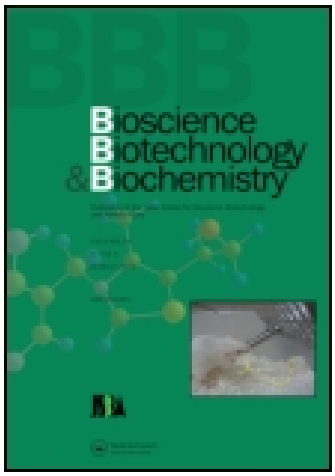

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Synthesis of the 5-Demethyl-6-deoxy Analogue of Sporogen AO-1, a Sporogenic Substance of Aspergillus oryzae

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Note

Synthesis of the 5-Demethyl-6-deoxy Analogue of Sporogen AO-1, a Sporogenic Substance of *Aspergillus oryzae*

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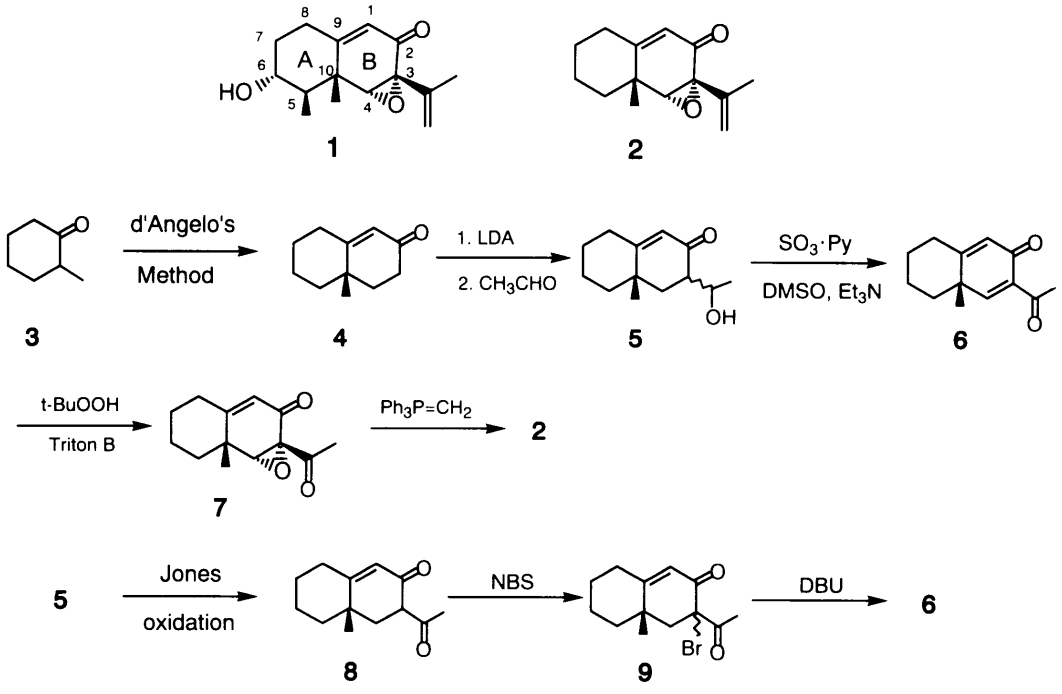
Sporogen AO-1 is a sporulation-stimulating substance isolated from *Aspergillus oryzae*. We speculated that ring B would be important for the activity and designed a compound without functional groups in ring A. We synthesized the compound from 2-methylcyclohexanone *via* chiral octalone. The compound showed activity at 200 μg/disc. The functional groups on ring B are suggested to be important for the activity.

Key words: sporulation; *Aspergillus oryzae*; sporogen AO-1

Sporogen AO-1 (**1**) has been isolated as an active substance, which stimulated the sporulation of *Aspergillus oryzae*,[1] one of the most important fungi in the Japanese fermentation industry. Sporulation is very important, because the quality and yield of fermented products are believed to be dependent on the extent of their sporulation.[2] Although the sporulation-stimulating activity of natural and synthetic sporogen AO-1 (**1**)[3] has been evaluated, there is no information about what moiety of this substance is important for its activity. We speculated that ring B would be important for the activity and designed model compound **2** without methyl and hydroxy groups in ring A, because the functional groups of sporogen AO-1 (**1**) could be concentrated in its ring B. We describe here, the synthesis of model compound **2**, 5-demethyl-6-deoxy sporogen AO-1, the procedure of which is shown in the Scheme.

We prepared chiral octalone **4** with 76%*e.e.* from 2-methylcyclohexanone **3** as the starting material, according to the d'Angelo's method.[4] Optical purity was evaluated by comparing the specific rotation value with that in the literature.[5] After deprotonating with LDA (lithium diisopropylamide), octalone **4** was coupled with freshly distilled acetaldehyde to give alcohol **5** as a diastereomeric mixture in an 83% yield. Successive Jones oxidation, NBS (*N*-bromosuccinimide) bromination, and HBr elimination with DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) gave diene-dione **6** in a 9% yield from **5**. After some trials with other oxidizing reagents on **5**, SO_3·Py-DMSO oxidation[6] in the presence of triethylamine was revealed to be most efficient, giving **6** in one step in a good yield (78%). Treatment of diene-dione **6** with *tert*-butylhydroperoxide in the presence of Triton B gave epoxide **7** with the desired stereochemistry. In the ^{1}H-NMR spectrum, the chemical shift of the proton (3.31 ppm) at C4 in epoxide **7** was very close to that of sporogen AO-1 (**1**; 3.22 ppm). The reagent attacked the more-electrophilic double bond in diene-dione **6** from the less-hindered α-side. Wittig methylenation of the less-hindered and more-electrophilic carbonyl group in epoxide **7** gave 5-demethyl-6-deoxy sporogen AO-1 (**2**). In the ^{1}H-NMR spectrum of 5-demethyl-6-deoxy sporogen AO-1 (**2**), the characteristic proton signals (experimental) showed good



Scheme

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agreement with those of sporogen AO-1 (**1**).[1)]

The sporulation-stimulating activity of 5-demethyl-6-deoxy sporogen AO-1 (**2**) was effective at a dose of 200 μg/disc by the sporogenic bioassay described.[1)] This sporulation-stimulating activity of 5-demethyl-6-deoxy sporogen AO-1 (**2**) was about 5 times lower than that of natural sporogen AO-1 (**1**), but this result shows that the functionalities on ring B are very important for the sporulation-stimulating activity. In other words, the functional groups on ring A wee not crucial for the activity.

Experimental

^{1}H-NMR spectra were recorded with TMS as an internal standard at 200 MHz and 100 MHz by JEOL FX 200 and JEOL FX 100 spectrometers, respectively. IR spectra were measured by a JASCO IR-3A infrared spectrometer. Mass spectra were recorded by a JEOL D-100 spectrometer at 70 eV. Fuji-Devison BW-300 was used for silica gel column chromatography.

(S)-(+)-10-Methyl-1(9)-octal-2-one (**4**). A solution of 2-methylcyclohexanone **3** (100 g, 0.89 mol) and D-phenylethylamine (120 g, 0.99 mol) together with *p*-toluenesulfonic acid (a few crystals) in 500 ml of benzene was heated to reflux overnight with continuous azeotropic removal of water by Dean Stark apparatus. The mixture was diluted with water, and the organic layer was separated. This organic layer was washed successively with aqueous sodium bicarbonate, water and brine, and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a crude imine which was purified by distillation (3 mmHg, 110–115 C with Kugelrohr apparatus) to give 185 g (97%) of the imine. ^{1}H-NMR (100 MHz, CD_2Cl_2) δ: 1.07 (3H, d, J=7 Hz), 1.08 (3H, d, J=7 Hz), 1.33 (3H, d, J=7 Hz), 1.34 (3H, d, J=7 Hz), 1.2–2.9 (9H, m), 4.71 (2H, q, J=7 Hz), 7.0–7.6 (10H, m); MS *m/z*: 215 (M^+), 200, 126, 106, 105, 104, 103, 91, 83, 79, 77.

A solution of the imine (185 g, 0.86 mol) and freshly distilled methyl vinyl ketone (150 ml, 1.72 mol) in dry benzene (2 liters) was kept at 60 C for 6 h. The mixture was then diluted with water, and the organic layer was separated. This organic layer was successively washed with water and brine, and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a crude oily product which was then diluted with 500 ml of THF and 100 ml of aq. HCl (10%). After 3 h at ambient temperature, the mixture was diluted with water and extracted with ether. The ethereal extract was successively washed with water and saturated brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to give 200 g of a crude oily product. A solution of this crude oily product (200 g) and MeONa (46 g, 0.86 mol) in methanol (1.5 liters) was kept for 2 h at 60 C. The reaction mixture was then diluted with aqueous NH_4Cl and extracted with ether. The extract was successively washed with water and saturated brine, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was distilled under reduced pressure (0.15 mmHg, 80–85 C) to give 65 g of (+)-octalone **4** (44% yield in 4 steps from **3**). $[\alpha]_D^{20}$ = +158.7° (c=1.0, EtOH) [lit. Johnson $[\alpha]_D^{20}$ = +208.3° (c=1.0, EtOH)][5)]; ^{1}H-NMR (100 MHz, $CDCl_3$) δ: 1.24 (3H, s), 1.20–1.50 (2H, m), 1.55–1.98 (6H, m), 2.18–2.60 (4H, m), 5.70 (1H, s); IR ν_{max} (CCl_4) cm^{-1}: 3020, 2940, 2860, 1675, 1670; MS *m/z*: 164 (M^+), 136, 122, 107.

(10S)-(+)-3-(1-Hydroxyethyl)-10-methyl-1(9)-octal-2-one (**5**). To a solution of LDA (prepared from 2.5 ml of diisopropylamine and 10 ml of 1.55 M methyllithium) in THF, octalone **4** (500 mg, 3 mmol) in dry THF (10 ml) was added dropwise at −78 C under nitrogen. After 1 h, freshly distilled acetaldehyde (0.25 ml) in THF (4 ml) was added dropwise to this solution, and the mixture was kept for 1 h. The mixture was then diluted with aqueous NH_4Cl and extracted with ether. The extract was successively washed with water and saturated brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, 30% EtOAc in hexane) to give 520 mg of product **5** as a diastereomeric mixture in an 83% yield. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.19 (3H, d, J=6.4 Hz), 1.21 (3H, d, J=6.8 Hz), 1.26 (3H, s), 1.28 (3H, s), 1.31–2.11 (*ca.* 16H, m), 2.12–2.45 (*ca.* 4H, m), 2.64 (1H, m), 3.12 (1H, m), 3.98 (1H, m), 4.25 (1H, m), 4.77 (1H, m), 5.71 (2H, s); IR ν_{max} (CCl_4) cm^{-1}: 3650, 3300, 2950, 2875, 1660, 1650, 1630, 1210; MS *m/z*: 208 (M^+), 190, 175, 164.

(10S,3R,4R)-(+)-3-Acetyl-10-methyl-1(9),3(4)-octal-2-one (**6**). To a solution of enone alcohol **5** (480 mg, 2.3 mmol) in 12 ml of dimethylsulfoxide (DMSO) containing Et_3N (5 ml, 35 mmol), SO_3·Py complex (2.7 g, 17 mmol) in DMSO (7 ml) was added at ambient temperature. After 1 h, the mixture was diluted with water and extracted with ether. The extract was successively washed with water and saturated brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, 20% EtOAc in hexane) to give 340 mg of diene-dione **6** in a 78% yield. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.32 (3H, s), 1.3 (1H, m), 1.6–2.1 (5H, m), 2.3–2.5 (2H, m), 2.39 (3H, s), 6.12 (1H, s), 7.39 (1H, s); IR ν_{max} (CCl_4) cm^{-1}: 2950, 2925, 1660, 1635, 1385, 1360, 1265, 1255; MS *m/z*: 204 (M^+), 189, 147.

(10S)-(+)-3-Acetyl-10-methyl-3(4)-oxo-1(9)-octal-2-one (**7**). To a solution of diene-dione **6** (200 mg, 0.98 mmol) in dry THF (5 ml) containing Triton-B (19.6 μmol), 0.6 ml of *tert*-butylhydroperoxide (1.8 M in 1,2-dichloroethane) was added at −78 C, and the mixture was kept for 1 h. The mixture was diluted with water and extracted with ether. The extract was successively washed with water and saturated brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, 20% EtOAc in hexane) to give 71 mg of epoxide **7** in a 33% yield. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.33 (3H, s), 1.6–2.0 (2H, m), 2.33 (3H, s), 2.28–2.48 (2H, m), 3.31 (1H, s), 5.77 (1H, d, J=2 Hz); IR ν_{max} (CCl_4) cm^{-1}: 2940, 2855, 1725, 1675, 1630, 1360, 1130, 1110; MS *m/z*: 220 (M^+), 177, 150, 84, 79.

5-Demethyl-6-deoxy sporogen AO-1 (**2**). To a solution of epoxide **7** (220 mg, 0.9 mmol) in dry THF (5 ml), a suspension of methylenetriphenylphosphorane (0.98 mmol) in THF (1 ml) was added dropwise at −78 C under nitrogen. After 1 h, the mixture was diluted with aqueous NH_4Cl and extracted with ether. The extract was successively washed with water and saturated brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, 30% EtOAc in hexane) to give 82 mg of 5-demethyl-6-deoxy sporogen AO-1 (**2**) in a 42% yield. $[\alpha]_D^{20}$ = +152.5° (c, 0.15, $CHCl_3$); ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.31 (3H, s), 1.12–1.52 (2H, m), 1.89 (3H, s), 1.64–1.96 (6H, m), 3.34 (1H, s), 5.04–5.16 (2H, m), 5.74 (1H, d, J=2 Hz); IR ν_{max} (CCl_4) cm^{-1}: 2960, 2890, 1685, 1640, 1135, 1110, 910; MS *m/z*: 218 (M^+), 203, 189, 185.

(10S)-(+)-3-Acetyl-10-methyl-1(9)-octal-2-one (**8**). To an ice-cooled solution of enone alcohol **5** (2.58 g, 12.4 mmol) in acetone (260 ml), Jones reagent (2.6 M) was added at 5 C while the color of the solution changed from brown to green. The mixture was quenched with isopropyl alcohol, diluted with water, and extracted with ether. The extract was successively washed with water and saturated brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, 30% EtOAc in hexane) to give 1.62 g of ene-dione **8** in a 64% yield. ^{1}H-NMR data was complicated due to the equilibration between α-Ac, β-Ac and enol forms, major signals being as follows: (200 MHz, $CDCl_3$) δ: 1.06 (6H, s), 1.1–2.6 (*ca.* 16H, m), 2.06 (6H, s), 2.10–2.44 (4H, m), 3.46 (1H, dd, J=14.3, 6.6 Hz), 3.58 (1H, dd, J=14.3, 4.5 Hz), 5.72 (2H, s).

(10S)-(+)-3-Acetyl-3-bromo-10-methyl-1(9)-octal-2-one (**9**). To a solution of ene-dione **8** (101 mg, 0.49 mmol) in DMF (3.5 ml) and water (0.1 ml), *N*-bromosuccinimide (180 mg, 0.98 mmol) was added at 0 C. After stirring for 3 h, the mixture was diluted with water and extracted with ether. The extract was successively washed with water, saturated brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, 15% EtOAc in hexane) to give 67 mg of bromide **9** in a 48% yield. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.16 (3H, s), 1.24–2.04 (6H, m), 2.16–2.36 (2H, m), 2.22 (3H, s), 2.47 (1H, d, J=12.8 Hz), 2.96 (1H, d, J=12.8 Hz), 5.92 (1H, s); MS *m/z*: 286 (M^+), 284 (M^+), 244, 242, 229, 227, 201, 199.

Dehydrobromination of **9** *to* **6**. A solution of bromoenone **9** (180 mg, 0.63 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (100 mg, 0.66 mmol) in THF (6 ml) was stirred overnight at ambient temperature. The mixture was diluted with water and extracted with ether. The extract was successively washed with water and saturated brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was purified by column chromatography (silica gel, 20% EtOAc in hexane) to give 37 mg of a diene-dione in a 29% yield. The ^{1}H-NMR spectrum of this product was identical with that of compound **6** synthesized from enone-alcohol **5**.

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