

PIERICIDINS: NATURALLY OCCURRING INHIBITORS AGAINST MITOCHONDRIAL RESPIRATION

SHIGEO YOSHIDA and NOBUTAKA TAKAHASHI

Department of Agricultural Chemistry, The University of Tokyo, 113, Tokyo

1. Discovery

It has been well-known that numerous antibiotics had been discovered in metabolites of microorganisms through vast systematic screening research. However, there were few screening researches to find insecticidal substances among metabolites of microorganisms. Tamura et al.¹ found that silkworm larvae were killed by topical application of the cultured broth of a certain *Streptomyces*, which was identified as a new species and named *Streptomyces mobaraensis* Nagatsu et Suzuki, and finally two potent insecticidal substances were isolated from the mycelial extract of this microbe and named piericidins A and B.

Later, some *Streptomyces* species were found to produce piericidin-like substances but most of them were identified as the known piericidins A or B. Quite recently, Takahashi et al.^{2,3} found that many kinds of new piericidins were produced by *Streptomyces pactum*, and sixteen piericidins including piericidins A and B were isolated and systematically named piericidins A_n, B_n, C_n and D_n (n=1,2,3 and 4).

2. Isolation

The essentially same coarse fractionation was used in both cases of *S. mobaraensis*¹ and *S. pactum*³, although in the latter case, more improved techniques were needed for the separation of many piericidins with similar chemical properties, as illustrated in Fig. 1. To obtain seed culture, *S. mobaraensis* and *S. pactum* were precultured in media I and II respectively. Composition of these media is shown in Table 1. The seed culture thus obtained was then transferred into a fermentation tank containing the same medium. Incubation was continued for two days in the case of *S. mobaraensis* and for four days in the case of *S. pactum* at 27°C with aeration and agitation. The cultured broth was mixed with appropriate amount of Celite and filtered. The obtained mixture of mycelia and filter aid was stirred with acetone for extraction. The acetone solution was evaporated under reduced pressure, and the aqueous residue was repeatedly extracted with n-hexane at pH 5. The combined extracts were washed successively with aqueous sodium bicarbonate and brine. The n-hexane layer was dried over anhydrous sodium sulfate. Evaporation of the solvent to dryness under reduced pressure yielded crude piericidins.

The product was dissolved in benzene and applied onto a silica gel column, which was eluted successively with benzene and benzene-ethyl acetate mixture with increasing content of ethyl acetate by 0.5% in each step. Nonpolar impurities were eluted with benzene containing 0 - 1.0% of ethyl acetate, and the eluate containing piericidins was separated into three fractions; Fractions I, II and III.

Table 1. Composition of Culture Medium (g/liter)

I for <i>S. mobaraensis</i>				II for <i>S. pactum</i>			
Glucose	20	Brewers yeast	4	Starch	25	(NH ₄) ₂ SO ₄	2
Starch	10	NaCl	2	Soybean meal	15	CaCO ₃	4
Meat extract	10	K ₂ HPO ₄	0.5	Ebios	2		
Soybean powder	25	10% NaOH	3ml	NaCl	5		

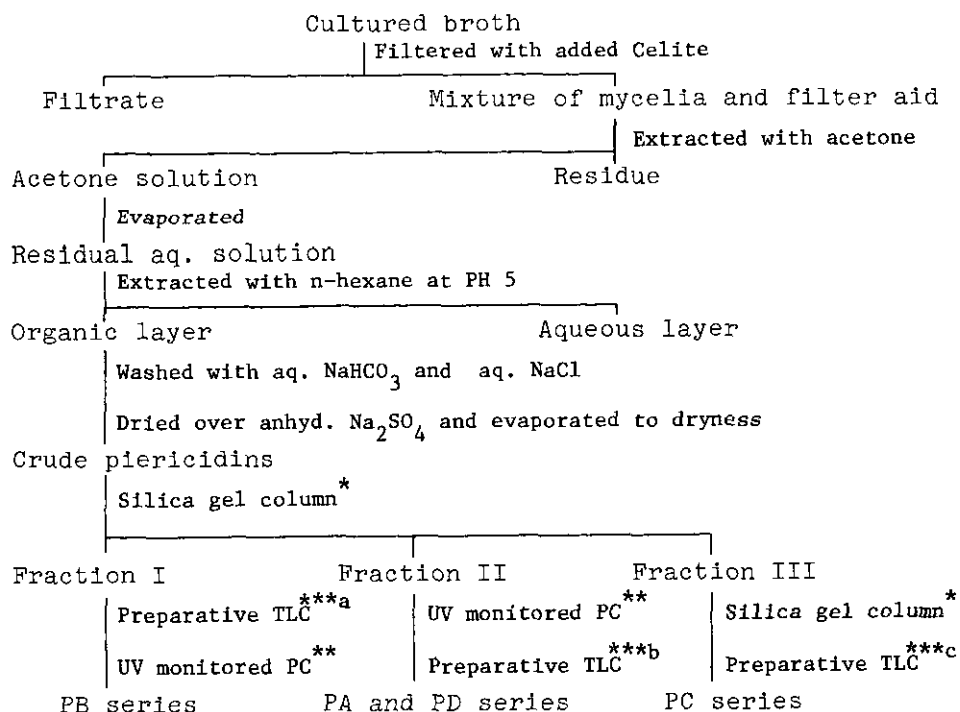


Fig. 1 Isolation procedure of piericidins.

- * Silica gel chromatography eluted with benzene-ethyl acetate.
- ** Prepacked silica gel column chromatography with pumping system:
solvent, n-hexane-tetrahydrofuran; UV monitor, 280 nm; flow rate,
240 or 300 ml/hr
- *** In these procedure, multiple development was usually needed.
solvent system: a, n-hexane-tetrahydrofuran (6 : 1);
b, benzene-ethyl acetate (5 : 1); c, benzene-ethyl acetate (4 : 1)

Fraction I containing PB series, which was eluted with benzene containing 1.5 - 2.0% of ethyl acetate, was further purified by preparative silica gel thin layer chromatography (TLC) developed with n-hexane-tetrahydrofuran mixture (Hex-THF, 6:1). The developed zone of piericidin B series was divided into five fractions and elution of the highest and the lowest zones gave pure PB₄ and PB₁ respectively. The residual middle zones composed of complex mixtures of piericidin B series were combined and applied onto a prepacked silica gel column system, which consisted of a pumping instrument and UV detector. The elution with Hex-THF (99:1) solvent system yielded pure PB₁, PB₂, PB₃ and PB₄.

Fraction II eluted with benzene containing 2.5 - 5.0% of ethyl acetate, which was composed of a complex mixture of piericidins A and D series, was rechromatographed through the prepacked column for further fractionation. The elution with Hex-THF (95:5) solvent system was able to separate Fraction II into nine fractions containing one or two kinds of piericidins which were readily separable to pure state by preparative TLC developed with benzene-ethyl acetate (Bz-EA, 5:1). These successive procedures afforded pure PA₁, PA₂, PA₃, PA₄, PD₁, PD₂, PD₃ and PD₄.

Fraction III eluted with benzene containing 5.5 - 8.0% of ethyl acetate, which consisted of piericidin C series, was also rechromatographed through a silica gel column. The eluate with Bz-EA (94:6) was finely separated into fractions containing only one or two kinds of piericidins. Two-component fractions were further purified by preparative TLC developed with Bz-EA (4:1). Thus PC₁, PC₂, PC₃ and PC₄ were clearly separated.

The yield of each piericidin was variable, depending on the fermentation period. For example, in the case of *S. pactum*, the standard cultivation, for four-day incubation, afforded the metabolite with the ratio of PD₁ to PC₁ in 1:5, while that ratio at seventh day changed to ca. 1:2. Table 2 shows approximate yields of piericidins produced by these microbes under the standard cultivation.

Table 2. Yield of Piericidins in Cultured Broth

Piericidin	Yield(mg/l)	Piericidin	Yield(mg/l)
A ₁	0.78 (800 - 900)*	B ₁	0.56 (2.0 - 4.0)*
A ₂	0.23	B ₂	0.04
A ₃	0.91	B ₃	0.26
A ₄	0.41	B ₄	0.11
C ₁	28.67	D ₁	6.22
C ₂	9.83	D ₂	0.13
C ₃	26.89	D ₃	1.56
C ₄	18.67	D ₄	0.24

3. Properties

All piericidins are pale yellow, viscous oils and readily soluble in most kinds of organic solvents but insoluble in water. They are unstable in air and in nonpolar solvents, but are mode-

* The yield of piericidins produced by *S. mobaraensis*.

rately stable in polar solvents such as ethanol and chloroform under darkness at low temperature.

On silica gel TLC, all piericidins are detectable by spraying with Dragendorff reagent or with 3% aqueous potassium permanganate, and these compounds are readily visualized as dark shadows under UV light (2537 Å) using silica gel GF₂₅₄ plates. The *R_f* value of piericidins A and D series on TLC are very close, although the change of solvent system from Bz-EA to Hex-THF causes much more *R_f* value shifts in the case of piericidin D series than A series. On the other hand, piericidin B series (less polar) and C series (more polar) are easily separated from the other two series.

The behavior of piericidins on high performance liquid chromatography (HPLC) corresponds to that on TLC. The partition chromatography using chemically bonded silica gel columns is most suitable for analysis of these compounds. Especially, octadecyl silanized silica gel column is efficient for separation of PB series. Chromatographic behavior of all piericidins is summarized in Table 3.

The typical UV, IR and mass spectra of piericidins are shown in Fig. 2, 3 and 4 respectively. The UV spectra of piericidins are classified into two types: 1) odd suffixed piericidins show $\lambda_{\max}$ at 239 nm (ca. ϵ 40,000), and 2) even suffixed piericidins show $\lambda_{\max}$ at 225 nm (ca. ϵ 20,000). Every piericidin shows very similar IR spectra. And in mass spectra (MS), odd suffixed piericidins show characteristically intense peaks at *m/e* 330 and 331, while in even suffixed ones, these peaks are replaced by ions at

Table 3. Chromatographic Behavior of Piericidins.

Piericidin	HPLC (retention time, min) ^a			TLC (Rf value) ^c	
	column ^b	CN	Si-10	RP	solvent ^d A B
B ₄		4.1 ^e		11.7 ⁱ	0.58 0.67
B ₃		4.8 ^e		8.3 ⁱ	0.56 0.64
B ₂		4.8 ^e		5.7 ⁱ	0.56 0.64
B ₁		5.6 ^e		3.9 ⁱ	0.54 0.62
D ₄		3.2 ^f	6.6 ^h	9.9 ^j	0.45 0.57
D ₃		3.7 ^f	7.5 ^h	7.4 ^j	0.37 0.54
D ₂		4.5 ^f	9.5 ^h	4.8 ^j	0.34 0.46
D ₁		5.7 ^f	11.4 ^h	3.3 ^j	0.30 0.40
A ₄		5.1 ^f	9.9 ^h	11.0 ^j	0.54 0.55
A ₃		6.3 ^f	13.2 ^h	8.2 ^j	0.48 0.49
A ₂		7.2 ^f	15.3 ^h	5.2 ^j	0.41 0.43
A ₁		8.7 ^f	18.4 ^h	3.7 ^j	0.34 0.33
C ₄		6.8 ^g		9.5 ^k	0.16 0.22
C ₃		8.8 ^g		6.5 ^k	0.14 0.18
C ₂		10.0 ^g		3.8 ^k	0.12 0.16
C ₁		13.4 ^g		3.0 ^k	0.10 0.12

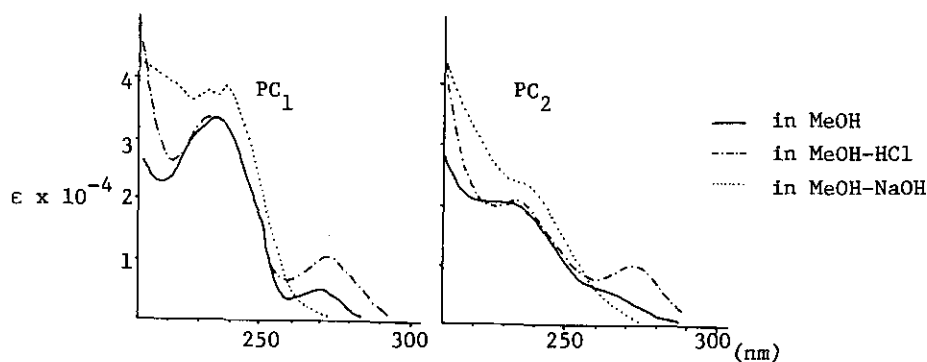


Fig. 2. UV Spectra of Piericidins

a. HPLC conditions: flow rate, 60 ml/hr; column temp., ambient or 60°C (RP); UV monitor, 240 nm. *b.* CN = Micro pak CN, Si-10 = Micro pak Si-10, RP = Vydac RP. *c.* Silica gel TLC. *d.* Solvent system: A = benzene-ethyl acetate (93 : 3), B = hexane-THF (4 : 1). *e - k.* Solvent system: Hexane-THF (*e*=97:3, *f*=96:4, *g*=91:9, *h*=95:5), Methanol-Water (*i*=7:3, *j*=35:65, *k*=4:6).

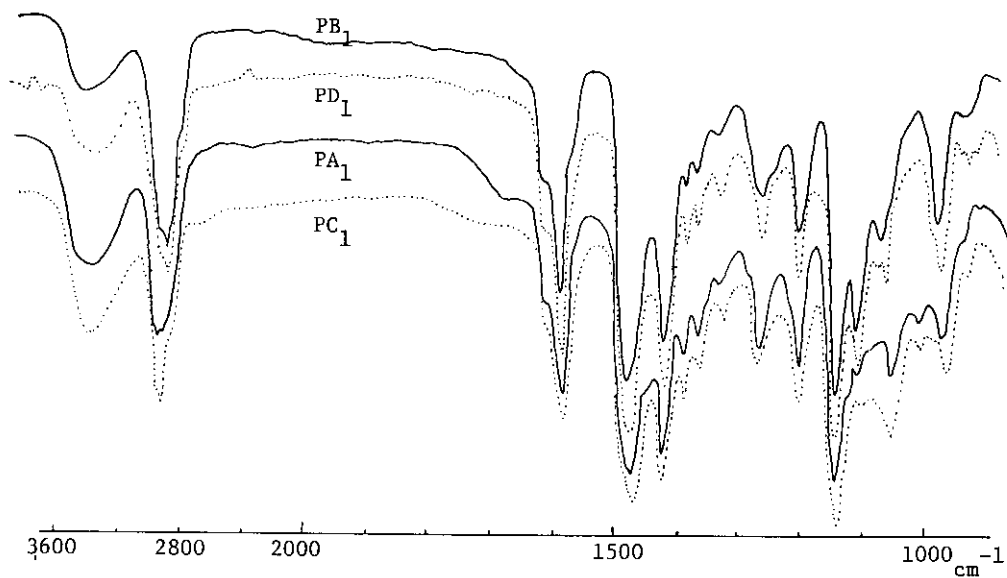


Fig. 3. IR Spectra of Piericidins

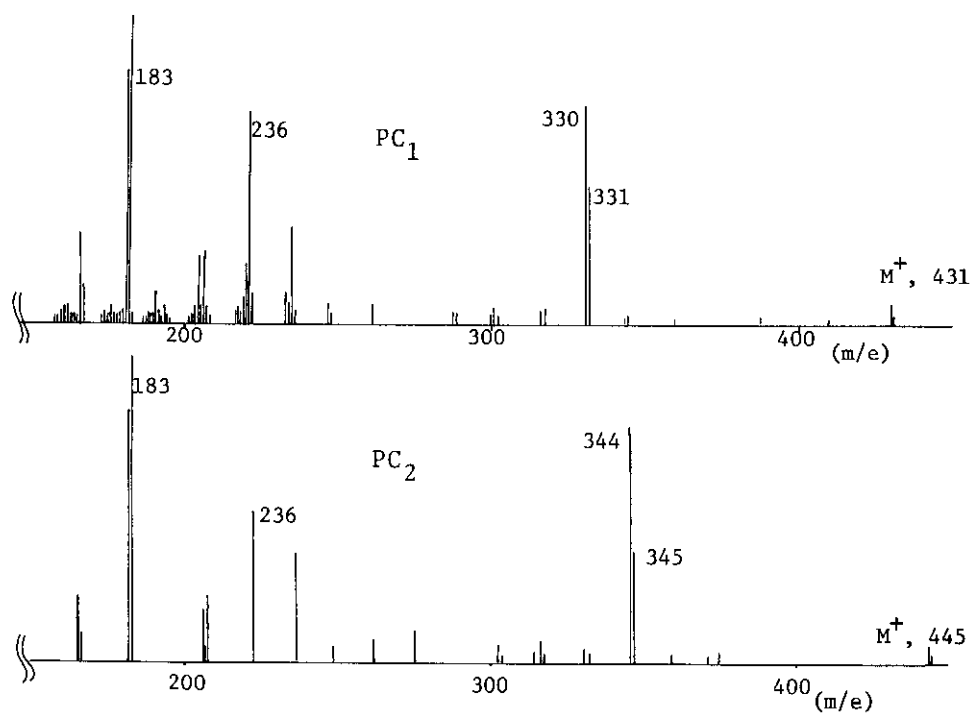


Fig. 4. Mass Spectra of piericidins

m/e 344 and 345. Further, piericidin B, C and D series exhibit rather strong molecular ion peaks, but A series show very weak ones in their MS. The molecular formula of every piericidin was assigned on the basis of high resolution MS, as listed in Table 4.

Table 4. Molecular Formulas of Piericidins

Piericidin	Mw(obs.)	Mw(calc.)	Molecular Formula
A ₁	415.2714	415.2722	C ₂₅ H ₃₇ NO ₄
A ₂	429.2866	429.2879	C ₂₆ H ₃₉ NO ₄
A ₃	443.3057	443.3035	C ₂₇ H ₄₁ NO ₄
A ₄	457.3156	457.3192	C ₂₈ H ₄₃ NO ₄
B ₁	429.2894	429.2879	C ₂₆ H ₃₉ NO ₄
B ₂	443.3032	443.3035	C ₂₇ H ₄₁ NO ₄
B ₃	457.3235	457.3192	C ₂₈ H ₄₃ NO ₄
B ₄	471.3369	471.3348	C ₂₉ H ₄₅ NO ₄
C ₁	431.2645	431.2668	C ₂₅ H ₃₇ NO ₅
C ₂	445.2784	445.2825	C ₂₆ H ₃₉ NO ₅
C ₃	459.2977	459.2981	C ₂₇ H ₄₁ NO ₅
C ₄	473.3125	473.3138	C ₂₈ H ₄₃ NO ₅
D ₁	445.2766	445.2825	C ₂₆ H ₃₉ NO ₅
D ₂	459.2924	459.2981	C ₂₇ H ₄₁ NO ₅
D ₃	473.3112	473.3138	C ₂₈ H ₄₃ NO ₅
D ₄	487.3267	487.3294	C ₂₉ H ₄₅ NO ₅

4. Chemical Structures

The structures of piericidins A₁ (PA₁) and B₁ (PB₁), which

were produced by *S. mobaraensis*, were established as (1) and (5) respectively by Takahashi et al.⁴⁻⁸ mainly on the basis of extensive degradation studies. On the other hand, recent development in various spectrometries often enables us to determine structures of complex molecules without any chemical degradation studies. The chemical structures of piericidins except PA₁ and PB₁ were elucidated by Yoshida et al.⁹ and Takahashi² by means of MS (Table 5), ¹H- and ¹³C-nuclear magnetic resonances (¹H- and ¹³C-NMR, Table 6 and 7).

Table 5. Relative Intensity (%) of Ion Peaks in MS of Piericidins

Ion Peak	A ₁	A ₂	A ₃	A ₄	B ₁	B ₂	B ₃	B ₄
M ⁺	trace*	trace	trace	trace	19	20	24	20
M-43	-	-	trace	trace	-	-	trace	2
345	-	100	-	100	-	52	-	50
344	-	97	-	88	-	100	-	100
331	100	-	100	-	54	-	50	-
330	77	-	90	-	100	-	100	-
316	12	19	24	9	8	5	8	12
262	10	11	3	5	4	4	3	5
248	10	4	3	2	4	6	5	5
236	56	67	54	67	27	36	32	44
222	39	50	43	50	32	38	36	38
208	52	43	41	62	24	26	20	24
183	88	96	88	80	76	86	88	82
182	86	83	80	74	58	72	76	68

* These peaks are very weak but surely observed.

Table 5. (Continued)

Ion Peak(m/e)	C ₁	C ₂	C ₃	C ₄	D ₁	D ₂	D ₃	D ₄
M ⁺	9	7	10	10	23	20	28	31
M-43	-	-	5	5	-	-	2	2
345	-	37	-	43	-	24	-	57
344	-	77	-	79	-	100	-	100
331	45	-	43	-	35	-	49	-
330	72	-	75	-	100	-	100	-
316	5	16	5	18	6	4	1	1
262	7	8	8	8	12	6	5	6
248	9	5	7	5	7	3	6	2
236	32	36	43	43	31	20	26	25
222	69	48	71	45	16	29	20	28
208	22	24	30	31	16	14	11	14
183	100	100	100	100	71	73	76	71
182	82	82	73	77	78	87	74	64

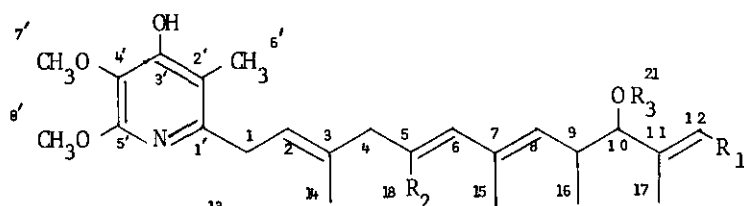
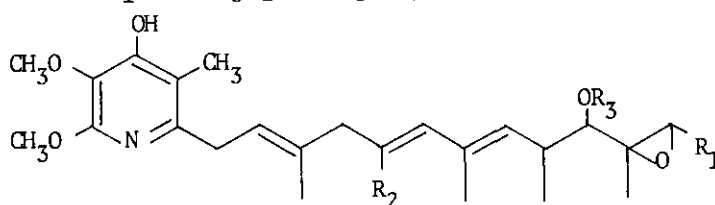
(1) $R_1 = \text{CH}_3$, $R_2, R_3 = \text{H}$ (2) $R_1, R_2 = \text{CH}_3$, $R_3 = \text{H}$ (3) $R_1 = (\text{CH}_3)_2\text{CH}$, $R_2, R_3 = \text{H}$ (4) $R_1 = (\text{CH}_3)_2\text{CH}$, $R_2 = \text{CH}_3$, $R_3 = \text{H}$ (5) $R_1, R_3 = \text{CH}_3$, $R_2 = \text{H}$ (6) $R_1, R_2, R_3 = \text{CH}_3$ (7) $R_1 = (\text{CH}_3)_2\text{CH}$, $R_3 = \text{CH}_3$, $R_2 = \text{H}$ (8) $R_1 = (\text{CH}_3)_2\text{CH}$, $R_2, R_3 = \text{CH}_3$ (9) $R_1 = \text{CH}_3$, $R_2, R_3 = \text{H}$ (10) $R_1, R_2 = \text{CH}_3$, $R_3 = \text{H}$ (11) $R_1 = (\text{CH}_3)_2\text{CH}$, $R_2 = \text{H}$, $R_3 = \text{H}$ (12) $R_1 = (\text{CH}_3)_2\text{CH}$, $R_2 = \text{CH}_3$, $R_3 = \text{H}$ (13) $R_1, R_3 = \text{CH}_3$, $R_2 = \text{H}$ (14) $R_1, R_2, R_3 = \text{CH}_3$ (15) $R_1 = (\text{CH}_3)_2\text{CH}$, $R_2 = \text{H}$, $R_3 = \text{CH}_3$ (16) $R_1 = (\text{CH}_3)_2\text{CH}$, $R_2, R_3 = \text{CH}_3$

Table 6. Proton Chemical Shifts of Piericidins on $^1\text{H-NMR}$

Proton No.	A ₁	A ₂	A ₃	A ₄	B ₁	B ₃	C ₁	C ₂	C ₃	C ₄	D ₁
1	3.30(d)	3.28(d)	3.28(d)	3.31(d)	3.28(d)	3.31(d)	3.26(d)	3.28(d)	3.29(d)	3.28(d)	3.30(d)
2	5.30(t)	5.30(t)	5.35(t)	5.35(t)	5.33(t)	5.32(t)	5.35(t)	5.31(t)	5.32(t)	5.31(t)	5.31(t)
4	2.73(d)	2.64(s)	2.74(d)	2.65(s)	2.72(d)	2.73(d)	2.70(d)	2.62(s)	2.73(d)	2.61(s)	2.47(d)
5	5.45(m)	-	5.45(m)	-	5.45(m)	5.45(m)	5.45(m)	-	5.45(m)	-	5.45(m)
6	6.00(d)	5.58(s)	6.02(d)	5.63(s)	6.00(d)	6.00(d)	5.95(d)	5.58(s)	6.00(d)	5.58(s)	5.98(d)
8	5.12(d)	4.98(d)	5.13(d)	5.03(d)	5.13(d)	5.12(d)	5.15(d)	4.98(d)	5.16(d)	5.01(d)	5.16(d)
9	2.66(m)	2.55(m)	2.65(m)	2.55(m)	2.60(m)	2.60(m)	2.50(m)	2.47(m)	2.51(m)	2.47(m)	2.50(m)
10	3.53(d)	3.49(d)	3.50(d)	3.51(d)	3.05(d)	3.09(d)	2.80(d)	2.78(d)	2.81(d)	2.77(d)	2.72(d)
12	5.35(q)	5.35(q)	5.15(d)	5.13(d)	5.30(q)	5.12(d)	2.40(q)	2.40(q)	2.38(d)	2.37(d)	2.40(q)
13	1.57(d)	1.60(d)	2.55(m)	2.55(m)	1.64(d)	2.55(m)	1.26(d)	1.27(d)	2.30(m)	2.30(m)	1.24(d)
14	1.70(s)	1.73(s)	1.76(s)	1.76(s)	1.72(s)	1.72(s)	1.70(s)	1.72(s)	1.70(s)	1.70(s)	1.73(s)
15	1.54(s)	1.64(s)	1.60(s)	1.66(s)	1.50(s)	1.52(s)	1.70(s)	1.64(s)	1.72(s)	1.64(s)	1.68(s)
16	0.76(d)	0.76(d)	0.79(d)	0.79(d)	0.74(d)	0.77(d)	0.86(d)	0.87(d)	0.91(d)	0.91(d)	0.87(d)
17	1.68(s)	1.71(s)	1.71(s)	1.66(s)	1.68(s)	1.66(s)	1.20(s)	1.23(s)	1.24(s)	1.24(s)	1.14(s)
18	-	1.64(s)	-	1.66(s)	-	-	-	1.64(s)	-	1.63(s)	-
19	-	-	0.90(d)	0.93(d)	-	0.92(d)	-	-	0.95(d)	0.94(d)	-
20	-	-	0.95(d)	0.97(d)	-	0.97(d)	-	-	1.07(d)	1.05(d)	-
21	-	-	-	-	3.03(s)	3.05(s)	-	-	-	-	3.45(s)
6'	2.00(s)	2.04(s)	2.04(s)	2.05(s)	2.04(s)	2.05(s)	2.01(s)	2.03(s)	2.04(s)	2.02(s)	2.04(s)
7'	3.78(s)	3.80(s)	3.80(s)	3.80(s)	3.84(s)	3.84(s)	3.78(s)	3.80(s)	3.78(s)	3.82(s)	3.80(s)
8'	3.85(s)	3.87(s)	3.87(s)	3.88(s)	3.90(s)	3.90(s)	3.86(s)	3.88(s)	3.88(s)	3.87(s)	3.89(s)

The splittings of signals are expressed in bracket. These spectra were obtained in CCl_4 , and chemical shifts are expressed in ppm from TMS.

Table 7. ^{13}C Chemical Shifts of Piericidins (CDCl_3 , ppm)

Carbon No	A ₁	A ₃	A ₄	C ₁	C ₂	C ₃	C ₄	D ₁
1	34.5(t)	34.4(t)	34.5(t)	34.4(t)	34.5(t)	34.5(t)	34.5(t)	34.5(t)
2	122.4(d)	122.4(d)	123.5(d)	122.3(d)	123.5(d)	122.3(d)	123.6(d)	122.3(d)
3	134.7(s)	134.8(s)	134.8(s)	134.9(s)	134.6(s)	134.6(s)	134.2(s)	134.7(s)
4	43.2(t)	43.1(t)	51.2(t)	43.1(t)	51.1(t)	43.1(t)	51.1(t)	43.1(t)
5	126.7(d)	126.5(d)	134.6(s)	126.5(d)	134.7(s)	126.4(d)	134.2(s)	125.8(d)
6	135.9(d)	135.7(d)	131.0(d)	135.7(d)	131.0(d)	135.8(d)	131.4(d)	136.1(d)
7	134.7(s)	134.8(s)	134.0(s)	134.6(s)	133.7(s)	134.6(s)	133.7(s)	134.7(s)
8	133.2(d)	133.3(d)	130.8(d)	132.5(d)	130.0(d)	132.6(d)	130.2(d)	133.5(d)
9	37.0(d)	37.0(d)	37.1(d)	36.2(d)	36.4(d)	36.1(d)	36.2(d)	36.2(d)
10	82.9(d)	82.9(d)	82.9(d)	81.8(d)	81.8(d)	81.8(d)	81.8(d)	91.2(d)
11	135.9(s)	137.1(s)	137.1(s)	62.6(s)	62.6(s)	63.5(s)	63.5(s)	61.9(s)
12	123.3(d)	132.8(d)	132.7(d)	58.4(d)	58.4(d)	68.7(d)	68.8(d)	55.0(d)
13	13.9(q)	27.3(d)	27.3(d)	13.3(q)	13.3(q)	27.7(d)	27.7(d)	13.2(q)
14	13.1(q)	13.0(q)	17.3(q)	13.0(q)	17.3(q)	13.0(q)	17.3(q)	12.9(q)
15	16.6(q)	16.6(q)	17.1(q)	16.6(q)	17.1(q)	16.6(q)	17.2(q)	16.6(q)
16	17.5(q)	17.5(q)	17.5(q)	17.4(q)	17.5(q)	17.4(q)	17.5(q)	18.0(q)
17	10.7(q)	11.0(q)	10.9(q)	11.0(q)	10.9(q)	11.2(q)	11.2(q)	11.4(q)
18	-	-	15.8(q)	-	15.8(q)	-	15.8(q)	-
19	-	18.8(q)	18.8(q)	-	-	18.6(q)	18.5(q)	-
20	-	20.6(q)	20.6(q)	-	-	20.3(q)	20.3(q)	-
21	-	-	-	-	-	-	-	58.6(q)
1'	150.8(s)	150.8(s)	150.8(s)	150.8(s)	150.8(s)	150.7(s)	150.8(s)	150.7(s)
2'	112.2(s)	112.2(s)	112.1(s)	112.2(s)	112.1(s)	112.2(s)	112.2(s)	112.1(s)
3'	154.3(s)	154.3(s)	154.3(s)	154.3(s)	154.2(s)	154.2(s)	154.3(s)	154.3(s)
4'	128.0(s)	128.0(s)	127.9(s)	127.9(s)	128.0(s)	128.0(s)	128.0(s)	128.0(s)
5'	153.7(s)	153.6(s)	153.6(s)	153.6(s)	153.6(s)	153.7(s)	153.7(s)	153.7(s)
6'	10.5(q)	10.5(q)	10.4(q)	10.5(q)	10.5(q)	10.5(q)	10.5(q)	10.5(q)
7'	60.5(q)	60.4(q)	60.5(q)	60.5(q)	60.5(q)	60.5(q)	60.5(q)	60.4(q)
8'	53.0(q)	53.0(q)	53.0(q)	53.0(q)	53.0(q)	53.0(q)	53.0(q)	52.9(q)

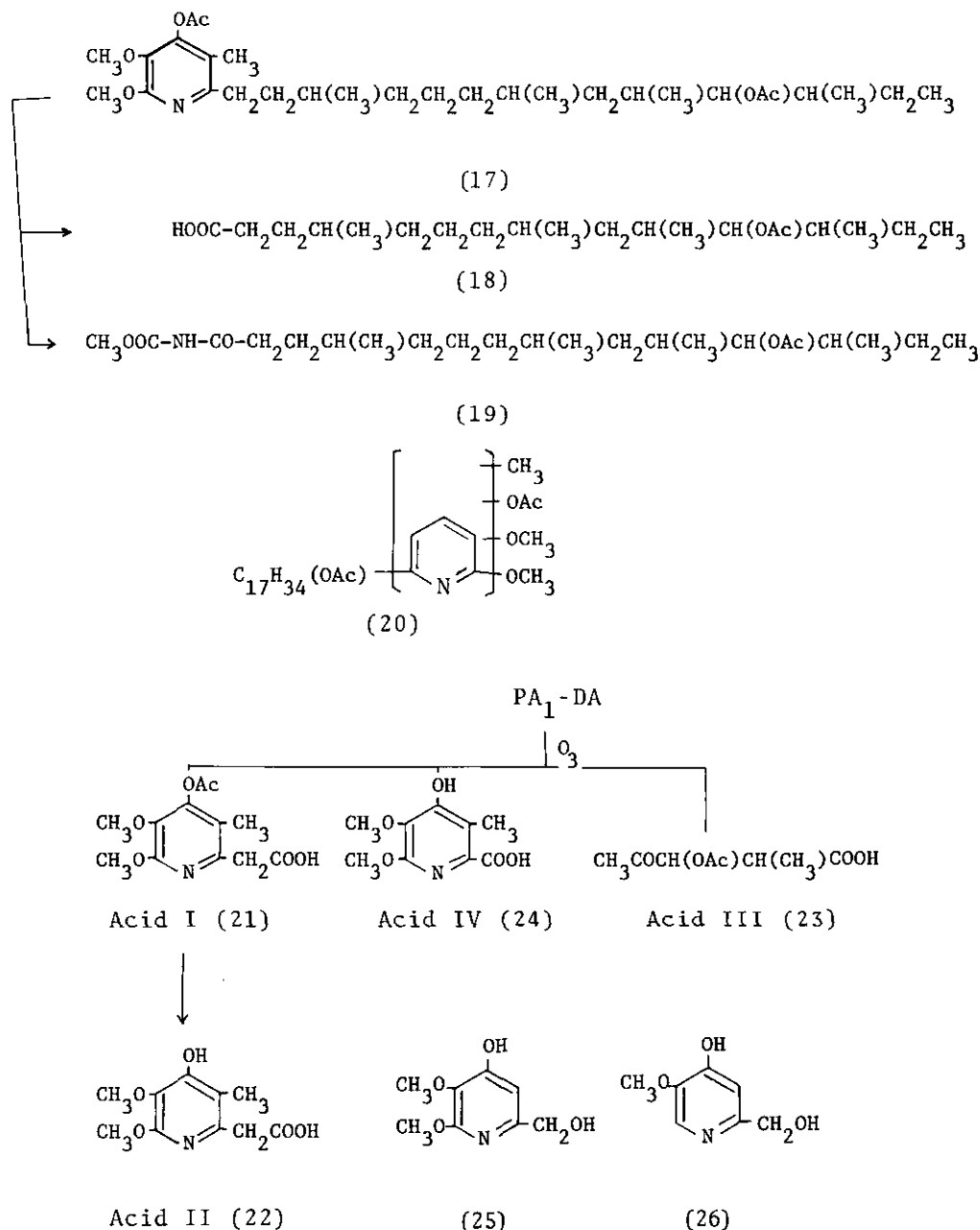
Splittings of signals in off-resonance mode are shown in brackets.

A. The Structure of Piericidin A₁.

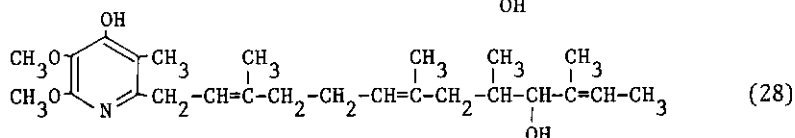
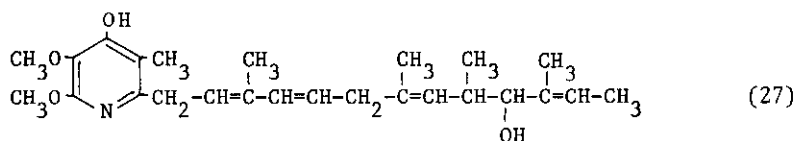
The structure of PA₁, which is considered to be fundamental for structural elucidations of other piericidins, was proposed as (1) on the basis of the following evidence. On ozonolysis of octahydropiericidin A₁ diacetate (H₈PA₁-DA, 17) derived from PA₁ by means of hydrogenation and subsequent acetylation, the heteroaromatic ring was cleaved to afford 10-acetoxy-3,7,9,11-tetramethyltridecane-1-carboxylic acid (18) and its N-methoxycarbonyl amide (19). The structures of these products were established by extensive degradation studies⁴⁻⁷. The formation of (18) and (19) indicates that the heteroaromatic ring must be a fully substituted pyridine ring as illustrated in Fig. 5 and in formula (20), because an acetoxyl, a methyl and a methoxyl residues contained in (17) were completely removed through this procedure.

On the other hand, PA₁ diacetate (PA₁-DA) was subjected to the controlled ozonolysis to give an acetaldehyde, "acid I" (21), "acid II" (22), "acid III" (23) and "acid IV" (24) as shown in Fig. 5. Acidic compounds (21), (22) and (24) containing a nitrogen atom, were ascertained to retain the original heteroaromatic ring structure, by comparison of their UV spectra with those of octahydrogenated piericidin A₁ and its derivatives. The structural determinations of (21), (22) and (24) were accomplished by comparison of the spectral data obtained from their analogues, (25) and (26), which were derived from kojic acid. Thus, the feature of substitutions on the pyridine ring in PA₁ has been established. Quite recently, this was confirmed by total synthesis of the ring system reported by Schmidtchen et al.¹⁰ The struc-

Fig. 5. Ozonolysis of PA₁-related Compounds.



ture of (23), originated in the side chain of PA_1 , was elucidated by synthetic studies (see the chapter of stereochemistry). All results of these degradative works suggested the structure of PA_1 as (1) or (27), both of which are completely compatible with the 1H -NMR study on this compound. This problem was remaining unsolved, but recently, Yoshida et al.¹¹ have settled it to determine the real structure (1) by selective hydrogenation to obtain dihydrogenated derivative (28), and by the analysis of MS and ^{13}C -NMR combined with the biosynthetic study (see the chapter of biosynthesis). Thus, PA_1 has been shown to have structure (1), and structures of other piericidins were elucidated on the basis of chemical and spectral comparison with this compound.



B. The Structure of Piericidin A_2 .

The molecular formula of PA_2 , $C_{26}H_{39}NO_4$, indicates that this compound contains one more carbon and two more hydrogens than PA_1 . In MS of PA_2 , characteristically intense peaks were not observed at m/e 330 and 331 but at m/e 344 and 345, and the fragmentation pattern between the molecular ion peak and these two peaks coincided with that of PA_1 . This spectral feature suggested that a methyl or a methylene should be introduced

somewhere between C-1 and C-9 of PA_1 . 1H -NMR spectrum of PA_2 showed signals of five allyl methyls and four olefinic protons. The result indicates that a methyl group must be attached at one of the double bonds in the side chain of PA_1 . Since the doublet (δ 4.98), the triplet (5.30) and the quartet (5.35) due to olefinic protons were readily assigned to H-8, H-2 and H-12 respectively based on the spectral comparison of PA_1 and PA_2 , the remaining olefinic proton signal (5.58, singlet) have to be assigned to H-6. Accordingly, the new methyl should be situated on C-5 as shown in the structure (2).

C. The Structure of Piericidin A_3 .

The structure of PA_3 was also determined by means of various spectral comparisons between PA_1 and PA_3 . The fragmentation patterns of PA_1 and PA_3 in MS were quite similar in the mass region below the characteristically intense peaks at m/e 330 and 331, suggesting that PA_3 has the same structure from C-1 to C-9 as PA_1 and differs in the terminal structure of the side chain. The elemental composition of this terminal moiety, $C_7H_{13}O$, was deduced from the high resolution MS. ^{13}C -chemical shifts and signal splittings on the off-resonance ^{13}C -NMR spectrum of PA_3 revealed that the terminal part contains three methyl, two sp^2 , a methine and a carbonyl carbons. On the other hand, the signals due to protons in the terminal part were observed at δ 0.90 (3H, d), 0.95 (3H, d), 1.71 (3H, s), 2.55 (1H, m), 3.50 (1H, d) and 5.15 (1H, d) in the 1H -NMR spectrum of PA_3 . A pair of the methyl doublets and the broad methine signal indicate the presence of an isopropyl. The allyl methyl signal at 1.71 corresponded to that due to H-17

in PA_2 and the lowest signal due to an olefinic proton was shown to be coupled with the methine proton in the isopropyl by means of spin decoupling studies. The remaining doublet at 3.50 was easily assigned to H-10 by the chemical shift, and indeed it was shown to be coupled with H-9. The consideration of these findings readily leads the structure of PA_3 to the formula (3).

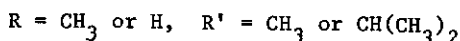
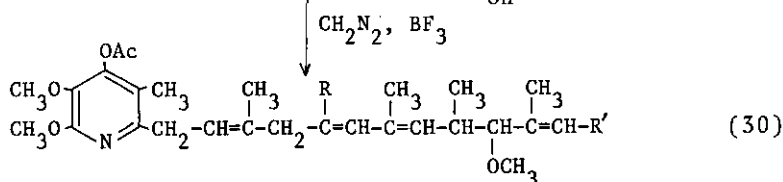
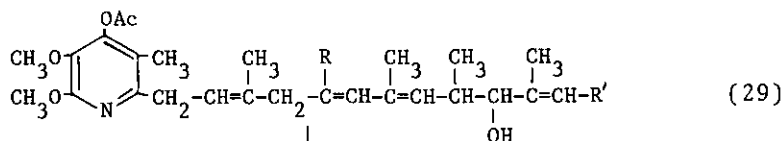
D. The Structure of Piericidin A_4 .

The chemical structure of PA_4 was deduced to be the formula (4) after the structural elucidations on PA_1 , PA_2 and PA_3 , since the mass spectral feature of PA_4 resembles that of PA_2 in the low mass region below the characteristic peaks at m/e 344 and 345, and of PA_3 in the high mass field above the peaks. This was confirmed by the 1H -NMR spectrum of PA_4 . The spectrum shows signals due to the same terminal moiety as in PA_3 at δ 0.93 (3H, d), 0.97 (3H, d), 1.66 (3H, s), 2.55 (1H, m), 3.51 (1H, d) and 5.13 (1H, d), and due to the same three trisubstituted olefins as PA_2 at 5.35 (1H, t), 5.63 (1H, s) and 5.03 (1H, d) to be assigned as H-2, H-6 and H-8 respectively.

E. The Structures of Piericidin B Series.

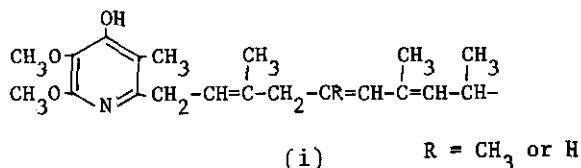
It was easy to determine the structures of PB series, since their molecular formulas obtained by high resolution MS and additional methoxy signals in 1H -NMR suggested that PB series should be mono O-methylated PA series. This was directly confirmed by methylating the C-10 hydroxyl in the side chain of monoacetyl PA series (29) with diazomethane and boron trifluoride etherate to afford acetyl PB series (30). Thus, the structures of PB_1 , PB_2 , PB_3 and PB_4 were completely established as (5), (6), (7) and (8).

respectively.



F. The Structures of Piericidin C Series.

The high resolution MS of this series indicates that these piericidins have one more oxygen than the respective PA members. The MS patterns of the odd and even suffixed members of both series are quite similar in the region below the intense peak at m/e 330/331 and 344/345 respectively. This suggests that both series have the same partial structure (i), because the intense fragment peaks are generated by cleavage of the bond between C-9 and C-10. In the ^{13}C -NMR spectra of PC series, the signal patterns are very similar to the case of PA series except two signals due to C-11 and C-12. The two carbons of PC series were considered to have been oxygenated, from their ^{13}C -chemical shifts. These findings and considerations suggest the presence of an epoxide in the structures of PC series. Indeed, the chemical correlation between PA_1 and PC_1 was demonstrated in following two ways.



The reduction of PC₁ by "Cornforth reaction"¹² gave PA₁ in 25% yield, and the epoxidation of PA₁ with *m*-chloroperbenzoic acid afforded PC₁ in 30% yield. Thus the chemical structures of PC series have been established as formulas (9) - (12).

G. The Structures of Piericidin D Series.

The molecular formula of each member of the PD series based on the high resolution MS shows the presence of one more carbon and two more hydrogens than the respective PC members, and only one more oxygen than the respective PB members. Therefore, the PD members can be regarded as O-methylated PC members. In fact, mass and NMR spectra of them indicate the structures to be assigned to (13) for PD₁, (14) for PD₂, (15) for PD₃ and (16) for PD₄. Further, this assignment was confirmed by methylation of acetyl PC members to give respective acetyl PD members.

H. The Relation between Structures and Spectroscopic Properties in Piericidins.

As above described, the correlation between structures and spectroscopic properties is quite useful in structural elucidation for piericidins. They can be summarized as follows. In MS, the intensity of molecular ion peaks is related to the terminal structure of the side chain, namely PA series containing an allylic alcohol at C-10 shows a very weak peak of molecular ion, while PB and PD series containing a methyl ether show a rather strong one and PC series containing an epoxide and an alcohol shows that with moderate intensity. Furthermore, MS of piericidins show the characteristic fragment peaks due to cleavage of the bond between C-9 and C-10 reflecting the nature of the carbon skeleton between

the substituted pyridine ring and C-9; the peaks at m/e 330 and 331 are observed in MS of odd suffixed piericidins (no methyl at C-5) and those at m/e 344 and 345 in MS of even suffixed ones (a methyl at C-5). In ^{13}C -NMR spectra, the signal due to C-4 methylene suffers a down field shift (8.0 ppm) by the methyl substituent at C-5, and the C-10 carbonyl carbon signal also suffers a down field shift (10 ppm) by the methylation of C-10 alcohol. From ^1H -NMR spectra, the presence of a terminal isopropyl can be recognized easily by a couple of doublets appeared at δ 0.9 - 1.1 ppm. UV spectra are informative on the presence of methyl on C-5 in the conjugated diene system, namely the C-5 methylated piericidins show the maximum absorption (λ_{max} 225 nm, ϵ 20,000) in almost half intensity of those of piericidins without C-5 methyl (λ_{max} 239 nm, ϵ 40,000).

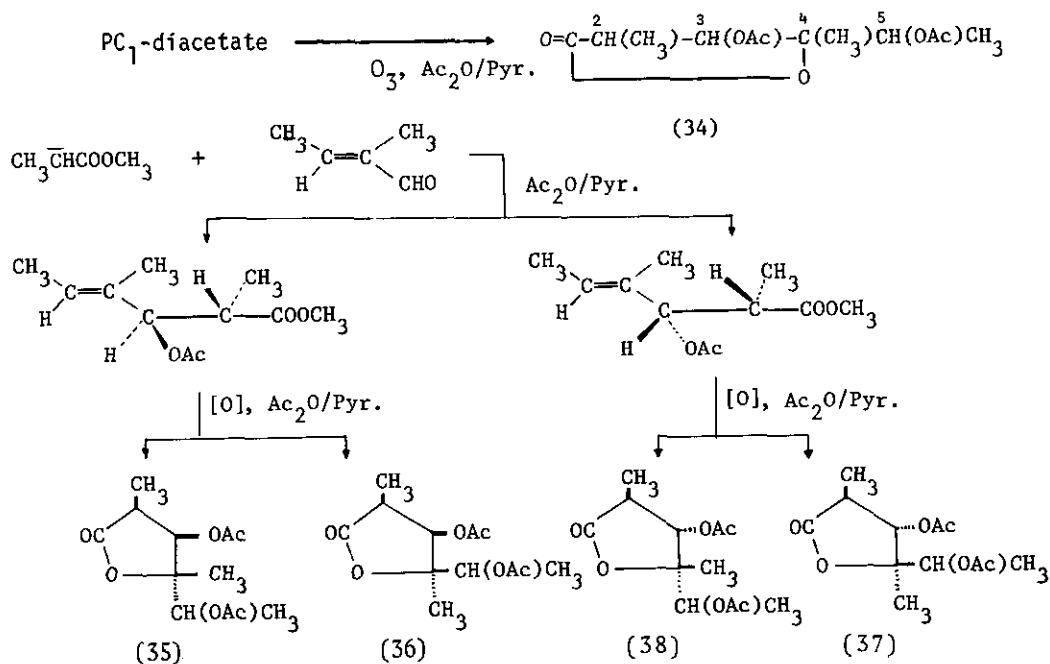
These empirical rules will be useful for structural elucidation for new unknown piericidins, which may be present in metabolites of other microorganisms.

5. Stereochemistry

In each molecule of PA and PB series, there are two asymmetric centers at C-9 and C-10. On the other hand, there are four asymmetric centers at C-9, C-10, C-11 and C-12 in each molecule of PC and PD series. Takahashi et al.¹³ have clarified the absolute configurations of C-9 and C-10 contained in piericidin A_1 as follows. Since these centers are retained in the ozonolysis product (23) as C-2 and C-3, the elucidation of the stereochemistry was conducted using this acid. Relative configurations of C-2 and

On the other hand, the determination of absolute configurations on the four neighboring centers of PC and PD members were attempted by Yoshida et al.¹⁴ Ozonolysis of PC₁-DA followed by oxidation and acetylation gave a lactone (34), whose chemical structure has been readily determined by mass and ¹H-NMR spectroscopies. In the lactone (34), three asymmetric centers of PC₁ (C-9, C-10 and C-12) should be retained as C-2, C-3 and C-5, but the configuration of C-4 (C-11 in PC₁) is considered to be reversed by back side attack of the carboxylate ion to the epoxide carbon (C-11) at the stage of lactonization. Diastereomeric isomers of the lactone (34) were synthesized from tiglyl aldehyde and propionate by the procedure shown in Fig. 8. Only four iso-

Fig. 8 Procedures to Obtain The Tetrasubstituted Lactone.



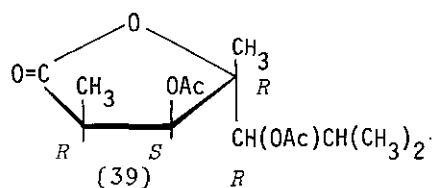
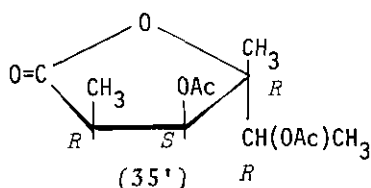
mers were formed by this method because of the stereochemical restriction in the epoxidation on the *E*-olefin with *m*-chloroperbenzoic acid. Fortunately, one of the isomers showed the same spectral feature as (34) in NMR and IR measurement, and this fact indicates that the relative configuration of C-11 and C-12 must be *R-R* or *S-S*. On the other hand, the epoxidation on PA₁ to afford PC₁ (see the chapter of chemical structures) revealed that absolute configurations of C-9 and C-10 in PC₁ should be same as PA₁. At this stage, the remaining problem is to determine the relative configurations of C-10 and C-11. Now four stereochemical expressions for the synthetic lactone, namely (35), (36), (37) and (38) are possible and the natural lactone should be assigned to (35) or (36), because they have the correct configurations concerning C-2 and C-3. In ¹H-NMR spectra of these lactones (Table 7), the signals due to C-2 methyl of (35) and (36) appear at upper field than that of (37) and (38) by the effect of *cis*-acetoxyl on C-3. Further *cis*-relation between C-3 acetoxyl and C-4 methyl in (35) and (37), was suggested since the signals due to C-4 methyl in (35) and (37) appear at upper field than in (36) and (38).

These results were further confirmed by the observation of nuclear overhauser effect (NOE) between C-2 methyl and H-3 in (37) and (38), and that between C-4 methyl and H-3 in (36) and (37). The evidences above cited allow to assign the relative configurations of C-2, C-3, C-4 and C-5 in the synthetic lactone (35) as *R-S-R-R* or *S-R-S-S*. Since the spectral features of the natural lactone (34) were identical with those of (35), the absolute configurations of C-2, C-3, C-4 and C-5 in (34) should be assigned

as *R-S-R-R* as shown in formula (35'), and consequently C-9, C-10, C-11 and C-12 in PC₁ should be assigned as *S-S-R-R*.

Table 8. Proton Chemical Shifts of The Lactones (in CDCl₃, δ ppm)

Proton	(35)=(35')	(36)	(37)	(38)	Splitting
2-CH ₃	1.18	1.14	1.39	1.41	d
4-CH ₃	1.32	1.44	1.37	1.51	s
5-CH ₃	1.32	1.32	1.26	1.32	d
2-H	3.13	3.06	2.77	2.79	m
3-H	5.57	5.44	5.33	5.08	d
5-H	5.01	5.23	5.01	5.20	q
CH ₃ CO-	2.07	1.98	2.04	2.03	s
	2.15	2.06	2.13	2.08	s

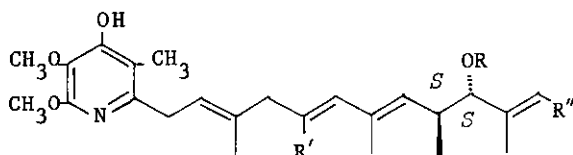


The absolute configurations of other members of PC and PD series are considered to be same as PC₁ because of their biogenetic relation to PC₁. This was supported by ¹H-NMR spectral similarity of (35') to (39), which were obtained by ozonolysis of PC₃. However, more strict examination to determine the stereochemistry of them are now in progress.

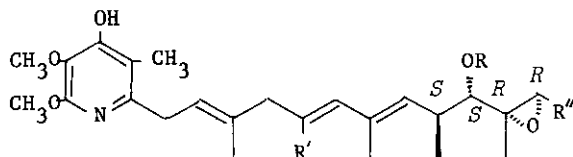
Configurations of the conjugated diene in the side chain of odd suffixed piericidins were assigned as *E-E* based on their strong UV absorption at 239 nm as well as on the large coupling constant

(16 Hz) between H-5 and H-6 in the ^1H -NMR spectra. Also the tetrasubstituted diene of even suffixed piericidins seems to have *E-E* configurations based on the UV spectra. Each isolated double bond was proposed as *E* configuration from the comparison of the ^1H -NMR data with those of model compounds.

PA and PB series



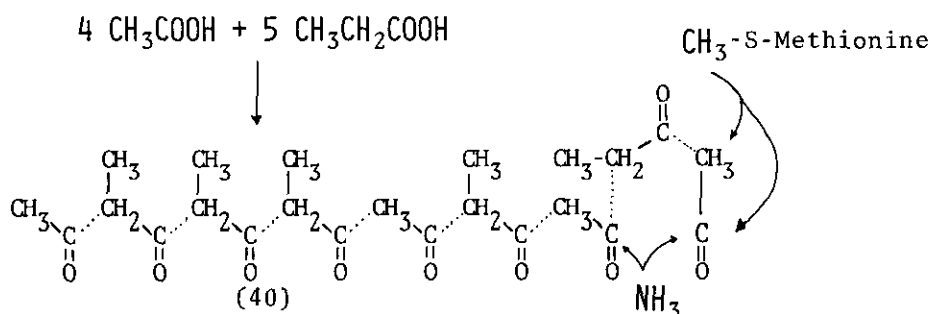
PC and PD series



6. Biosynthesis

The biosynthesis of PA_1 and PB_1 by *S. mobaraensis* was studied by Kimura et al.¹⁵ using ^{14}C -tracing technique. Incorporation ratios of various ^{14}C -labeled precursors into PA_1 indicated that PA_1 should be biosynthesized from acetate, propionate and methionine. To determine the disposition of the precursors in the molecule, the degradation reactions of PA_1 derivatives labeled with ^{14}C were examined. The result revealed that the carbon skeleton of PA_1 should be biosynthesized via a polyketide intermediate (40), and that the S-methyl of methionine was incorporated into the two methoxys attached to the pyridine ring. Furthermore, these experiments pointed out the possibility for the conversion of

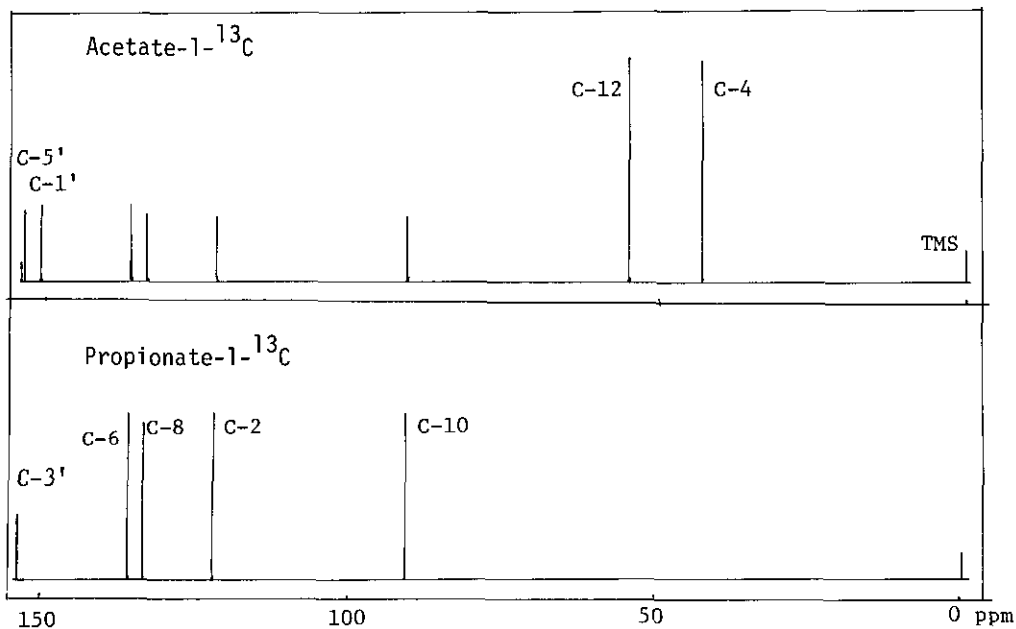
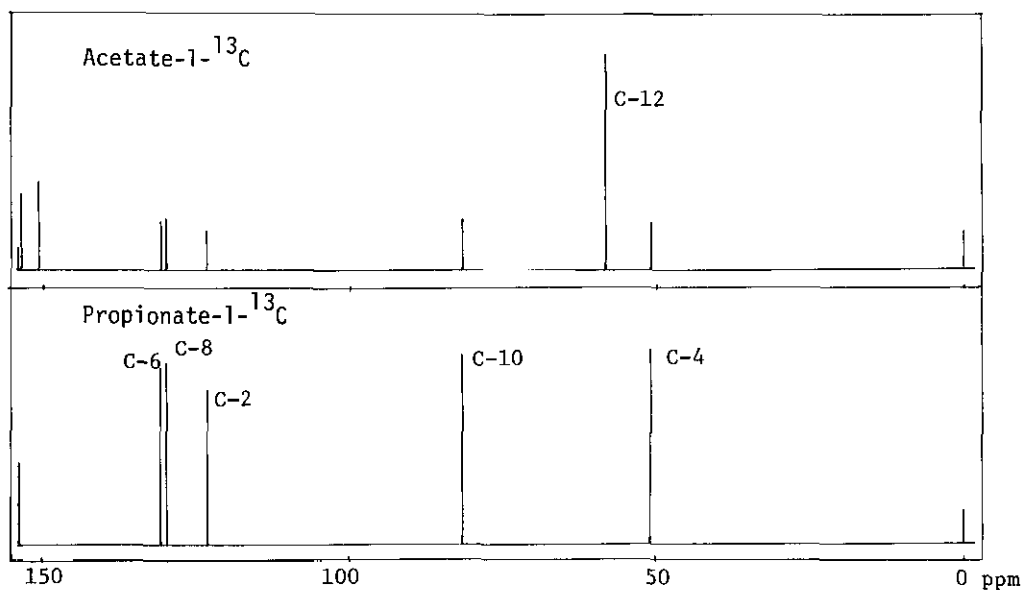
acetate into propionate via TCA cycle, since the extensive randomization of isotopes in PA_1 obtained from acetate-1- ^{14}C and -2- ^{14}C , was observed. The biosynthesis of PB_1 was also established by ^{14}C -tracing experiments where PA_1 was confirmed to be biologically methylated at C-10 hydroxyl with S-methyl of methionine to form PB_1

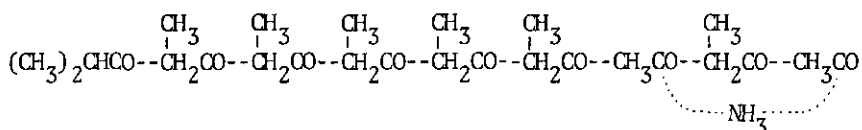


Recently, biosynthetic studies on metabolites of microorganisms have been improved very much by application of ^{13}C -tracing technique, which needs not to decompose the labeled products. As the preliminary application of this technique, Tanabe et al.¹⁶ tried to confirm the biosynthesis of PA_1 , and they found that C-3 methyl of propionate was incorporated into the methyl groups at C-3, 7, 9, 11 and 2' of PA_1 . Later, Yoshida et al.^{11,17} fully applied ^{13}C -tracing technique on the biosynthetic investigation of piericidins obtained from both *S. mobaraensis* and *S. pactum*. In the course of these studies, the situation of the conjugated diene in the side chain was confirmed by the incorporation of ^{13}C labeled precursors. Namely, ^{13}C -signal due to the methylene between the conjugated diene and the isolated double bond was enhanced not by propionate-1- ^{13}C but by acetate-1- ^{13}C . This result

clearly indicates that the methylene should not be assigned to C-6 but to C-4, favoring the structure (1) better than (27).

It is remarkable that *S. pactum*, a single strain, biosynthesized such a large variety of piericidins, and it is an interesting problem to clarify whether the first and/or the fifth acetyl units in the polyketide intermediate (40) may be attacked by C_1 source, or may be replaced with other two kinds of precursors. *S. pactum* was fermented in a medium containing the ^{13}C -labeled acetate or propionate. As shown in Fig. 9, the ^{13}C -NMR spectra of PD_1 obtained from acetate-1- ^{13}C and propionate-1- ^{13}C indicate that piericidins having the same carbon skeleton as PA_1 are produced through the intermediate (40). The ^{13}C -NMR spectra of PC_2 also fed by the acetate and the propionate (Fig. 10) obviously indicate that the fifth acyl unit is not derived from acetate followed by C_1 attacking but from propionate, since the signal due to C-4 was enhanced by propionate-1- ^{13}C . These observations allow to propose an intermediate (41) for the 2-suffixed piericidins. The next problem is to clarify the origin of the terminal isopropyl moiety of 3- and 4-suffixed piericidins. Both acetate-1- ^{13}C and propionate-1- ^{13}C were not incorporated into C-12 of PC_3 (Fig. 11), while isobutyrate-1- ^{13}C was specifically incorporated into PC_3 , indicating that 3-suffixed piericidins must be biosynthesized via a nona-ketide intermediate derived from three acetate, five propionate and an isobutyrate units, as shown in the formula (42). In the same way, it was confirmed that 4-suffixed piericidins were produced by this microbe via an intermediate (43) derived from two acetate, six propionate and an isobutyrate units. The

Fig. 9. ^{13}C -NMR Spectra of ^{13}C -labeled PD_1 Fig. 10. ^{13}C -NMR Spectra of ^{13}C -labeled PC_2 



(43)

origin of isobutyrate in biosynthesis of piericidins was confirmed to be valine by specific incorporation of universally ^{14}C -labeled valine into PC_3 and PC_4 .

Thus, the existence of four individual biosynthetic pathways which allow to produce the four skeletal variety by this single strain has been proved. It is quite interesting challenge to solve whether the polyketide synthetase in *S. pactum* has a loose substrate specificity, or this strain has four kinds of specific polyketide synthetase. It is difficult to answer for the question, but the trial to select a correct possibility is under way.

7. Biological Activity

A. Respiratory Inhibition.

As cited in the chapter of discovery, piericidins were found as naturally occurring insecticides. The structural resemblance between piericidins and ubiquinone (coenzyme Q, 44) prompted Hall et al.¹⁸ and Jeng et al.¹⁹ to examine their inhibitory activity on respiratory electron transport system, where ubiquinone plays an important role. Their experiments revealed that PA₁ and PB₁ inhibit NADH oxidation at very low concentration. Mitsui et al.²⁰ also observed that both piericidins A₁ and B₁ strongly inhibit electron transport system in mitochondria of insects and mammals at almost the same level. Horgan et al.²¹ demonstrated the rela-

tion between piericidins and the active site of the enzyme by means of extensive quantitative studies using radio-active compounds. Their studies established that piericidins, rotenoids and amytal each react at the same site, namely ubiquinone site, and that affinity of piericidin A₁ to the enzyme is the most potent among them.

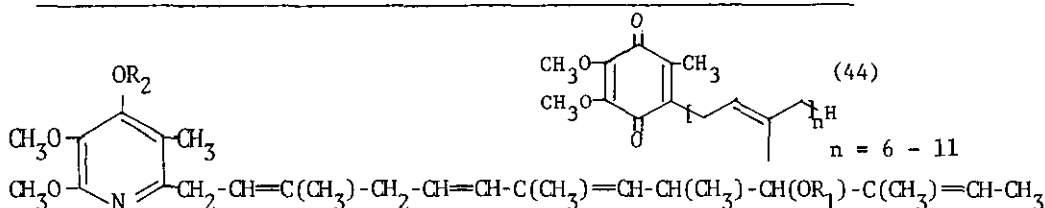
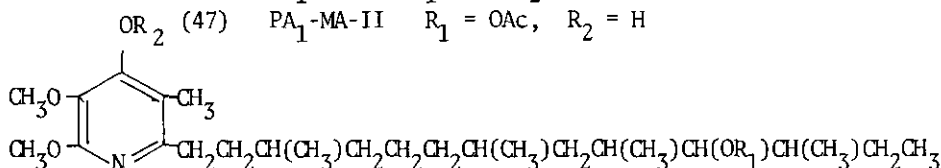
Thus, it has been doubtlessly established that piericidins block the site between NADH dehydrogenase flavoprotein and ubiquinone specifically. Recently, Takahashi² reported the respiratory inhibition caused by various piericidins obtained from *S. pactum* as listed in Table 9. In spite of the structural difference in the side chains, these piericidins equally show potent inhibitory activity, suggesting that the structural part from C-4 to the terminal of the side chain may be not so important for the respiratory inhibition but the nuclei of piericidins should be essential parts. The structure-activity relationship between piericidins and respiratory inhibition was preliminarily investigated by Mitsui et al.²² Effects of PA₁ derivatives (17, 45 - 50) on the mitochondrial respiration were observed to

Table 9. I₅₀ (10⁻¹ mole/liter) Value of Piericidins on Respiration of Rat Liver Mitochondria.

Compound	I ₅₀	Compound	I ₅₀	Compound	I ₅₀
A ₁	1.2	C ₁	1.2	D ₁	1.8
A ₃	1.5	C ₂	1.5	D ₃	2.5
A ₄	1.9	C ₃	1.3	rotenone	1.7
B ₁	1.1	C ₄	2.0		

Table 10. I_{50} (10^{-7} mole/liter) Value of PA_1 -related Compounds on Respiration of Rat Liver Mitochondria

Compound	I_{50}	Compound	I_{50}
PA_1 -DA (45)	16	H_8PA_1 (48)	43
PA_1 -MA-I (46)	5.8	H_8PA_1 -DA (17)	1000
PA_1 -MA-II (47)	1.0	H_8PA_1 -MA-I (49)	280
PA_1	1.2	H_8PA_1 -MA-II (50)	18

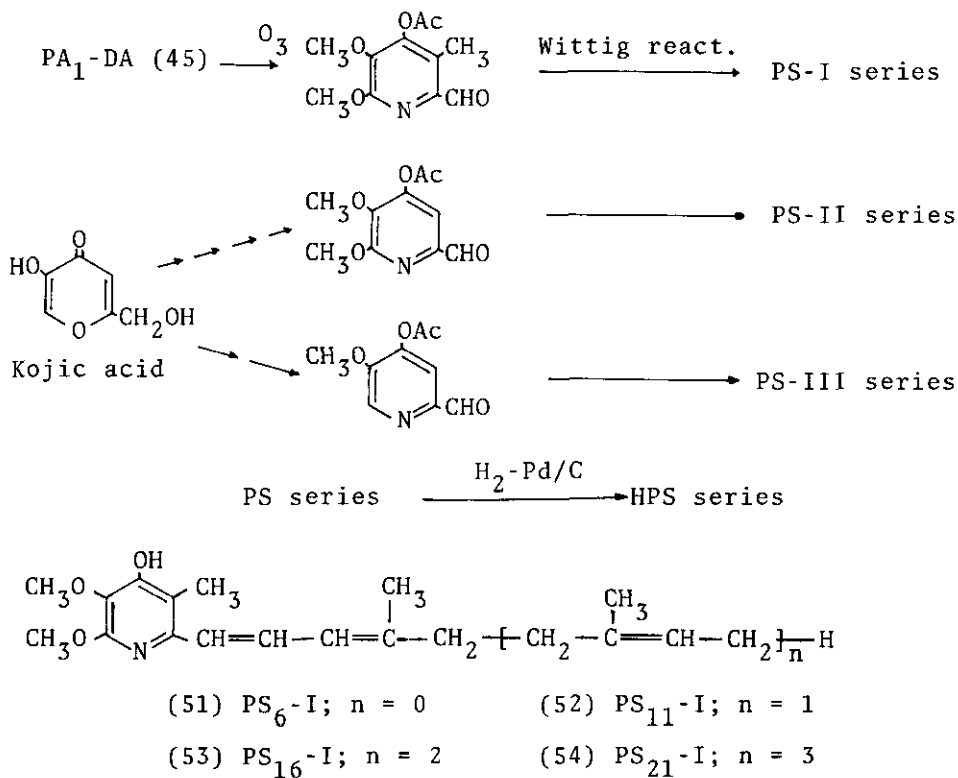
(45) PA_1 -DA, $R_1, R_2 = OAc$ (46) PA_1 -MA-I, $R_1 = H, R_2 = OAc$ (47) PA_1 -MA-II $R_1 = OAc, R_2 = H$ (48) H_8PA_1 , $R_1, R_2 = H$ (17) H_8PA_1 -DA, $R_1, R_2 = OAc$ (49) H_8PA_1 -MA-I, $R_1 = H, R_2 = OAc$ (50) H_8PA_1 -MA-II, $R_1 = OAc, R_2 = H$

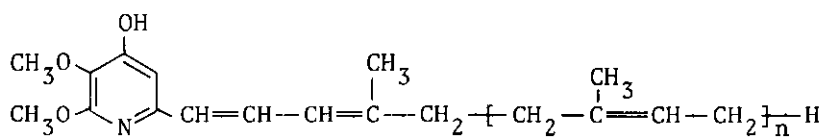
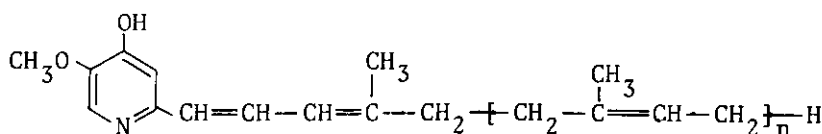
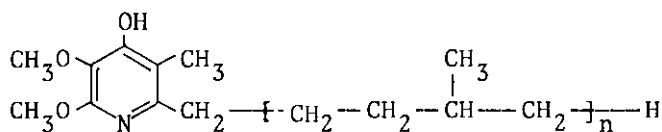
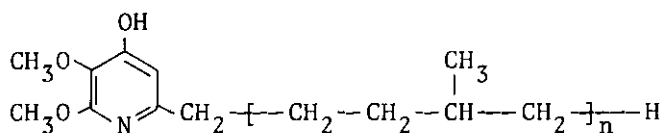
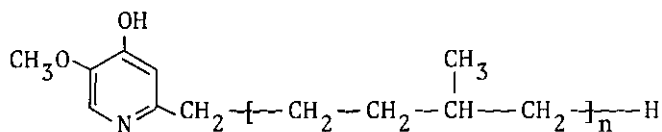
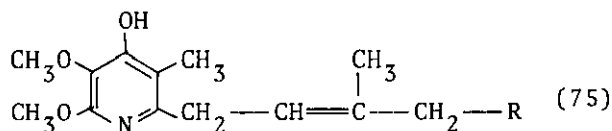
be compared with that of PA_1 . As shown in Table 10, the result indicates that two functionalities are necessary for exhibiting the activity, namely, unsaturations in the side chain and a free phenolic hydroxyl in the pyridine ring. Yoshida et al.²³ further investigated on the structure-activity relationship by means of the comparison of the effect among piericidin derivatives and

Table 11. I_{50} (10^{-7} mole/liter) Value of Piericidin Related Compounds on Respiration of Cockroach Muscle Mitochondria

Compound	I_{50}	Compound	I_{50}	Compound	I_{50}
PA_1	3.2	HPS_{16} -I (65)	170	HPS_{21} -II (70)	>1000
H_2PA_1 (28)	2.8	HPS_{21} -I (66)	470	PS_6 -III (59)	>1000
H_8PB_1	8.0	PS_6 -II (55)	>1000	PS_{11} -III (60)	31
PS_6 -I (51)	>1000	PS_{11} -II (56)	580	PS_{16} -III (61)	5.8
PS_{11} -I (52)	96	PS_{16} -II (57)	>1000	PS_{21} -III (62)	>1000
PS_{16} -I (53)	350	PS_{21} -II (58)	>1000	HPS_6 -III (71)	>1000
PS_{21} -I (54)	260	HPS_6 -II (67)	>1000	HPS_{11} -III (72)	7.9
HPS_6 -I (63)	98	HPS_{11} -II (68)	>1000	HPS_{16} -III (73)	7.5
HPS_{11} -I (64)	30	HPS_{16} -II (69)	>1000	HPS_{21} -III (74)	330

Fig. 12. Preparation of Piericidin Related Compounds.



(55) PS₆-II; n = 0 (56) PS₁₁-II; n = 1(57) PS₁₆-II; n = 2 (58) PS₂₁-II; n = 3(59) PS₆-III; n = 0 (60) PS₁₁-III; n = 1(61) PS₁₆-III; n = 2 (62) PS₂₁-III; n = 3(63) HPS₆-I; n = 1 (64) HPS₁₁-I; n = 2(65) HPS₁₆-I; n = 3 (66) HPS₂₁-I; n = 4(67) HPS₆-II; n = 1 (68) HPS₁₁-II; n = 2(69) HPS₁₆-II; n = 3 (70) HPS₂₁-II; n = 4(71) HPS₆-III; n = 1 (72) HPS₁₁-III; n = 2(73) HPS₁₆-III; n = 3 (74) HPS₁₆-III; n = 4

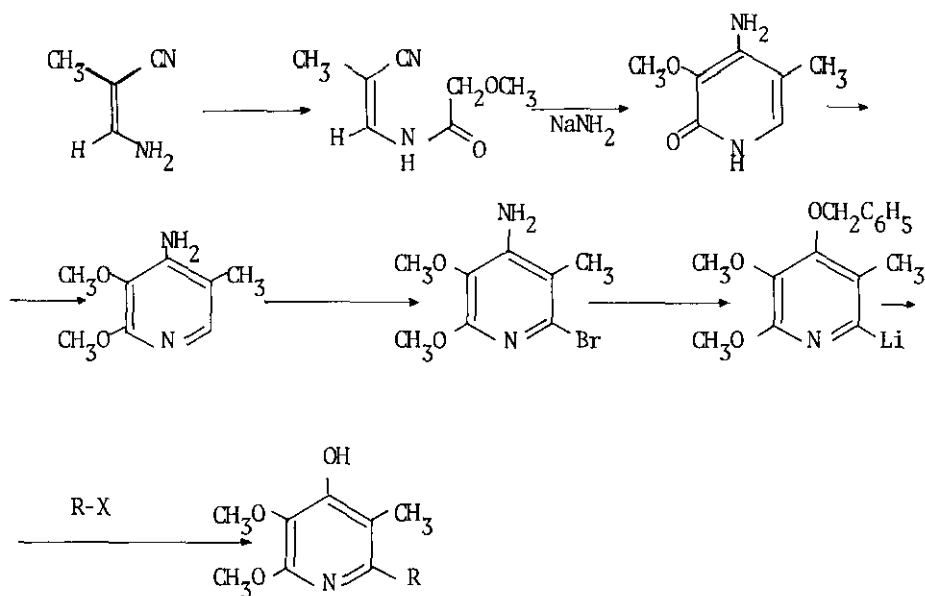
synthetic analogues (51 - 74), which were prepared by the method illustrated in Fig. 12. The result, as listed in Table 11, suggests that the conjugated diene of piericidins does not contribute to the inhibition activity, because the effect of 5,8-dihydro PA_1 (H_2PA_1) is as strong as of PA_1 . Since H_8PA_1 , H_8PB_1 , PS-I series and HPS-I series show lower activity than natural piericidins, it is obvious that the unsaturation between C-2 and C-3 is important to keep high activity. The comparison of the activity among octahydrogenated piericidins and HPS-I series suggests that branched methyls of the side chain, especially C-3 methyl is necessary to cause strong inhibition. On the other hand, the comparison of the activity among synthetic analogues suggests that the inhibition needs the fully substituted pyridine ring as in piericidins. It is a remarkable fact that rather strong activity is observed in PS-III and HPS-III series with the trisubstituted pyridine ring. Since chemical and physical characters of these compounds are quite different from those of piericidins, active sites of these analogues are uncertain. Based on facts above cited, Takahashi² have proposed the structure essential to inhibit the mitochondrial respiratory chain as illustrated in the formula (75). Quite recently, Gutman et al.²⁴ have shown the correctness of this speculation. They measured respiratory inhibition of "ubichidins"; totally synthetic products, which were synthesized by Schmidtchen et al.¹⁰ through the procedure as shown in Fig. 13. All "ubichidins" exhibit strong inhibition to the electron transport system as were expected, and it is interesting that the result (Table 12) shows the necessity of suitable length of side chain for the activity,

which is in good agreement with the speculation.

Table 12. I_{50} (10^{-10} mole/mg protein) Value of "Ubicidins" on Respiration of Beef Heart Mitochondria

Compound	I_{50}	Compound	I_{50}
PA ₁	0.6	Ubicidin 1 (76)	900
Ubicidin 2 (77)	2.3	Ubicidin 3 (78)	1.7
Ubicidin 3 (79)	200	Ubicidin 10 (80)	200

Fig. 13. Synthetic Route of "Ubicidin".



- (76) Ubicidin 1; R = prenyl
 (77) " 2; R = geranyl
 (78) " 3; R = farnesyl
 (79) " 4; R = phythyl
 (80) " 10; R = solanesyl

B. Effect on Insects.

Insecticidal activity of PA_1^{1a} and PB_1^{1c} , the metabolites of *S. mobaraensis*, on various insects is shown in Table 13. Both compounds are significantly active against housefly (*Musca domestica vicina* Macquart) and silkworm (*Bombyx mori* L.), however they are not so effective against German cockroach (*Blattella germanica* L.), green caterpillar (*Pieris rapae crucivora* Boisduval) and rice stem borer (*Chilo simplex* Butler). Concerning other piericidins of *S. pactum*, Takahashi² compared insecticidal activity of PC series with that of PA_1 (Table 14). Interestingly, only PC_2 shows the same toxicity against azuki bean weevil (*Callosobruchus chinensis* L.) as PA_1 , while other piericidins are almost inactive. On the other hand, all of PC members exhibit strong insecticidal activity against green rice leafhopper (*Nephotettix cincticeps* Uhler.) as well as PA_1 does. In spite of that piericidins equally show strong inhibition against mitochondrial respiration, their insecticidal action spectra are different, probably this may be due to the difference of penetrating property through cuticle.

Table 13. LD_{50} (μ g/body) of PA_1 and PB_1 on Insects

Insect	PA_1	PB_1	Antimycin A	Me-parathion
Housefly	0.8	1.2	0.35	
Cockroach	5.0	12.0	8.0	
Rice stem borer	14.5	16.0		0.17
Silkworm	1.5	1.6		0.38
Green caterpillar	16.0	27.0		2.2

Table 14. Insecticidal Activity of PC Series

(1) Azuki bean weevil*					
Piericidin	Dry film method, % mortality in 48 hr				
	1	10	100	500	1000 $\mu\text{g}/\text{tube}$
A ₁	0	16.7	18.3	58.3	90.0
C ₁	0	0	0	0	1.7
C ₂	0	0	13.3	40.0	83.3
C ₃	0	0	0	0	1.7
C ₄	0	3.3	3.3	1.7	3.3
(2) Green rice leafhopper**					
Piericidin	Topical application, % mortality in 24 hr				
	1.65	6.6	26.3	52.6	$\mu\text{g}/\text{g insect}$
A ₁	22.2	57.7	70.0	90.0	
C ₁	5.6	26.3	45.0	85.0	
C ₂	0	42.1	80.0	95.5	
C ₃	5.6	36.8	55.0	95.0	
C ₄	11.1	73.7	70.0	80.0	

C. Mammalian Toxicity

The mammalian toxicity of piericidins was examined on mice by intraperitoneal dosage. As summarized in Table 15, PA₁ is the most toxic among them. Although PC series is less toxic than

* 30 insects/tube were used in each concentration.

** Twenty insects were used in each dosage. Average weight of insects was 3.8 mg.

PA₁, their toxicity is still rather high.

Table 15. Toxicity of Piericidins to Mice

Piericidin	LD ₅₀ (mg/kg)
A ₁	0.87
C ₁	5.24
C ₂	2.26
C ₃	2.26
C ₄	3.62
D ₁	3.62

D. Antimicrobial Activity

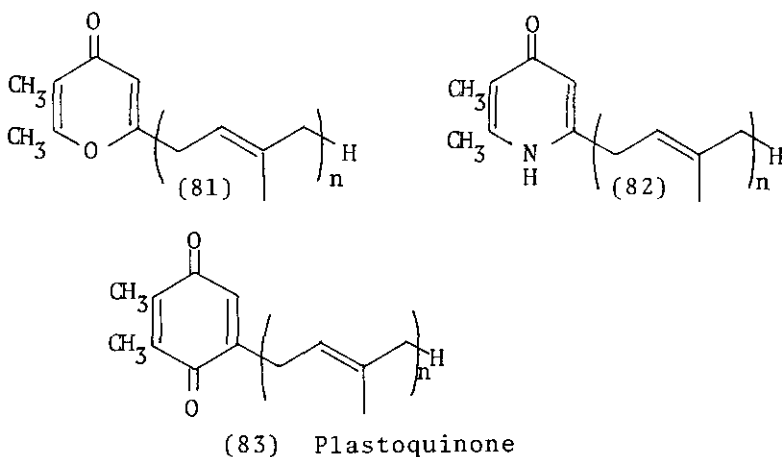
Antimicrobial activities of piericidins A₁ and C₁₋₄ against bacteria and fungi were tested according to the usual manner. The result listed in Table 16 indicates that all of them are very active against some fungi, *Trycophyton*s and *Cryptococcus neoformans* at 0.2 - 5 µg/ml, while they are inactive against other fungi and bacteria below 20 µg/ml concentration.

Table 16. Antimicrobial Activity of Piericidins

Test organisms	Minimum inhibitory concentration µg/ml				
	PA ₁	PC ₁	PC ₂	PC ₃	PC ₄
<i>Trycophyton mentagrophytes</i>	5	5	5	2	1
<i>T. rubrum</i>	5	5	5	2	1
<i>Cryptococcus neoformans</i>	0.5	1	0.2	0.5	0.5
<i>Piricularia oryzae</i>	20	20	5	20	10

9. Discussion

Although piericidins were discovered originally as naturally occurring insecticides, they have not been used as pest control chemicals because of their high mammalian toxicity and chemical instability. Nevertheless, the studies on piericidins may give a good chance for finding new types of pesticides or other biologically active substances, since the investigation on the relationship between their structures and activity suggests that the site where benzoquinone type coenzyme acts, may be blocked by heterocyclic compounds having the structure similar to the coenzyme. Indeed, Yoshida et al.²⁵ found that synthetic pyrones (81) and pyridones (82), of which structures are resembled to plastoquinone (83), show fairly strong inhibition against photosynthesis in chloroplast, where plastoquinone acts an important role as coenzyme in the electron transport system. It is an interesting approach to search novel inhibitors in other system, where quinones are playing roles, from academic and practical point of view.



References

1. a) S. Tamura, N. Takahashi, S. Miyamoto, R. Mori, S. Suzuki and J. Nagatsu, *Agric. Biol. Chem.*, 27, 576 (1963). b) S. Tamura and N. Takahashi, *Naturally Occurring Insecticides* (M. Jacobson and D. G. Crosby, ed.), Marcel Dekker Inc., U. S. A., 1971, p. 499. c) N. Takahashi, A. Suzuki, Y. Kimura, S. Miyamoto, S. Tamura, T. Mitsui and J. Fukami, *Agric. Biol. Chem.*, 32, 1115 (1968).
2. N. Takahashi, *Proc. 1st Intern. Congr. of the International Association of Microbiological Society, Tokyo, 1974*, p. 472.
3. S. Yoshida, K. Yoneyama, S. Shiraishi, A. Watanabe and N. Takahashi, *Agric. Biol. Chem.*, 41, 849 (1977).
4. N. Takahashi, A. Suzuki and S. Tamura, *J. Am. Chem. Soc.*, 87, 2066 (1965).
5. N. Takahashi, A. Suzuki and S. Tamura, *Agric. Biol. Chem.*, 30, 1 (1966).
6. A. Suzuki, N. Takahashi and S. Tamura, *Agric. Biol. Chem.*, 30, 13 (1966).
7. A. Suzuki, N. Takahashi and S. Tamura, *Agric. Biol. Chem.*, 30, 18 (1966).
8. N. Takahashi, A. Suzuki, Y. Kimura, S. Miyamoto and S. Tamura, *Tetrahedron Letters*, 1961 (1967).
9. S. Yoshida, K. Yoneyama, S. Shiraishi, A. Watanabe and N. Takahashi, *Agric. Biol. Chem.*, 41, 854 (1977).
10. F. P. Schmidtchen, H. Rapoport, *J. Am. Chem. Soc.*, 99, 7014 (1977).
11. a) S. Yoshida, S. Shiraishi, K. Fujita and N. Takahashi, *Tetrahedron Letters*, 1863 (1975). b) S. Yoshida, S. Shiraishi and N. Takahashi, *Agric. Biol. Chem.*, 41, 587 (1977).
12. J. W. Cornforth, (Mrs.) Cornforth and K. K. Mathew, *J. Chem. Soc.*, 1959, 122.
13. N. Takahashi, S. Yoshida, A. Suzuki and S. Tamura, *Agric. Biol. Chem.*, 32, 1108 (1968).

14. S. Yoshida, K. Yoneyama and N. Takahashi, in preparation.
15. Y. Kimura, N. Takahashi and S. Tamura, *Agric. Biol. Chem.*, **33**, 1507 (1969).
16. M. Tanabe and H. Seto, *J. Org. Chem.*, **35**, 2087 (1970).
17. a) S. Yoshida and N. Takahashi, *Abstract Vol. 1, 26th Intern. Congr. of I. U. P. A. C., Tokyo, 1977*, p. 347. b) S. Yoshida, K. Yoneyama, S. Shiraishi and N. Takahashi, in preparation.
18. C. Hall, M. Wu, F. L. Crane, N. Takahashi, S. Tamura and K. Folkers, *Biochem. Biophys. Res. Commun.*, **25**, 373 (1966).
19. M. Jeng, C. Hall, F. L. Crane, N. Takahashi, S. Tamura and K. Folkers, *Biochemistry*, **7**, 1311 (1968).
20. T. Mitsui, J. Fukami, K. Fukunaga, S. Sagawa, N. Takahashi and S. Tamura, *Botyu-Kagaku*, **34**, 126 (1969).
21. a) D. J. Horgan and T. P. Singer, *Biochem. J.*, **104**, 50c (1967). b) D. J. Horgan, T. P. Singer and J. E. Casida, *J. Biol. Chem.*, **243**, 834 (1968). c) D. J. Horgan and J. E. Casida, *Biochem. J.*, **108**, 153 (1968). d) D. J. Horgan, H. Ohno, T. P. Singer and J. E. Casida, *J. Biol. Chem.*, **243**, 5967 (1968).
22. T. Mitsui, T. Sagawa, J. Fukami, K. Fukunaga, N. Takahashi and S. Tamura, *Botyu-Kagaku*, **34**, 135 (1969).
23. S. Yoshida and N. Takahashi, Unpublished work and Jap. pat. 7308108.
24. M. Gutman, S. Kliatchko, *Febs Letters*, **67**, 348 (1976).
25. S. Yoshida, H. Date and N. Takahashi, Jap. pat. 52087171.

Received, 4th October, 1978