 2929, 2858, 1745, 1392, 1250, 1188, 1124, 1076, 835, 787, 584; ^1H NMR (270 MHz, $CDCl_3$) δ 0.13, 0.19 (s, 3H each, CH_3), 0.93 (s, 9H, *t*-Bu), 1.51 (s, 3H, 4-Me), 2.82 (bs, 1H, OH), 3.41 (s, 1H, H-3), 3.90, 4.11 (bd, 1H each, $J = 12.4$ Hz, H-6), 4.48 (bs, 1H, H-5), 4.91 (s, 1H, H-2); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 170.0, 79.6, 69.0, 61.9, 61.3, 56.9, 25.5, 18.3, 17.9, –4.4; HRMS [FAB, *m*-NBA] *m*/*z* $(M+H)^+$ 289.1454; calcd for $C_{13}H_{25}O_5Si$: 289.1471; anal. calcd for $C_{13}H_{24}O_5Si$: C, 54.14; H, 8.39; found: C, 53.89; H, 8.50.
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