Short Communication

Pyrichalasin H, a New Phytotoxic Metabolite Belonging to the Cytochalasans from *Pyricularia grisea* (Cooke) Saccardo

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In continuing chemical studies of metabolites produced by the fungi belonging to the genus *Pyricularia*,¹⁾ a new phytotoxic metabolite named pyrichalasin H (**1**)²⁾ was isolated from the fungus *Pyricularia grisea* (Cooke) Saccardo IFO 7287. Pyrichalasin H was found to strongly inhibit the growth of rice seedlings and at 1 $\mu\text{g}/\text{ml}$ (1.9×10^{-6} M), characteristic curling of the shoot was observed as shown in Fig. 1. In this communication, the isolation and structural determination of pyrichalasin H is reported.

The fungus IFO 7287 was cultured in a soy sauce-sucrose medium using a jar fermenter for 10 days. The filtered culture broth was

passed through a column of XAD-2, the column being washed with methanol. The methanol washings were combined and concentrated to an aqueous suspension, which was then extracted with ethyl acetate. The ethyl acetate extract was subjected to silica gel column chromatography with a solvent system of ethyl acetate-*n*-hexane. The fractions containing pyrichalasin H as indicated by TLC monitoring (silica gel 60 F₂₅₄ aluminum sheet (Merck), chloroform-methanol=10:1 (v/v), *R*_f=0.47) were combined, and after rotary evaporation, the residue was crystallized from ethyl acetate to give crude crystals, which afforded upon recrystallization colorless plates, mp 207~209°C, $[\alpha]_D^{23} -18.4^\circ$ ($c=1.05$, CHCl_3),³⁾ in a yield of 54 mg/l of medium.

Elemental and mass spectral analyses indicated the molecular formula $\text{C}_{31}\text{H}_{41}\text{NO}_6$ for pyrichalasin H (Found: C, 70.86; H, 7.95; N, 2.47. Calcd. for $\text{C}_{31}\text{H}_{41}\text{NO}_6$: C, 71.10; H, 7.89; N, 2.67%. M^+ (m/z) 523). On the basis of the following evidence the structure **1** was assigned for pyrichalasin H: Its UV spectrum (ethanol) exhibited intense absorption maxima at 203 nm (ϵ 22000) and 224 (17000), with inflections at 276 (2000) and 283 (1640), which indicated the presence of several isolated double bonds and of a substituted aromatic ring. The IR spectrum (chloroform) showed ab-

FIG. 1. Effects of Pyrichalasin H on the Growth of Rice Seedlings (cultivar: Sasanishiki).

FIG. 2. CD Spectra Taken in Ehtanol of Pyrichalasin H (—) and Cytochalasin H (-----).

FIG. 3. Structures of 1 and 2.

sorption bands at 3590, 3540 and 3410 (OH and NH); 1735 (ester CO); 1690 (amide CO); 1610, 1580 and 1512 (benzene ring); and 965 (E-CH=CH-) cm^{-1} . The ^1H NMR spectrum (Table I), whose assignment is based on the results of 2D homonuclear correlation spectroscopy (COSY),⁴⁾ indicated the presence of a *para*-substituted benzene ring, two disubstituted double bonds, and an exocyclic methylene, two secondary methyl, and tertiary methyl groups. The ^{13}C NMR spectrum (Table I), whose assignment was based on the results of 2D heteronuclear COSY that included long-range C-H COSY,⁵⁾ confirms these functional assignments. The analysis of these 2D NMR spectra led to the construction of the structure 1 for pyrichalasin H. This structure is very similar to cytochalasin H (2),

TABLE I. ^1H (400 MHz) AND ^{13}C (100 MHz) NMR DATA FOR PYRICALASIN H (1)

Position	δ_{C}	δ_{H}^a
1	174.18	
2		5.56 s
3	53.85	3.21 m
4	50.31	2.11 dd (4, 5)
5	32.84	2.77 m
6	148.01	
7	69.73	3.83 d (10)
8	47.19	2.94 dd (10, 10)
9	51.77	
10	44.70	2.59 dd (14, 10) 2.80 dd (14, 5)
11	14.08	0.99 d (7)
12	113.99	5.11 br.s 5.35 br.s
13	127.15	5.74 dd (15, 10)
14	138.64	5.40 m
15	42.74	2.04 m 1.80 m
16	28.43	1.79 m
17	53.69	1.88 dd (14, 3) 1.57 dd (14, 2)
18	74.28	
19	125.98	5.87 dd (15, 2)
20	138.08	5.53 dd (15, 2)
21	77.47	5.51 d (2)
22	26.42	1.05 d (7)
23	31.14	1.35 s
1'	129.37	
2', 6'	129.99	7.06 d (8)
3', 5'	114.32	6.85 d (8)
4'	158.68	
Ac	170.06	
	20.82	2.24 s
OMe	55.29	3.79 s

^a s=singlet; d=doublet; dd=double doublet; m=multiplet; br.s=broad singlet. The values in parentheses are coupling constants expressed in Hz.

whose structure has been established by chemical and spectroscopic as well as by X-ray crystallographic studies.⁶⁾ The chemical shifts of the carbons from C-1 to C-23 of pyrichalasin H were of almost identical values ($\Delta\delta_{\text{C}} < 1.1$ ppm) to the respective carbon chemical shifts of cytochalasin H.⁷⁾ The chemical shift differences can be seen in the signals coming from the substituents at C-10, i.e., a *p*-methoxyphenyl group for pyrichalasin H and phenyl group for cytochalasin H. The chemical shift differences observed in these carbon sig-

nals are similar to the chemical shift differences observed between the signals of the aromatic carbons of phenylalanine and tyrosine.⁸⁾ The fragmentation pattern⁹⁾ in the EI-MS spectrum of pyrichalasin H reflects this structural difference when compared to those of the reported fragmentation pattern of cytochalasin H.¹⁰⁾ The comparison of the proton-proton coupling constants for cytochalasin H and pyrichalasin H indicates that the relative stereochemistry of both compounds should be the same, and a comparison of the CD curves (Fig. 2) of both compounds shows that the absolute stereochemistry of both compounds should also be the same. Therefore, the structure of pyrichalasin H was established to be (7*S*,16*S*,18*R*,21*R*)-21-acetoxy-7,18-dihydroxy-16,18-dimethyl-10-*p*-methoxyphenyl-[11]cytochalasin-6(12),13(*E*),19(*E*)-triene-1-one. This is the first reported case of a *p*-methoxyphenyl group being substituted at C-10, the preceding metabolites belonging to the cytochalasins containing either one phenyl, indolyl, or isopropyl group.¹¹⁾ Tyrosine may be a possible biogenetic origin, with subsequent polyketide cyclization and methylation leading to pyrichalasin.

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REFERENCES AND NOTES

- 1) M. Nukina, T. Sassa, M. Ikeda, T. Umezawa and H. Tasaki, *Agric. Biol. Chem.*, **45**, 2161 (1981).
- 2) Since the name "cytochalasin" is mainly used for fungal metabolites possessing a phenyl group at C-10, the name "aspochalasin" for metabolites having an isopropyl group, and the name "chaetoglobosin" for those having an indolyl group,¹¹⁾ the name "pyrichalasin" is introduced for those metabolites having a *p*-methoxyphenyl group which is biogenetically considered to be derived from tyrosine.
- 3) Pyrichalasin H shows marked solvent dependence for its optical rotation; i.e., $[\alpha]_D^{23} + 59^\circ$ ($c = 1.05$, MeOH). Similar behavior for cytochalasin H (kodo-cytochalasin-1) and cytochalasin D has been reported: S. A. Patwardhan, R. C. Pandey, S. Dev and G. S. Pendse, *Phytochemistry*, **13**, 1985 (1974).
- 4) In the 2D homonuclear COSY spectrum of **1**, cross peaks were observed between the following pairs of signals: ($\delta_H - \delta_H$) 7.06 ~ 6.85, 2.59 ~ 2.80, 2.59 ~ 3.21, 2.80 ~ 3.21, 3.21 ~ 2.11, 2.11 ~ 2.77, 2.77 ~ 0.99, 3.83 ~ 2.94, 2.94 ~ 5.74, 5.74 ~ 5.40, 5.40 ~ 2.04, 5.40 ~ 1.80, 2.04 ~ 1.80, 2.04 ~ 1.79, 1.79 ~ 1.05, 1.79 ~ 1.88, 1.57 ~ 1.88, 5.53 ~ 5.87 and 5.87 ~ 5.51, affording the connectivities of five carbons from C-10 to C-11 through C-3, -4 and -5, and of eight carbons from C-7 to C-22 through C-8, -13, -14, -15, -16 and -17. In the 2D homonuclear long-range COSY spectrum of **1**, cross peaks appeared between the following pairs: 3.79 ~ 6.85, 7.06 ~ 2.80, 7.06 ~ 2.59 and 5.53 ~ 1.35, giving the connectivity of the *p*-methoxyphenyl group at C-10, and a tertiary methyl group to C-18 being adjacent to C-19.
- 5) In the 2D heteronuclear C-H long-range COSY spectra of **1**, cross peaks were observed between the following pairs of signals: ($\delta_H - \delta_C$) 2.94 ~ 174.18, 2.24 ~ 170.06, 3.79 ~ 158.68, 6.85 ~ 158.68, 0.99 ~ 148.01, 2.11 ~ 148.01, 2.04 ~ 138.64, 2.94 ~ 138.64, 5.51 ~ 138.08, 1.57 ~ 138.08, 1.35 ~ 138.08, 6.85 ~ 129.99, 2.80 ~ 129.99, 2.59 ~ 129.99, 5.51 ~ 125.98, 5.35 ~ 125.98, 7.06 ~ 114.32, 5.87 ~ 77.47, 5.87 ~ 74.28, 1.57 ~ 74.28, 1.35 ~ 74.28, 1.35 ~ 53.69, 5.51 ~ 51.77, 2.94 ~ 51.77, 5.51 ~ 50.31, 0.99 ~ 50.31, 5.51 ~ 47.19, 1.88 ~ 42.74, 5.35 ~ 32.84, 1.80 ~ 28.43 and 1.57 ~ 26.42, which enabled to construct the structure **1**.
- 6) M. A. Beno, R. H. Cox, J. M. Wells, R. J. Cole, J. W. Kirksey and G. G. Christoph, *J. Am. Chem. Soc.*, **99**, 4123 (1977), and references cited therein.
- 7) Since the H-C heteronuclear COSY of pyrichalasin H clearly indicates that the carbon chemical shift of C-23 is δ_C 28.5 and that of C-16 is δ_C 31.2, the ^{13}C chemical shift assignments made by Beno *et al.*⁶⁾ of the corresponding two carbons in cytochalasin H should be interchanged.
- 8) E. Pretsch, J. Seible, W. Simon and T. Clerc, "Tables of Spectral Data for Structure Determination of Organic Compounds," Springer-Verlag, New York, 1983, p. C210.
- 9) In the EI-MS spectrum of pyrichalasin H, the following prominent ions were observed: m/z (relative intensity (%)) 523 (2%, M^+), 506 (2%, $\text{M}^+ - 17$ (OH)), 505 (2%, $\text{M}^+ - 18$ (H_2O)), 464 (4%, $\text{M}^+ - 59$ (OAc)), 463 (3%, $\text{M}^+ - 60$ (AcOH)), 446 (9%, $\text{M}^+ - 77$ (AcOH + OH or AcO + H_2O)), 445 (11%, $\text{M}^+ - 78$ (AcOH + H_2O)), 402 (12%, $\text{M}^+ - 121$ ($\text{MeOC}_6\text{H}_4\text{CH}_2$)), 385 (1%, $\text{M}^+ - 138$ ($\text{MeOC}_6\text{H}_4\text{CH}_2 + \text{OH}$)), 384 (5%, $\text{M}^+ - 139$ ($\text{MeOC}_6\text{H}_4\text{CH}_2 + \text{H}_2\text{O}$)), 343 (3%, $\text{M}^+ - 180$ ($\text{MeOC}_6\text{H}_4\text{CH}_2 + \text{OAc}$)), 342 (12%, $\text{M}^+ - 181$ ($\text{MeOC}_6\text{H}_4\text{CH}_2 + \text{AcOH}$)), 325 (9%, $\text{M}^+ - 198$ ($\text{MeOC}_6\text{H}_4\text{CH}_2 + \text{AcOH} + \text{OH}$)), 324 (33%, $\text{M}^+ - 199$ ($\text{MeOC}_6\text{H}_4\text{CH}_2 +$

- AcOH + H₂O)), 306 (8%, M⁺ - 217 (MeOC₆H₄CH₂ + 2H₂O + OAc)), and 121 (100%, MeOC₆H₄CH₂⁺ ion).
- 10) R. J. Bushway, D. F. McGann and A. A. Bushway, *J. Agric. Food Chem.*, **32**, 548 (1984).
- 11) W. B. Turner and D. C. Aldridge, "Fungal Metabolites II," Academic Press, London, 1983, pp. 459~466.
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