Communication to the Editor

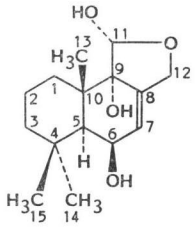
STRUCTURE OF NEW ANTIBIOTICS, PERENIPORINS A AND B, FROM A BASIDIOMYCETE

Sir:

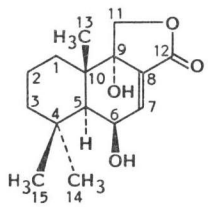
During the course of a screening search for new plant growth regulators among metabolites of microorganisms, *Perenniporia medullaepanis* Aj 8345 was found to produce plant growth inhibitors, named pereniporins A and B (Fig. 1). In this paper, we wish to report the isolation and structural elucidation of these metabolites.

The strain was shake cultured for 20 days at 27°C in Sakaguchi flasks containing the following medium in 1,000 ml of distilled water; soluble starch 10 g, glucose 20 g, $(NH_4)_2SO_4$ 5 g, KH_2PO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, NaCl 0.5 g, potato extract 20 g, 1 ml of a trace salt mixture, which contained $CuSO_4 \cdot 5H_2O$ 0.6 g, $FeSO_4 \cdot 7H_2O$ 0.11 g, $MnCl_2 \cdot 4H_2O$ 0.79 g and $ZnSO_4 \cdot 7H_2O$ 0.15 g per 100 ml.

Fig. 1. Structures of pereniporins A and B.



Pereniporin A (**1**)



Pereniporin B (**2**)

The culture filtrate (6 liters) was extracted with ethyl acetate. The organic solvent was evaporated *in vacuo*, and the residue was subjected to silica gel column chromatography (methanol - ethyl acetate, 5: 95). Active fractions were combined and evaporated *in vacuo* to dryness, dissolved in 5 ml of methanol and subjected to gel filtration on Sephadex LH-20 in methanol. Combined active fractions were concentrated *in vacuo* to 2 ml. Further purification by preparative HPLC (μBondapak C_{18}; mobile phase, 20% aqueous CH_3CN) gave two pure samples of new antibiotics, pereniporins A and B, in an overall yield of 19 mg and 6 mg, respectively.

The physico-chemical properties of pereniporins A (**1**) and B (**2**) are shown in Table 1. **2** contains two less hydrogen atoms than **1**. The IR spectra of these compounds showed characteristic bands ranging from 3440 to 3400 cm^{-1} attributable to hydroxyl groups. The UV absorption at 225 nm and the IR absorption at 1750 cm^{-1} of **2** suggested the presence of an α,β-unsaturated γ-lactone. The ^1H and ^{13}C NMR spectra of **1** and **2** taken in CD_3OD are shown in Table 2. The assignments of functional groups were made with the aid of INEPT and 2D C-H correlation spectra of **1** and **2**. Their spectra were very similar, particularly the ^1H and ^{13}C NMR data, the exception being the UV and IR data mentioned above.

The molecular formula of **1**, $C_{15}H_{24}O_4$, established from the FD-MS (m/z 268, M^+) and ele-

Table 1. Physico-chemical properties of pereniporins A and B.

		Pereniporin A	Pereniporin B
Appearance		White powder	White powder
MP (°C)		164~166	181~183
$[\alpha]_D^{24}$ (MeOH)		−181° (*c* 0.25)	−208° (*c* 0.05)
FD-MS m/z (M^+)		268	266
Formula		$C_{15}H_{24}O_4$	$C_{15}H_{22}O_4$
IR ν_{max}^{KBr} cm^{-1}		3400, 1050, 1030	3440, 1750, 1040
UV λ_{max}^{MeOH} nm		End absorption	225
Color reaction	Positive:	Potassium permanganate	Potassium permanganate
	Negative:	Ninhydrin	Ninhydrin
Silica gel TLC* Rf		0.73	0.86

* Using a solvent system of MeOH - EtOAc (5: 95).

mental analysis (*Anal* Found: C 63.59, H 8.39, N 0.06%. Calcd for $C_{15}H_{24}O_4 \cdot H_2O$: C 62.94, H 8.39, N 0%.), and the presence of one double bond (Table 2) oblige **1** to have three ring systems in the molecule. Analysis of the ^{13}C NMR spectrum accounted for 21 protons directly attached to carbons. The remaining three exchangeable protons are assumed to exist as hydroxyl groups from the IR spectrum and ^{13}C NMR spectral data of **1** (Table 2).

Table 2. Assignments of the chemical shifts in ^1H NMR and ^{13}C NMR spectra of pereniporins A and B.

	Pereniporin A	Pereniporin B
$-CH_3$	19.4 (1.14)	20.3 (1.12)
	25.1 (1.34)	24.9 (1.35)
	33.4 (1.08)	33.4 (1.12)
$-CH_2-$	19.3 (1.46, 1.70)	19.2 (1.51, 1.72)
	33.3 (1.27, 1.92)	33.3 (1.19, 2.05)
	45.9 (1.27, 1.34)	45.7 (1.32, 1.36)
$-CH_2O-$	67.8 (4.17, 4.53)	76.5 (4.17, 4.47)
$>CH-$	47.8 (1.83)	47.3 (1.87)
$>CHO-$	66.0 (4.45)	65.6 (4.60)
$-CH<^{O-}_{O-}$	99.3 (5.31)	
$=CH-$	124.8 (5.64)	140.8 (6.77)
$>C-$	35.0	35.0
	39.3	39.9
$>CO-$	78.5	78.2
$=C<$	140.1	130.8
$>C=O$		172.1

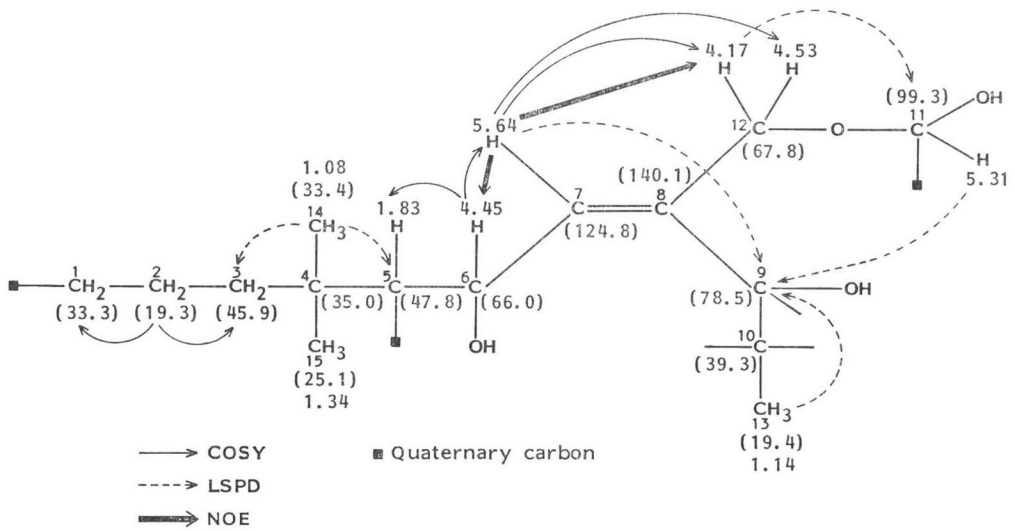
The chemical shifts of protons are given in parentheses.

The ^1H and ^{13}C NMR spectra were taken in CD_3OD with TMS as internal reference.

A triacetate (**3**), prepared by treatment of **1** with acetic anhydride in pyridine, was analyzed with FD-MS and the main peak was m/z 394 (M^+). The ^1H NMR spectrum of **3** showed signals due to three singlet methyls at δ_H 2.04, 2.06 and 2.07. These data indicated the presence of three hydroxyl groups in **1**. Acetylation shifts were observed with two methine protons (δ_H 4.45, 5.31 in **1**, δ_H 5.54, 6.30 in **3**). These data suggested that two secondary hydroxyl groups attached to the two methine carbons (δ_C 66.0, 99.3) and a tertiary alcohol were acetylated in **3**. The acetylation of the C-9 tertiary alcohol is explained by a reasonable C-11→C-9 acyl migration,[1] followed by ordinary acetylation of the secondary alcohol and this result, therefore, may imply that the two hydroxyl groups at these carbons are sterically close to each other.

Analysis of the 2D-COSY spectrum of **1** revealed straightforwardly the partial structure shown in Fig. 2. The oxymethine proton H-6 (δ_H 4.45) was coupled with the olefinic proton H-7 (δ_H 5.64) and the methine proton H-5 (δ_H 1.83). The methylene protons H-2 (δ_H 1.46, 1.70) were coupled with the methylene protons H-1 (δ_H 1.27, 1.92) and H-3 (δ_H 1.27, 1.34). The C-12 methylene carbon in the partial structure of **1** was connected to C-8, because long range coupling was observed between the olefinic pro-

Fig. 2. The partial structure of pereniporin A proved by the 2D-COSY spectrum, LSPD and NOE experiment.



ton H-7 (δ_H 5.64) and the oxymethylene protons H-12 (δ_H 4.17, 4.53) in the relayed COSY spectrum and NOE enhancement was observed between the olefinic proton H-7 (δ_H 5.64) and one of the oxymethylene protons H-12 (δ_H 4.17).

By long range selective proton decoupling (LSPD),[2] irradiating at both the C-7 olefinic proton (δ_H 5.64) and the C-13 methyl protons (δ_H 1.14), the C-9 carbon (δ_C 78.5) was collapsed to a sharp signal. Thus C-9 was connected to C-8 and C-10 was connected to C-9. Furthermore, it was concluded by LSPD that the quaternary carbon C-4 (δ_C 35.0) was connected to C-3 and C-5 by irradiating the C-14 methyl protons (δ_H 1.08) and that the C-11 hemiacetal carbon (δ_C 99.3) was connected to C-12 *via* an oxygen atom by irradiating the C-12 methylene protons (δ_H 4.17, 4.53). By LSPD irradiating at the C-11 methine proton (δ_H 5.31), the C-9 carbon (δ_C 78.5) was collapsed to a sharp signal. Thus C-11 was connected to C-9. Consequently, C-10 was connected to C-5 and C-1 to give the planar structure of **1** as shown in Fig. 1.

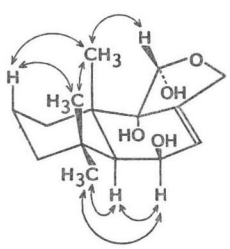
The structural elucidation of **2** was performed by comparing its ^{13}C NMR signals with those of **1**. As shown in Table 2, the ^{13}C NMR data of both compounds were almost identical except for the chemical shifts of carbons due to the five membered ether ring and one double bond. From these NMR spectral data and the evidence of the existence of an α,β-unsaturated γ-lactone moiety, the structure of **2** was determined as shown in Fig. 1. **2** was identical including optical rotation to $6\beta,9\alpha$-dihydroxydrimenine, which was chemically derived from mukaadial[3] by alkaline treatment. However, it is the first time that **2** has been isolated from nature.

The relative stereochemistry of **1** was determined by observation of the NOE enhancements shown in Fig. 3. In view of the co-occurrence of **1** and **2**, and their closely related structures, the absolute configuration of **1** is assumed to be as shown in Fig. 1.

Details of the structure elucidation of **1** and **2** will be reported elsewhere.

1 showed antimicrobial activity against *Bacillus subtilis* Aj 1316 in Davis minimum medium (MIC 6.25 μg/ml) but was inactive against Gram-negative bacteria. **1** inhibited the root elongation of lettuce at 100 ppm in the assay system described previously.[4] **1** and **2** showed cytotoxicity against Friend leukemia cells, F5-5, at 130 μg/ml and 3.91 μg/ml, respectively, in the assay system reported by MORIOKA *et al.*[5]

Fig. 3. Summarized results of ^1H-^1H NOE for pereniporin A.



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