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STRUCTURE OF SPOROGEN-AO 1, A SPOROGENIC SUBSTANCE OF *Aspergillus oryzae*

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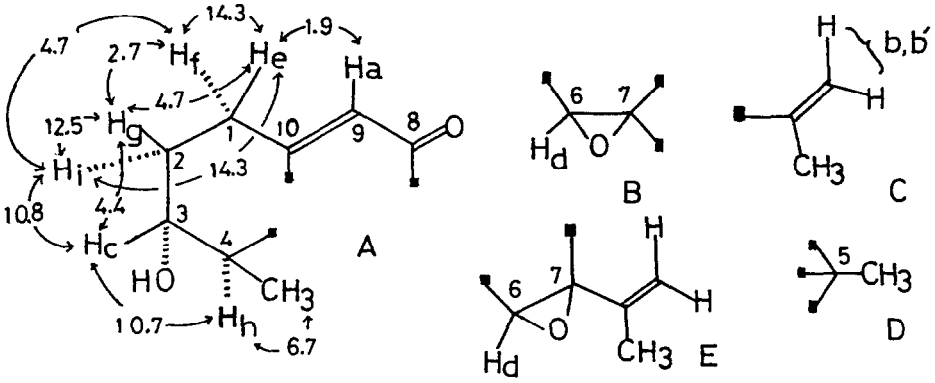
Summary: A sporogenic substance, sporogen-AO 1, isolated from the culture broth of *Aspergillus oryzae*(NOY-2) has been shown to have the structure 1. The structure was elucidated using spectroscopic methods, especially by long range selective proton decoupling in the gated decoupled ^{13}C nmr spectrum and with difference nuclear Overhauser effect techniques.

Aspergillus oryzae is one of the most important fungi in Japanese fermentation industry, which is widely used in Japanese traditional manufactures of sake, soy sause and miso. The fungus also is used for the production of various useful enzymes, e.g., amylase and protease.[1] As an extent of the sporulation, as well as physiological properties of the produced spores, affects the quality and yield of the fermented products, the nutritional and environmental conditions influencing the sporulation of *Asp. oryzae* have been extensively investigated. In our continuing research on asexual sporogenic substances of fungi,[2-5] we found that a rich-sporulating strain(NOY-2) of *Asp. oryzae* produced sporogenic substances in the culture broth, which stimulated dramatically phialospore[6] formation on the mycelial hyphae of a less-sporulating strain of this fungus. We have succeeded in isolating one of these sporogenic substances, named sporogen-AO 1,[7] and now describe the structure elucidation of the new active compound as 1. This is the first fungal metabolite with phialospore formation-stimulating activity.

The crude active compound extracted from the culture broth into the ethyl acetate soluble neutral fraction was purified by partition between carbon tetrachloride and aqueous methanol and by subsequent column chromatography of the aqueous methanol fraction on silica gel followed by HPLC(LiChrosorb Si-60 and Develosil C8-5) and preparative GC(3% OV-1 on Gas Chrom Q, 1 m, He 40 ml/min, 150-230°C, 5°C/min). The active compound thus isolated(yield 3 mg from 40 liters of the culture broth) showed significant sporogenic activity at a dose of 4.4 μg/disc.[7]

Sporogen-AO 1, 1, revealed the following physicochemical properties; colorless oil, $[\alpha]_D^{20}$ +214°(c=1.0, $CHCl_3$), $C_{15}H_{20}O_3$(HRMS: M^+ obsd. 248.1431, calcd. 248.1413), λ_{max}^{MeOH} 240 nm (ε=10500), ν_{max}^{KBr} 3440 and 1670 cm^{-1}, ^1H nmr(360 MHz, $CDCl_3$, δ): 1.22(3H, s, $-CH_3$), 1.26(3H, d, J=6.7 Hz, CH-CH_3), 1.44(1H, dddd, J=4.7. 10.8, 12.5, 14.3 Hz, H_i), 1.81(1H, dq, J=6.7, 10.8 Hz, H_h), 1.87(3H, bs, C=C-CH_3), 2.16(1H, dddd, J=2.7, 4.4, 4.7, 12.5 Hz, H_g), 2.34(1H, ddd, J=2.7, 4.7, 14.3 Hz, H_f), 2.52(1H, dddd, J=1.9, 4.7, 14.3, 14.3 Hz, H_e), 3.22(1H, s, H_d), 3.62(1H, ddd, J=4.4, 10.8, 10.8 Hz, H_c), 5.10(1H, q, J=1.5, $H_{b'}$), 5.11(1H, bs, H_b) and 5.76(1H, d, J=1.9, H_a). The ^{13}C nmr spectrum of 1(50 MHz, $CDCl_3$, δ): 11.4(q, $^1J_{^{13}C-H}$=126 Hz, $-CH_3$), 18.9(q, J=126, $-CH_3$), 19.8(q, J=128, $-CH_3$), 31.4(t, J=132, $-CH_2-$), 35.4(t, J=132, $-CH_2-$), 41.1(s, >C<), 44.5(d, J=129, >CH-), 63.6(s, >C-O-), 68.3(d, J=180, >CH-O-), 71.1(d, J=141, >CH-O-), 114.5(t, J=158, $>C=CH_2$), 121.3(d, J=162, >C=CH-), 139.2(s, >C=C), 163.2(s, >C=C) and 192.8(s, >C=O). The gated decoupled ^{13}C nmr of 1 showed that one of the two oxygen-bearing methine carbons had a large coupling constant($^1J_{^{13}C-H}$=180) characteristic for an epoxide ring.[8] Based on these spectral data an α,β-unsaturated ketone, a secondary hydroxyl and an epoxide group were suggested to be present in 1. The extensive spin decoupling experiments on the ^1H nmr spectrum indicated that 1 had the following four partial structures, A to D. H_a which had a lower chemical shift(5.76) than those of H_b and $H_{b'}$,(5.10 and 5.11) indicated that it was a vinyl proton connected to the α carbon of the unsaturated ketone. H_c(3.62) was assigned to a proton on a OH-bearing carbon



because it shifted to δ 4.86 upon acetylation. In the long range selective proton decoupling (LSPD) of 1, an irradiation of vinyl H_b and $H_{b'}$, sharpened both of a vinyl methyl carbon(δ 19.8) in the structure C and a quaternary epoxide C-7 carbon in the structure B. The latter result suggests that these vinyl protons are connected to the C-7 epoxide carbon through three bonds, thus, the partial structure B and C were extended to the structure E. The combination of this

C-7 epoxide carbon with the C-8 carbonyl carbon in the structure A was revealed by the aid of chemical manipulation. Hydrogenation of 1 on Pd/C gave a ketonic compound 2($C_{15}H_{26}O_2$, M^+ m/z 238), the mass spectrum of which showed typical fragmentation peaks via McLafferty rearrangement at m/z 196(44%, $M^+-C_3H_6$), 178(68%, $M^+-C_3H_6-H_2O$) and 108(100%, C_7H_8O), requiring the α-isopropyl ketone moiety in the structure 2. Thus, only one possible structure 1 for sporogen-AO 1 was deduced by combining the combined A-E with the structure D.

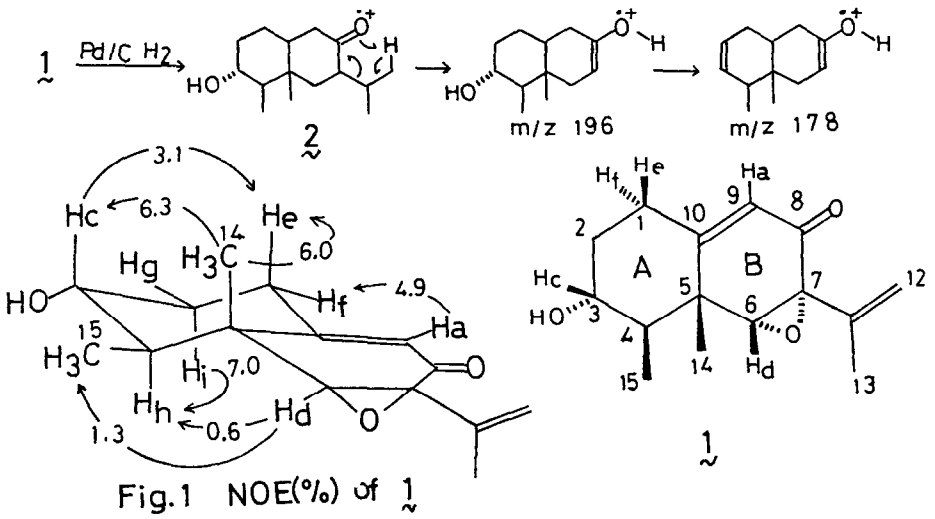


Fig.1 NOE(%) of 1

The relative stereochemistry of 1 was clarified by the proton coupling constants in the structure A and appropriate nuclear Overhauser effect(NOE) difference spectroscopy(Fig. 1). The coupling constants of the ring A protons indicated it to be in a chair conformation. The 1,3-diaxial relationships between the quaternary C-14 methyl and H_c and H_e were shown by large NOE enhancements of H_c and H_e upon irradiation of the C-14 methyl protons. All other NOE values observed (Fig. 1) were well in accord with the assigned structure. The absolute configuration of 1 was determined by the CD spectrum of 1 and by applying the exciton chirality method[9] to p-bromobenzoate of 1. The CD spectrum of 1 showed three Cotton effects, two of which were due to a π-π* transitions[220 nm(Δε -6.28) and 254(+2.56)] and the other one due to a n-π*[334(+3.20)]. Bergstahler and Barkhurst[10] have reported that in the CD spectra of the cyclic conjugated enone system the π-π* Cotton effect in the shorter wavelength(200 - 220 nm) reflects the chirality of a pseudoaxial bond on α'-carbon of the ketone group, and the longer

Cotton effect(230 - 260 nm) reflects that of the allylic axial substituents, as shown in Fig. 2. Thus, the negative Cotton effect at 220 nm in 1 showed that the epoxide C-7 oxygen should be situated pseudoaxially on the α-side of the structure 1. The positive Cotton effect at 254 nm showed that the allylic C-14 methyl group was on the β-side because the axial C-14 methyl has a contribution bigger than the smaller C-1 axial hydrogen(H_e), which shows an opposite contribution to the Cotton effect, as shown in the case of cholest-4-en-3-one.[10] Moreover, the CD spectrum of the p-bromobenzoate of 1 gave split type Cotton effects at 252 nm (Δε -4.06) and 237(+1.51) in MeOH. The negative first Cotton effect indicates that two chromophores, p-bromobenzoyloxy and the conjugated enone groups, are twisted in an anti-clockwise sense. Thus, the absolute configuration of sporogen-AO 1 was established as 1.

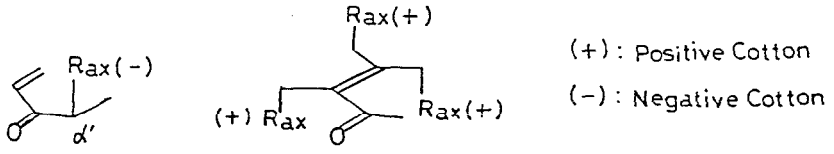


Fig. 2 α'-Pseudoaxial and allylic axial chirality contributions

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