Structure and Synthesis of Sporogenic Psi Factors from *Aspergillus nidulans*

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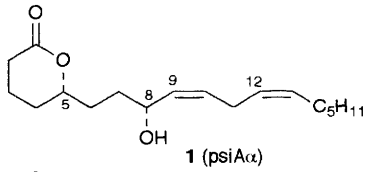
The structures of four hydroxylated unsaturated C_{18} fatty acids (psi factors) which induce premature sexual sporulation in *A. nidulans*, and the enantioselective synthesis of two of the components, psiBα and psiBβ, are described.

We have reported the characterization of endogenous factors, psiAα **1** and psiAβ **2**, which induce premature sexual sporulation in the ascomycetous fungus *Aspergillus nidulans*.[1,2] The characterization† of psiBα **3a**, psiBβ **4a**, psiCα **5a**, and psiCβ **6a**, with higher sporogenic activity, and the synthesis of psiBα **3a** and psiBβ **4a** are described below.

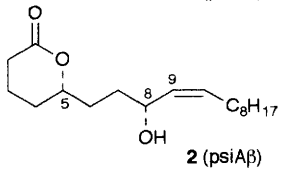
Crude psiB and psiC, IR ν 1710 cm^{-1} (CO_2H), were esterified with diazomethane and purified by HPLC (C_{18}, 80% MeCN–H_2O) to yield the methyl esters psiBα **3b**, psiBβ **4b**, psiCα **5b**, and psiCβ **6b**. Analysis of spectroscopic data of **3b** indicated that acid **3a** is identical with laetisaric acid (8-OH configuration undetermined), an allelopathic agent produced by the basidiomycete fungus *Laetisaria arvalis*.[3] The ^1H and ^{13}C NMR of psiBβ methyl ester **4b** showed the presence of secondary OH and a *cis*-disubstituted double bond ($J_{9,10}$ = 11.0 Hz). Location of 8-OH in the psiB's was confirmed by the mass spectrum of the perhydro trimethylsilyl ether derivative **7**, which showed peaks corresponding to cleavage at C-7–C-8 (*m/z* 243, $C_{14}H_{31}OSi$) and C-8–C-9 (*m/z* 245, $C_{12}H_{25}O_3Si$); the absolute configuration was established as (8*R*) from the CD[4] of the *p*-bromobenzoate of psiBβ methyl ester **4c**, $\lambda_{ext}(\Delta\varepsilon)$ 243 nm (−7.5) in MeCN. The structures of psiBα and psiBβ are thus (8*R*)-(*Z*,*Z*)-hydroxyoctadeca-9,12-dienoic acid and (8*R*)-(*Z*)-hydroxyoactadec-9-enoic acid, respectively.

PsiCs are readily converted to psiAs, particularly with acid, the products being identified with authentic psiAs by TLC and HPLC; furthermore, the conversion products yield psiC methyl esters upon methanolysis. This suggested psiCs **5a**, **6a** to be the acyclic forms of psiAs; a comparison of psiCα **5b** and psiCβ **6b** methyl esters with those of psiAα and psiAβ (prepared by acidic methanolysis) showed the compounds to be identical. Assuming that 8-OH retains its configurations in the psiA → psiC conversions, the structures of psiCα and psiCβ are established as (5*S*, 8*R*)-(*Z*,*Z*)-dihydroxyoctadeca-9,12-dienoic acid and (5*S*, 8*R*)-(*Z*)-dihydroxyoctadec-9-enoic acid, respectivley.

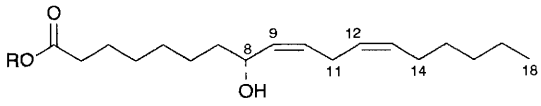
The psiB and psiC components, **3a/4a** and **5a/6a**, exhibit the highest sporogenic activity;[2] from the present results it



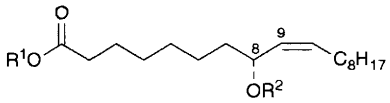
1 (psiAα)



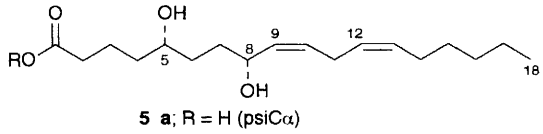
2 (psiAβ)



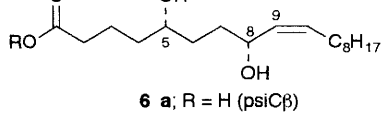
3 **a**; R = H (psiBα)
b; R = Me



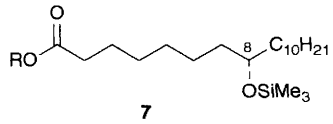
4 **a**; R^1 = H (psiBβ)
b; R^1 = Me, R^2 = H
c; R^1 = Me, R^2 = *p*-$BrC_6H_4CH_2$



5 **a**; R = H (psiCα)
b; R = Me



6 **a**; R = H (psiCβ)
b; R = Me



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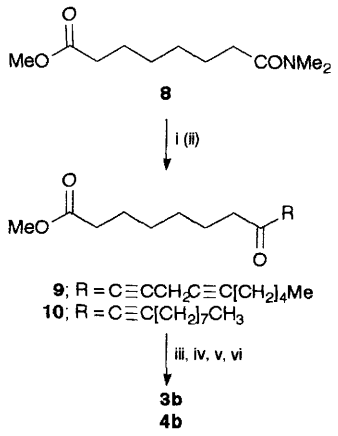
† Spectroscopic data for **3b**: high-resolution EI-MS (HRMS) *m/z* 310.2515 (M^+, $C_{19}H_{34}O_3$, calc. 310.2508); ^1H NMR (250 MHz, $CDCl_3$) δ 5.50–5.20 (4H, m, 9-, 10-, 12-, 13-H), 4.44 (1H, dt, *J* 8.5, 6.4 Hz, 8-H), 3.65 (3H, s, CO_2Me), 2.82 (2H, m, 11-H, H′), 2.28 (2H, t *J* 7.4 Hz, 2–H,H′), 2.03 (2H, dt, *J* 6.5, 7.0 Hz, 14-H, H′), 1.70–1.20 (16H, m) 0.86 (3H, t, *J* 6.7 Hz, 18-Me); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 174.2, 132.8, 130.9, 130.5, 127.1, 67.7, 51.4, 37.4, 34.1, 31.5, 29.7, 29.2, 29.1, 27.3, 26.1, 25.2, 24.9, 22.6, 14.0

For **4b**: HRMS *m/z* 312.2626 (M^+, $C_{19}H_{36}O_3$, calc. 312.2664); ^1H NMR (250 MHz, $CDCl_3$)δ 5.46 (1H, dt, *J* 11.0, 7.3 Hz, H-10), 5.34 (1H, ddt, *J* 11.0, 7.5, 1.5 Hz, 9-H), 4.40 (1H, dt, *J* 8.5, 6.4 Hz, 8-H), 3.65 (3H, s, CO_2Me), 2.28 (2H, t, *J* 7.4 Hz, 2-H, H′), 2.05 (2H, m, 11-H, H′), 1.70–1.20 (22H, m), 0.86 (3H, t, *J* 6.4 Hz, 18-Me); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 174.2, 132.6, 132.4, 67.7, 51.4, 37.5, 34.1, 31.9, 29.7, 29.4, 29.2, 29.1, 27.7, 25.2, 24.9, 22.7, 14.1.

For **5b**: HRMS *m/z* 309.2424 (M^+ − OH, $C_{19}H_{33}O_3$, calc. 309.2430); CI-MS, *iso*-butane, *m/z* 327 (M^+ + 1); ^1H NMR (400 MHz, $CDCl_3$): δ 5.45 (1H, dt, *J* 10.9, 7.1 Hz, 10-H), 5.45–5.35 (2H, m, 13, 9-Hs), 5.29 (1H, dtt, *J* 10.7, 7.2, 1.5 Hz, 12-H), 4.48 (1H, ddd, *J* 8.3, 7.6, 4.5 Hz, 8-H), 3.65 (3H, s, CO_2Me), 3.60 (1H, m, 5-H), 2.82 (2H, m, 11-H, H′), 2.33 (2H, t, *J* 7.6 Hz, 2-H, H′), 2.02 (2H, dt, *J* 6.9, 6.9 Hz, 14-H, H′), 1.85–1.55 (5H, m), 1.55–1.40 (3H, m), 1.40–1.20 (6H, m), 0.87 (3H, t, *J* 6.9 Hz, 18-Me); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 174.2, 132.5, 131.0, 130.4, 126.9, 71.4, 68.0, 51.6, 36.9, 34.1, 33.8, 33.7, 31.5, 29.2, 27.3, 26.0, 22.6, 21.0, 14.1.

For **6b**: high-resolution FAB-MS, nitrobenzyl alcohol, *m/z* 329.2652 (M^+ + 1, $C_{19}H_{37}O_4$, calc. 329.2692); ^1H NMR (400 MHz, $CDCl_3$): δ 5.46 (1H, dt, *J* 11.0, 7.3 Hz, 10-H), 5.38 (1H, ddt, *J* 11.0, 8.3, 1.4 Hz, 9-H), 4.44 (1H, ddd, *J* 8.3, 7.8, 4.5 Hz, 8-H), 3.65 (3H, s, CO_2Me), 3.60 (1H, m, 5-H), 2.33 (2H, t, *J* 7.2 Hz, 2-H, H′), 2.05 (2H, m, 11-H, H′), 1.80–1.40 (10H, m), 1.40–1.20 (10H, m), 0.86 (3H, t, *J* 6.8 Hz, 18-Me); ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 174.2, 132.5, 132.2, 71.4, 68.0, 51.5, 36.9, 34.1, 33.9, 33.8, 31.9, 29.7, 29.4, 29.3, 27.7, 22.7, 21.0, 14.1.



Scheme 1 *Reagents and conditions*: i, $LiC{\equiv}CCH_2C{\equiv}C[CH_2]$-Me (3.3 equiv.), $BF_3{\cdot}OEt_2$ (3.5 equiv.), tetrahydrofuran (THF), −78 °C, 30 min (76%); ii, $LiC{\equiv}C[CH_2]_7Me$ (3.3 equiv.), $BF_3{\cdot}OEt_2$ (3.5 equiv.), THF, −78 °C, 30 min (46%); iii, (+)-Alpine-Borane (2–4 equiv.; 97.5% e.e.), neat, room temp., 12 h; iv, EtCHO; v, H_2O_2, 3 mol dm^{-3} NaOH or $HOCH_2CH_2NH_2$ (72–86%; 90–92.5 e.e.); vi, H_2Pd–$BaSO_4$, quinoline, MeOH (55–68%; 90–91.5% e.e.)

appears that the weak activity of psiA is due to its conversion to psiC during the assay. It is noteworthy that while the psi factors induce sporulation in *A. nidulans*, laetisaric acid elicits a dramatically different fungicidal response by hyphal lysis (primarily in the phycomycetous fungi).[3]

The structures of psiBα **3a** and psiBβ **4a** were confirmed by an enantioselective synthesis (Scheme 1).‡ The ester-*N,N*-dimethylamide **8** was prepared from cyclooctene by (*i*) ozonolysis (92%),[5] (*ii*) oxidation with $KMnO_4$ (94%), (*iii*) formation of the monoacid chloride with $SOCl_2$ (77%), and (*iv*) amidation with 40% aqueous Me_2NH (50%). The amide **8** was coupled with the alkyne boranes prepared from the lithium acetylide of deca-1,4-diyne[6] or dec-1-yne and $BF_3{\cdot}Et_2O$[7] to afford the acetylenic ketones **9** (46%) and **10** (76%). Reduction of the ketones **9** and **10** with neat (+)-Alpine-Borane[8] {from 9-BBN (9-borabicyclo[3.3.1]-nonane) and (1*R*)-(+)-α-pinene; 97.5% enantiomeric excess (e.e.)} provided the corresponding (8*R*)-propynylic alcohols 72–86%, 90–93% e.e.§), which were hydrogenated over Pd–$BaSO_4$ poisoned with quinoline to afford methyl esters of psiBα **3b** and psiBβ **4b** (55–68%, 90–92% e.e.). Spectral data of synthetic methyl estrs, psiBα **3b**, $[\alpha]_D^{26}$ (synth. **3b**) +13.7° (*c* 0.033 g ml^{-1}, $CHCl_3$) and psiBβ **4b**, $[\alpha]_D^{26}$ (synth. **4b**) +13.7° (*c* 0.012 g ml^{-1}, $CHCl_3$) and those of natural samples were identical. Moreover, synthetic psiB acids **3a** and **4a** obtained by hydrolysis induced premature sexual sporulation in *A. nidulans* as with authentic compounds.

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‡ All synthetic material exhibited satisfactory ^1H NMR, ^{13}C NMR, IR, and MS data. Synthesis of psiAβ **2** and psiCβ **6a**: P. Mazur and K. Nakanishi, submitted for publication.

§ Determined by ^1H NMR or using Eu $(hfc)_3$, or from ^{19}F NMR.