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Penigequinolones A and B, Pollen-growth Inhibitors Produced by *Penicillium* sp., No. 410

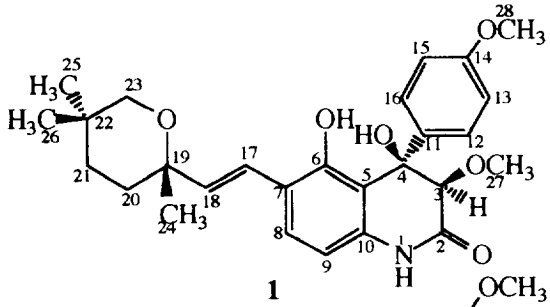
Yasuo Kimura †, Miyako Kusano, Hiroyuki Koshino*, Jun Uzawa*, Shozo Fujioka and Kiyotsugu Tani

Department of Agricultural Chemistry, Faculty of Agriculture, Tottori University, Koyama, Tottori 680, Japan

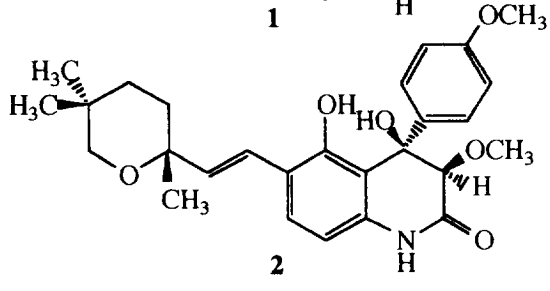
*The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama 351-01, Japan

Abstract: Penigequinolones A and B were isolated from the mycelial mats of Penicillium sp. No. 410 as new pollen-growth inhibitors, and their structures were established by NMR studies.

Pollen-growth inhibitors may be useful for developing new herbicides and as tools to analyze the reproductive functions in higher plants.[1] From these points of view, we have investigated pollen growth inhibitors among the fungal metabolites by means of the bioassay method[2] using tea pollen grains of *Camellia sinensis* O. Kuntze. As a result, we found the presence of active compounds in the mycelial mats of *Penicillium* sp. No. 410 and isolated a mixture of penigequinolones A (**1**) and B (**2**). Here we describe the structural elucidation of **1** and **2**.



1



2

The fungus was cultured stationarily in a malt medium at 24 °C for 21 days. The mycelial mats obtained after filtration of the culture broth were extracted three times with acetone. The combined solvents were concentrated *in vacuo* and the residue was chromatographed on a silica gel and sephadex LH-20 column. A multistep fractionation by chromatography afforded the active fraction as a yellow amorphous powder {$[\alpha]_D$ + 60°(c 1.0, MeOH)} in a yield of 11.6 mg/l , which showed a single peak on the HPLC using a ODS column with MeOH-H_2O. The molecular formula of the active fraction was established as $C_{27}H_{33}NO_6$ by HR-EIMS [M^+, *m/z* 467.2290 (-1.6 mmu. error)] and HR-FABMS [$(M+H)^+$, *m/z* 468.2373 (-1.3 mmu. error)]. The IR spectrum indicated the presence of hydroxyl, amido, and phenyl groups at ν_{max} 3306, 1690, and 1611 cm^{-1}. The ^{1}H-NMR spectrum in $CDCl_3$ indicated that the active fraction was acutually a 2:1 mixture of two closely related diastereoisomers (**1** and **2**). The ^{1}H-NMR data are summarized in the Table, and half of the signals appeared in pairs with a 2:1 ratio in integrational values. Two methoxy signals (C-27, 28), three aromatic signals (C-8, 12, 13), an olefinic signal (C-18), methylene signals (C-23), and a methine signal (C-3) appeared in pairs with a small difference in chemical shifts, but the distinction between the major and minor diastereoisomers was possible. The ^{1}H-NMR spectrum in DMSO-d_6 solution was also measured and the ratio of the mixture was not changed. In the experiments of NOE differential spectroscopy at 50 °C in DMSO-d_6 solution, there was no chemical exchange between any protons of the major and the corresponding protons of the minor diastereoisomers. Although the mixture was further heated to 80 °C in DMSO-d_6 solution, there was no change in their 2:1 ratio. These results indicated the relationship between the diastereomers and provided evidence that the mixture of **1** and **2** was not an exchangeable conformer or tautomer. The ^{13}C-NMR spectrum showed also the presence of a pair of signals for the aliphatic carbons of C-19, 23, 24, 25 and the aromatic carbons of C-7, 8 and the olefinic carbon of C-18. The assignments of ^{1}H- and ^{13}C-NMR shown in the Table were confirmed by the analyses of PFG-DQFCOSY[3] and PFG-HMQC[4]

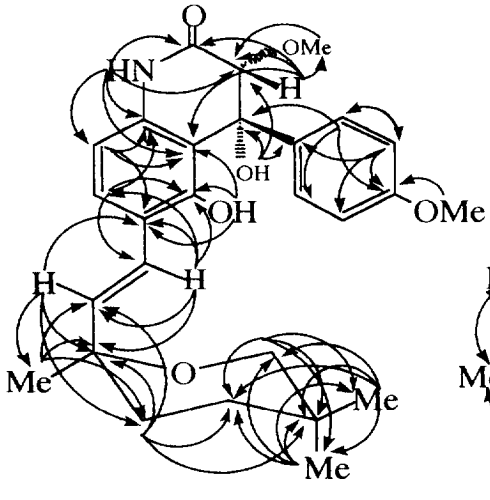


Fig. 1. PFG-HMBC experiments of **1**

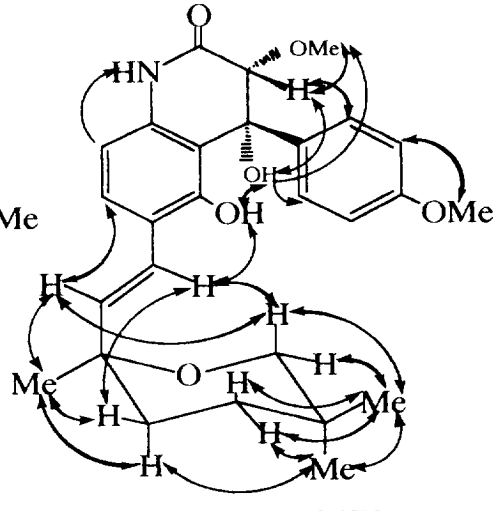


Fig. 2. Selective PFG-1D-ROESY experiments of **1**

data. These 2D spectral data suggested the presence of a tetra-substituted aromatic ring (C-5~C-10), a *p*-methoxyphenyl group (C-11~C-16), a (*E*)-double bond between C-17 and 18 (J=16.6 Hz), and a tetrahydropyranyl ring as partial structures.

The connectivities of each partial structure and assignments of all other quarternary carbons at C-4, 19, and C-22, and the carbonyl carbon at C-2 of the mixture were established by PFG-HMBC[5] data. Distinction of an amido proton at δ_H 8.44 from two other exchangeable hydroxyl protons at δ_H 4.60 (4-OH) and 9.12 (6-OH) was carried out by ^1H-^{15}N PFG-HMQC.[6] The amido proton was correlated to nitrogen at δ_N 109.6 which chemical shift was represented from external reference of $^{15}NH_4NO_3$ in DMSO-δ_6 at 0 ppm. From these results, the planar structure of the mixture was established to be the same.

By selective PFG-1D-ROESY experiments,[7] ROEs of the mixture were observed between the protons of a methoxy group (C-27) and the hydroxy proton (4-OH), the methine proton (H-3) and the hydroxy proton (4-OH), and the methine proton (H-3) and the aromatic proton at C-12 (C-16) of *p*-methoxyphenyl group. However, ROE was not observed between the protons of a methoxy group (C-27) and the aromatic proton at C-12 (C-16) of the *p*-methoxyphenyl group. These results account for the relative

Table 1. ^1H (600 MHz) and ^{13}C (100 MHz) NMR Data for Penigequinolones in $CDCl_3$

Carbon Number	^{13}C	^1H	Carbon Number	^{13}C	^1H
2	166.05		20	31.04	1.71(m) ax.
3	84.16	3.69(d, 1.5)M			1.81(m) eq.
		3.70(d, 1.5)m	21	33.48	1.33(m) eq.
4	78.66				1.45(m) ax.
5	110.75		22	29.66	
6	155.04		23	72.69 M	3.38(d, 11.2)M, ax.
7	121.94 M			72.72 m	3.37(d, 11.2)m, ax.
	121.89 m				3.23(dd, 2.0, 11.2)M, eq.
8	127.38 M	7.38(d, 8.3)M			3.22(dd, 2.0,11.2)m, eq.
	127.33 m	7.39(d, 8.3)m	24	29.10 M	1.30(s) eq.
9	106.96	6.36(d, 8.3)		29.15 m	
10	134.26		25	26.53 M	0.79(s) eq.
11	129.00			26.57 m	
12, 16	127.79	7.19(d, 8.8)M	26	24.00	1.00(s) ax.
		7.18(d, 8.8)m	27	58.88	3.610(s)M
13, 15	114.20	6.82(d, 8.8)M			3.612(s)m
		6.81(d, 8.8)m	28	55.23	3.750(s)M
14	160.19				3.747(s)m
17	123.15	6.73(d, 16.6)	1-NH		8.44(br.s)
18	134.31 M	6.14(d, 16.6)M	4-OH		4.60(s
	134.21 m	6.13(d, 16.6)m	6-OH		9.12(s)
19	74.32 M				
	74.37 m				

The s, d, and m, and the numbers in parentheses of ^1H NMR spectrum showed multiplicities and coupling constants. The M and m revealed the peaks with higher intensity and lower intensity, respectively. The ax. and eq. showed the axial and equatorial positions.

stereochemistry at C-3 and C-4 to be 3*R** and 4*R**, respectively. The conformation of the tetrahydropyranyl moiety was determined by selective PFG-1D-ROESY experiments. ROEs were observed between the axial proton at δ_H 3.38 (3.37) of a methylene group (C-23) and both of the olefinic protons of H-17 and H-18, and the axial proton at δ_H 1.71 of a methylene group (C-20) and the methyl protons at C-26. Other ROEs were shown in the Figure 2.

These ROEs data indicated that the conformation of the tetrahydropyranyl ring was a chair form and the double bond at C-18 was in axial orientation. All observed ROEs were common to both diastereoisomers, so the conformation of the diastereoisomers may be quite similar. Based on the spectral data mentioned above, the structures of penigequinolone A and B were determined to be **1** and **2** as depicted. In this study, the ratio of the mixture of penigequinolones A and B could not be determined as 2:1 or 1:2, because the chiral center at C-19 is separated from the other chiral centers at C-3 and C-4.

Thus, the structures of penigequinolones have a novel dihydro 2-quinolone skeleton and are quite different from those of hericerin,[8] emeniveol[9] and isofunicone[10] which have been isolated as pollen-growth inhibitors.

Biological activity of the penigequinolone mixture was examined in the growth of tea pollen tubes. Penigequinolones inhibited the pollen-growth by 40 % at 10 mg/l and achieved complete inhibition at 100 mg/l. Its activity was stronger than that of emeniveol, but weaker than that of hericerin and isofunicone. More detailed biological activities will be reported elsewhere.

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