# PIERICIDINS: NATURALLY OCCURRING INHIBITORS AGAINST MITOCHONDRIAL RESPIRATION

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## 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 *S. mobaraensis* | | | | II for *S. pactum* | | | |
|---|---|---|---|---|---|---|---|
| Glucose | 20 | Brewers yeast | 4 | Starch | 25 | $(NH_4)_2SO_4$ | 2 |
| Starch | 10 | NaCl | 2 | Soybean meal | 15 | $CaCO_3$ | 4 |
| Meat extract | 10 | $K_2HPO_4$ | 0.5 | Ebios | 2 | | |
| Soybean powder | 25 | 10% NaOH | 3ml | NaCl | 5 | | |

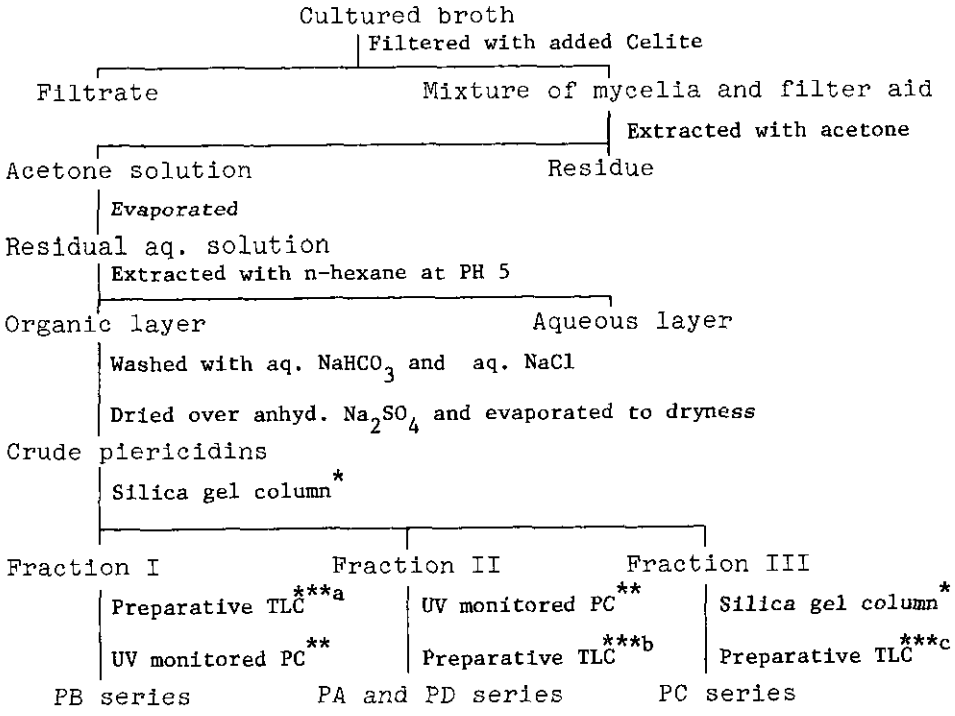


Fig. 1 Isolation procedure of piericidins.

* 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 $PB_4$ and $PB_1$ 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 $PB_1$, $PB_2$, $PB_3$ and $PB_4$.

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 $PA_1$, $PA_2$, $PA_3$, $PA_4$, $PD_1$, $PD_2$, $PD_3$ and $PD_4$.

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 $PC_1$, $PC_2$, $PC_3$ and $PC_4$ 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 $PD_1$ to $PC_1$ 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-

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* 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 redily visualized as dark shadows under UV light (2537 Å) using silica gel $GF_{254}$ plates. The *Rf* 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 *Rf* 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 |

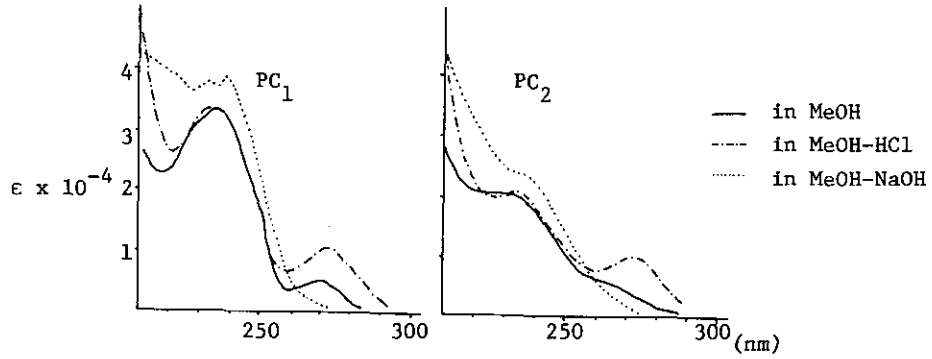


Fig. 2. UV Spectra of Piericidins

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*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).

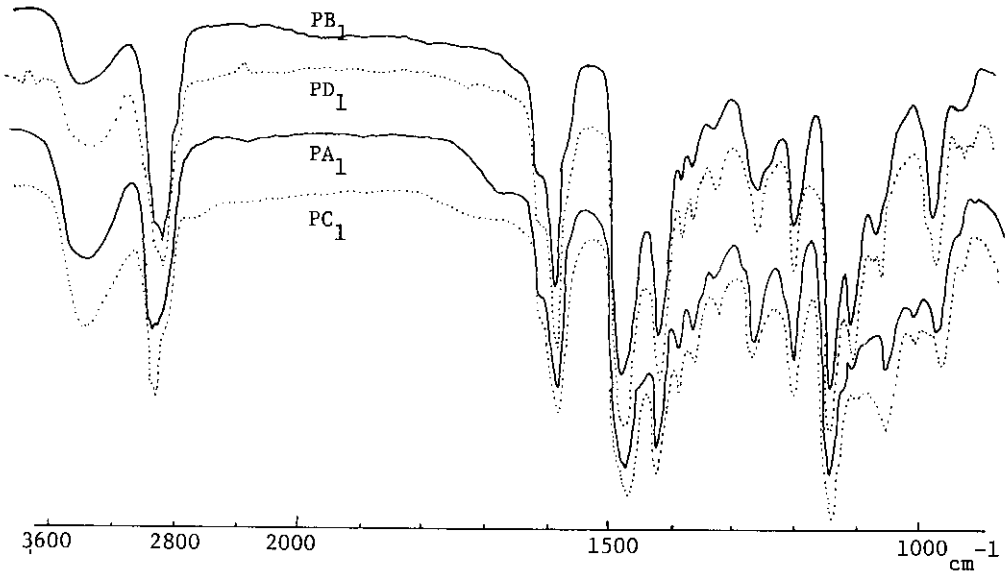



Fig. 3. IR Spectra of Piericidins

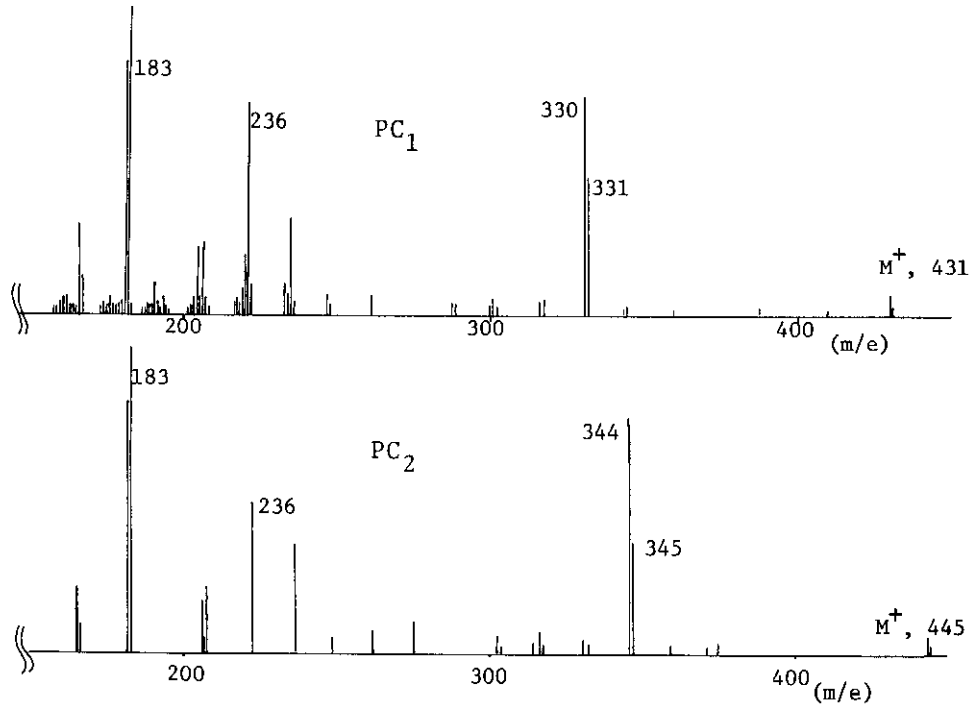



Fig. 4. Mass Spectra of piericidins

m/e 344 and 345. Further, piericidin B, C and D series exibit 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 $A_1$ ($PA_1$) and $B_1$ ($PB_1$), 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 $PA_1$ and $PB_1$ were elucidated by Yoshida et al.[9] and Takahashi[2] by means of MS (Table 5), $^1H$- and $^{13}C$-nuclear magnetic resonances ($^1H$- and $^{13}C$-NMR, Table 6 and 7).

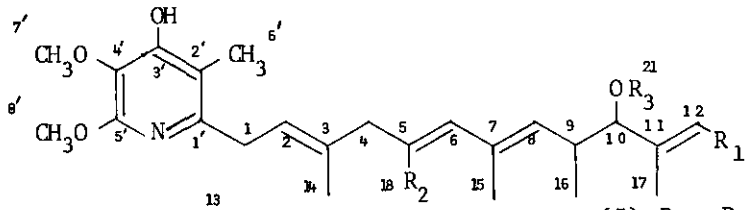
Table 5. Relative Intensity (%) of Ion Peaks in MS of Piericidins

| 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 |

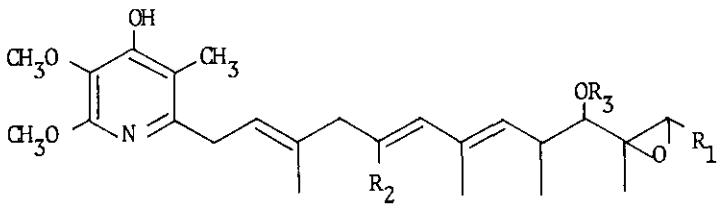
* These peaks are very weak but surely observed.

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) $R_1$ = $^{13}CH_3$, $R_2$,$R_3$ = H
(2) $R_1$,$R_2$ = $^{19,20}CH_3$, $R_3$ = H
(3) $R_1$ = $(CH_3)_2CH$, $R_2$,$R_3$ = H
(4) $R_1$ = $(CH_3)_2CH$, $R_2$=$CH_3$, $R_3$=H
(5) $R_1$, $R_3$ = $CH_3$, $R_2$ = H
(6) $R_1$,$R_2$,$R_3$ = $CH_3$
(7) $R_1$ = $(CH_3)_2CH$, $R_3$=$CH_3$, $R_2$=H
(8) $R_1$ = $(CH_3)_2CH$, $R_2$,$R_3$ = $CH_3$



(9) $R_1$ = $CH_3$, $R_2$,$R_3$ = H
(10) $R_1$,$R_2$ = $CH_3$, $R_3$ = H
(11) $R_1$ = $(CH_3)_2CH$, $R_2$ = H, $R_3$ = H
(12) $R_1$ = $(CH_3)_2CH$, $R_2$ = $CH_3$, $R_3$ = H
(13) $R_1$,$R_3$ = $CH_3$, $R_2$ = H
(14) $R_1$,$R_2$,$R_3$ = $CH_3$
(15) $R_1$ = $(CH_3)_2CH$, $R_2$ = H, $R_3$ = $CH_3$
(16) $R_1$ = $(CH_3)_2CH$, $R_2$,$R_3$ = $CH_3$

Table 6. Proton Chemical Shifts of Piericidins on $^1$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) |

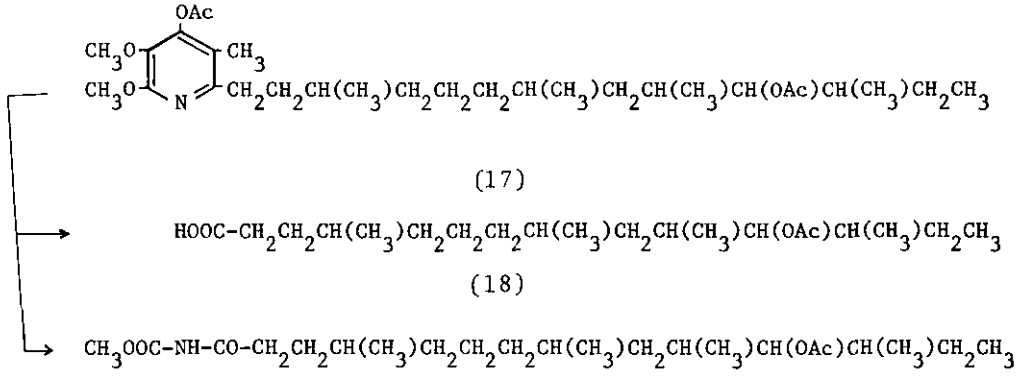
Splittings of signals in off-resonance mode are shown in brackets.

*A. The Structure of Piericidin $A_1$.*

The structure of $PA_1$, 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 $A_1$ diacetate ($H_8PA_1$-DA, 17) derived from $PA_1$ 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 studies[4-7]. 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, $PA_1$ diacetate ($PA_1$-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 $A_1$ 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 $PA_1$ 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 $PA_1$-related Compounds.



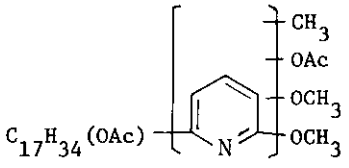
(17)



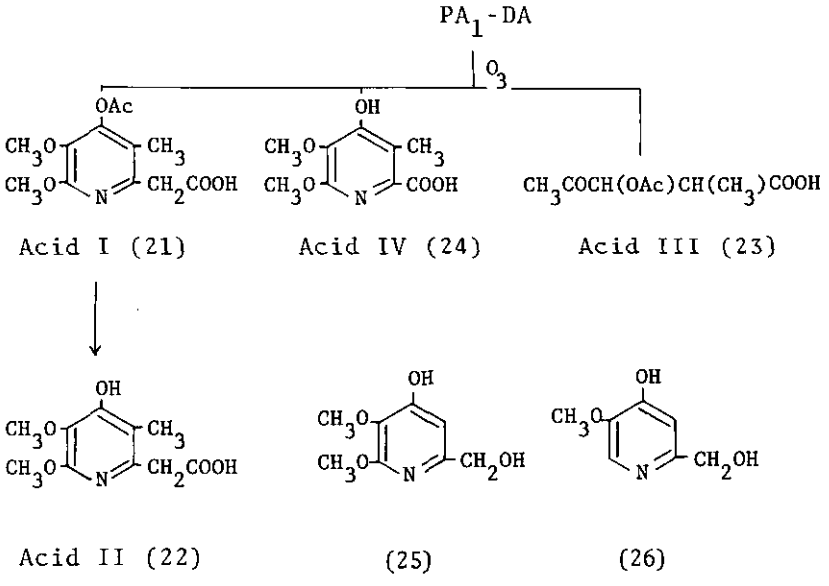
(18)



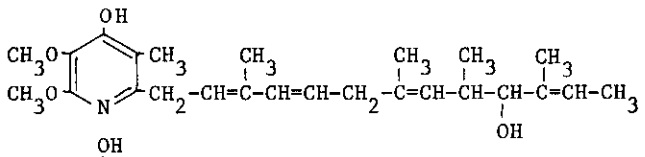
(19)



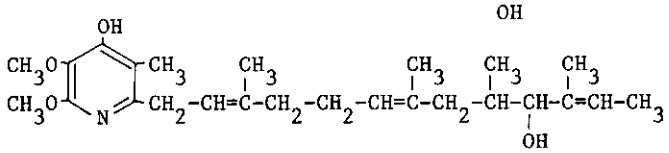
(20)



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 $^1$H-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.



(27)



(28)

*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 $PA_1$. $^1$H-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 carbinyl 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 $^1$H-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).

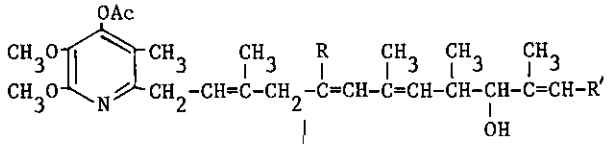
*D. The Structure of Piericidin $A_4$.*

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 $^1$H-NMR spectrum of $PA_4$. The spectrum shows signals due to the same terminal moiety as in $PA_3$ at $\delta$ 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.

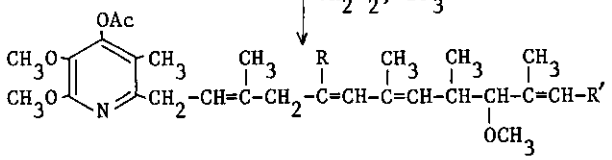
*E. The Structures of Piericidin B Series.*

It was easy to determine the structures of PB series, since their molecular formulas obtained by high resolution MS and additional methoxy signals in $^1$H-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.



(29)

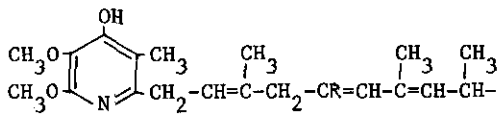


(30)

R = $CH_3$ or H, R' = $CH_3$ or $CH(CH_3)_2$

*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.



(i) R = $CH_3$ or H

The reduction of $PC_1$ by "Cornforth reaction"[12] gave $PA_1$ in 25% yield, and the epoxidation of $PA_1$ with *m*-chloroperbenzoic acid afforded $PC_1$ 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 $PD_1$, (14) for $PD_2$, (15) for $PD_3$ and (16) for $PD_4$. 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 carbinyl carbon signal also suffers a down field shift (10 ppm) by the methylation of C-10 alcohol. From $^{1}$H-NMR spectra, the presence of a terminal isopropyl can be recognized easily by a couple of doublets appeared at $\delta$ 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 ($\lambda_{max}$ 225 nm, $\varepsilon$ 20,000) in almost half intensity of those of piericidins without C-5 methyl ($\lambda_{max}$ 239 nm, $\varepsilon$ 40,000).

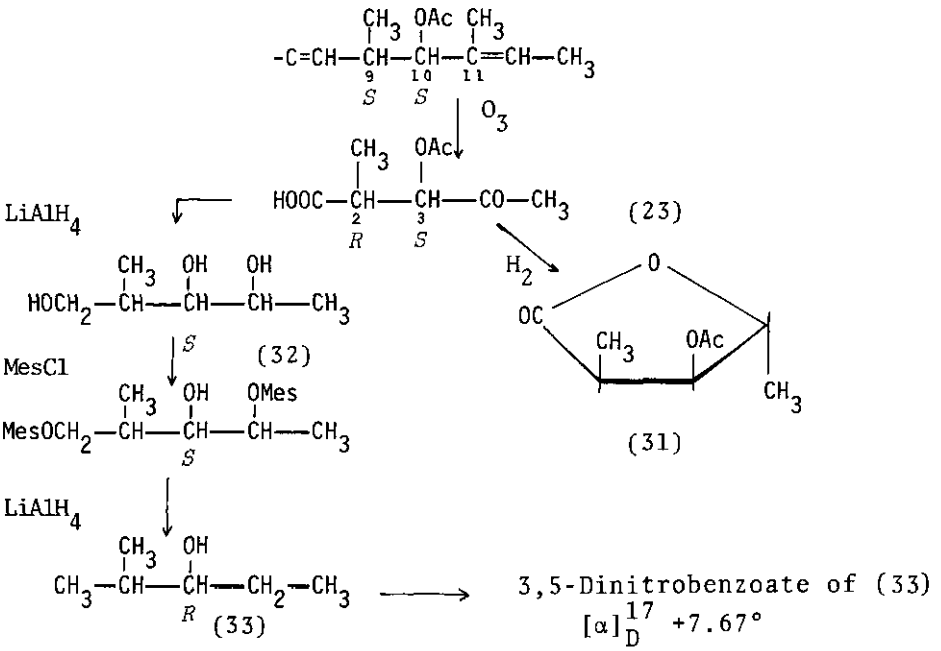
These empirical rules will be useful for structural elucidation for new unknown piericidins, which may be present in metabolites of other microorganisms.

## 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

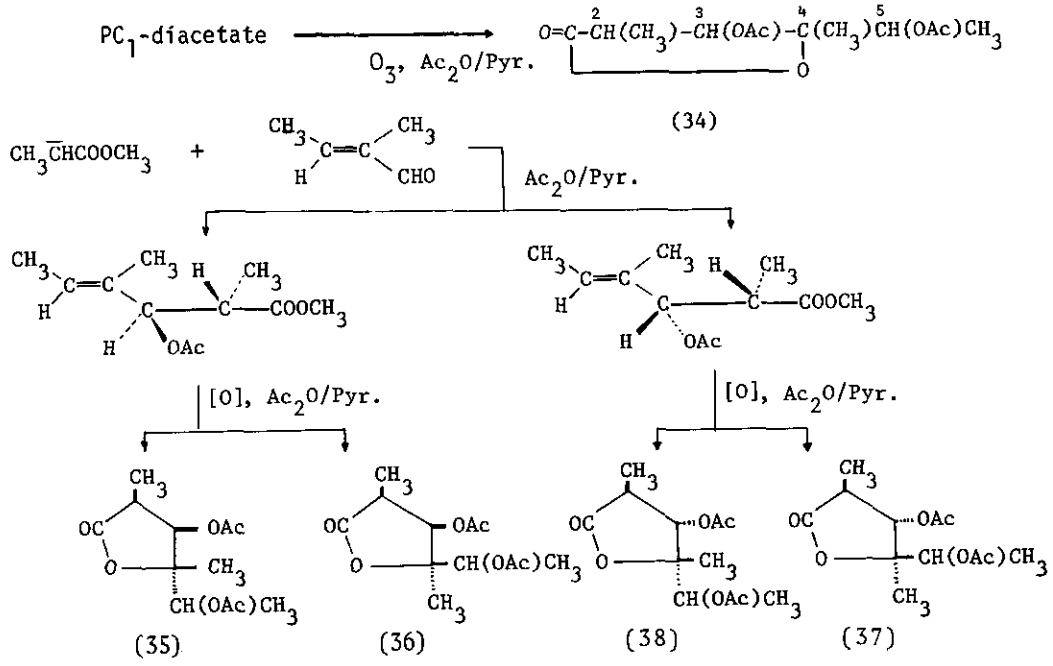
and C-3 in (23) were confirmed to be *R-S* or *S-R* by comparison of the lactone (31) obtained by reduction of the natural (23) with that from the synthetic (23), which was a diastereomeric mixture at C-2 and C-3. As shown in Fig. 7, the methyl ester of the natural (23) was successively converted to known (+)-2-methylpentan-3-ol (33). Since the absolute configuration of C-3 in the alcohol (33) has already been established as *R*, C-2 and C-3 in the acid (23) must be *R-S*, and therefore C-9 and C-10 in $PA_1$ are *S-S* on the basis of the stereochemical correlation to (23). Yoshida et al.[14] found that ozonolysis of other members of PA series gave the same product (23), and accordingly the all members of PA and PB series have been proved to have the same absolute configuration, *S-S*, at C-9 and C-10.

Fig. 7 Stereochemical correlation of C-10 in $PA_1$ (1) to C-3 in (+)-2-methyl-3-pentanol (33)



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 $PC_1$-DA followed by oxidation and acetylation gave a lactone (34), whose chemical structure has been readily determined by mass and $^1$H-NMR spectroscopies. In the lactone (34), three asymmetric centers of $PC_1$ (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 $PC_1$) 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.



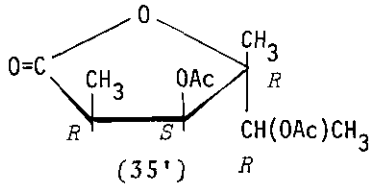
mers were formed by this method because of the stereochemical restriction in the epoxidation on the *E*-olefin with *m*-chloroperbenzoic acid. Fortunately, one of the isomers showed the same spectral feature as (34) in NMR and IR measurement, and this fact indicates that the relative configuration of C-11 and C-12 must be *R-R* or *S-S*. On the other hand, the epoxidation on $PA_1$ to afford $PC_1$ (see the chapter of chemical structures) revealed that absolute configurations of C-9 and C-10 in $PC_1$ should be same as $PA_1$. 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 $^1$H-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

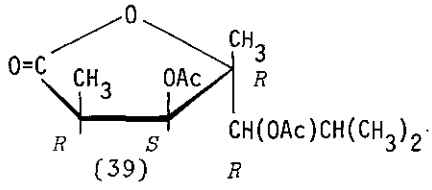
as *R-S-R-R* as shown in formula (35'), and consequently C-9, C-10, C-11 and C-12 in $PC_1$ should be assigned as *S-S-R-R*.

Table 8. Proton Chemical Shifts of The Lactones (in $CDCl_3$, $\delta$ 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 | NOE | 1.26 | NOE | 1.32 | NOE | 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 |
| $CH_3CO$- | 2.07 | 1.98 | | 2.04 | | 2.03 | | s |
| | 2.15 | 2.06 | | 2.13 | | 2.08 | | s |




(35')



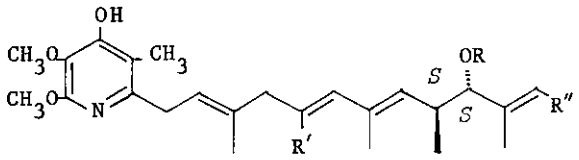

(39)

The absolute configurations of other members of PC and PD series are considered to be same as $PC_1$ because of their biogenetic relation to $PC_1$. This was supported by $^1H$-NMR spectral similarity of (35') to (39), which were obtained by ozonolysis of $PC_3$. However, more strict examination to determine the stereochemistry of them are now in progress.

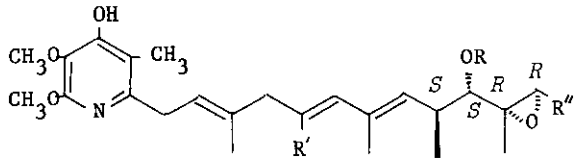
Configurations of the conjugated diene in the side chain of odd suffixed piericidins were assigned as *E-E* based on their strong UV absorption at 239 nm as well as on the large coupling constant

(16 Hz) between H-5 and H-6 in the $^{1}$H-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 $^{1}$H-NMR data with those of model compounds.

PA and PB series



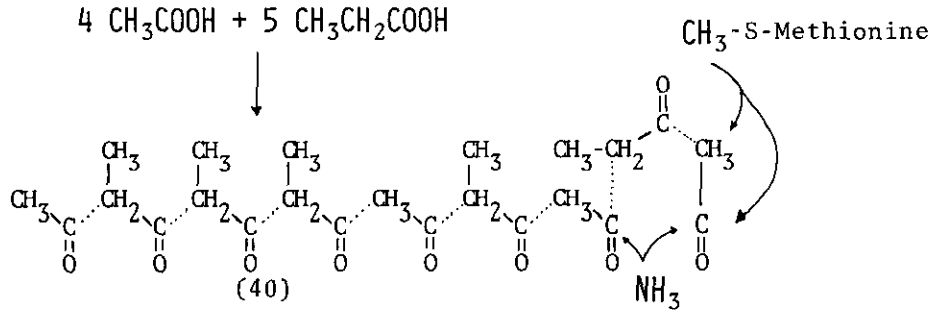

PC and PD series




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 methoxyls 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 $PB_1$




(40)

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 $C_1$ sourse, 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 $PD_1$

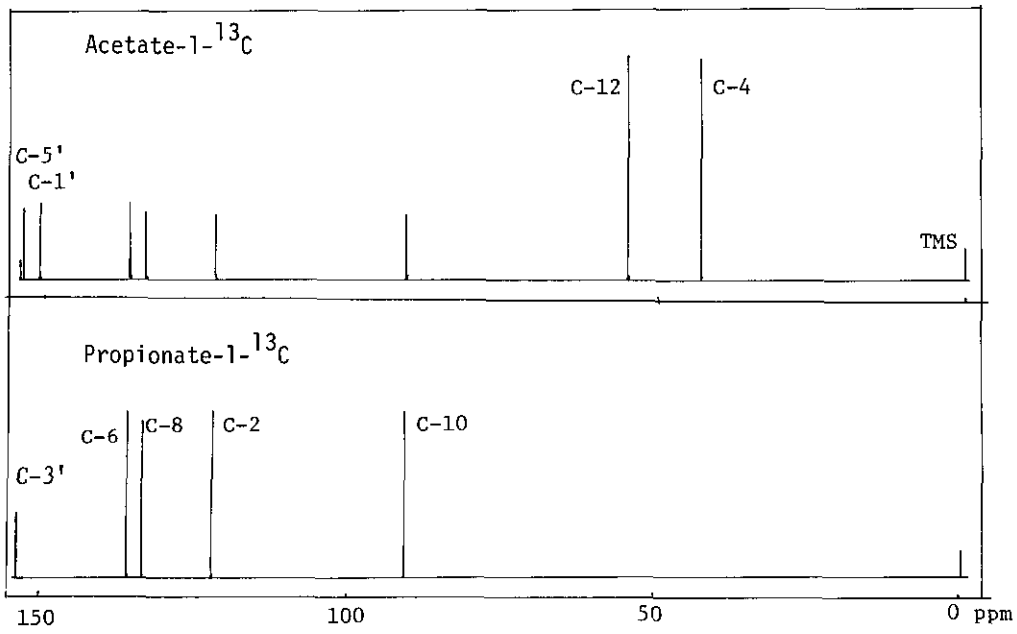



Fig. 10. $^{13}$C-NMR Spectra of $^{13}$C-labeled $PC_2$

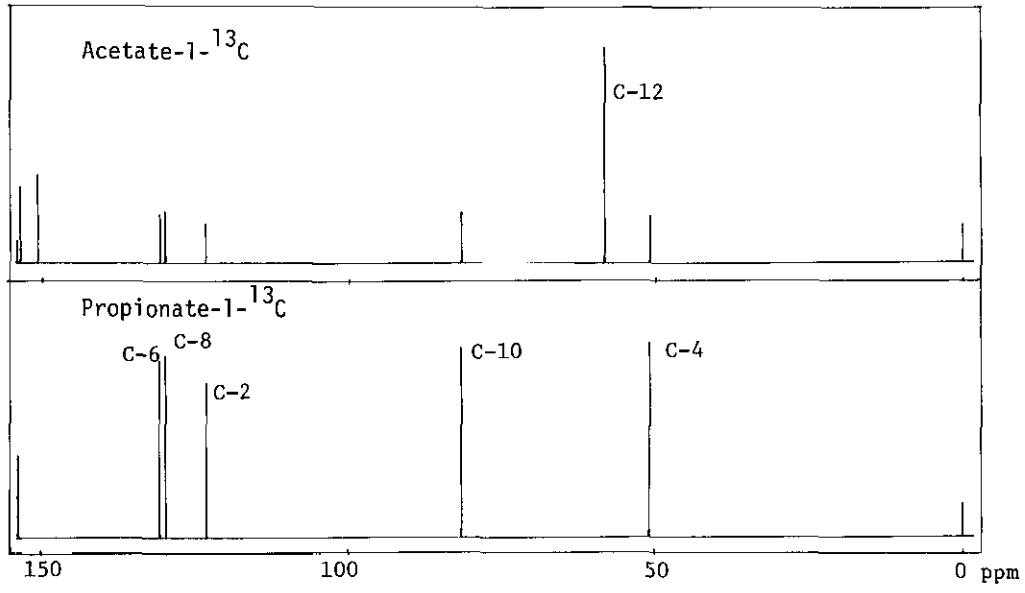
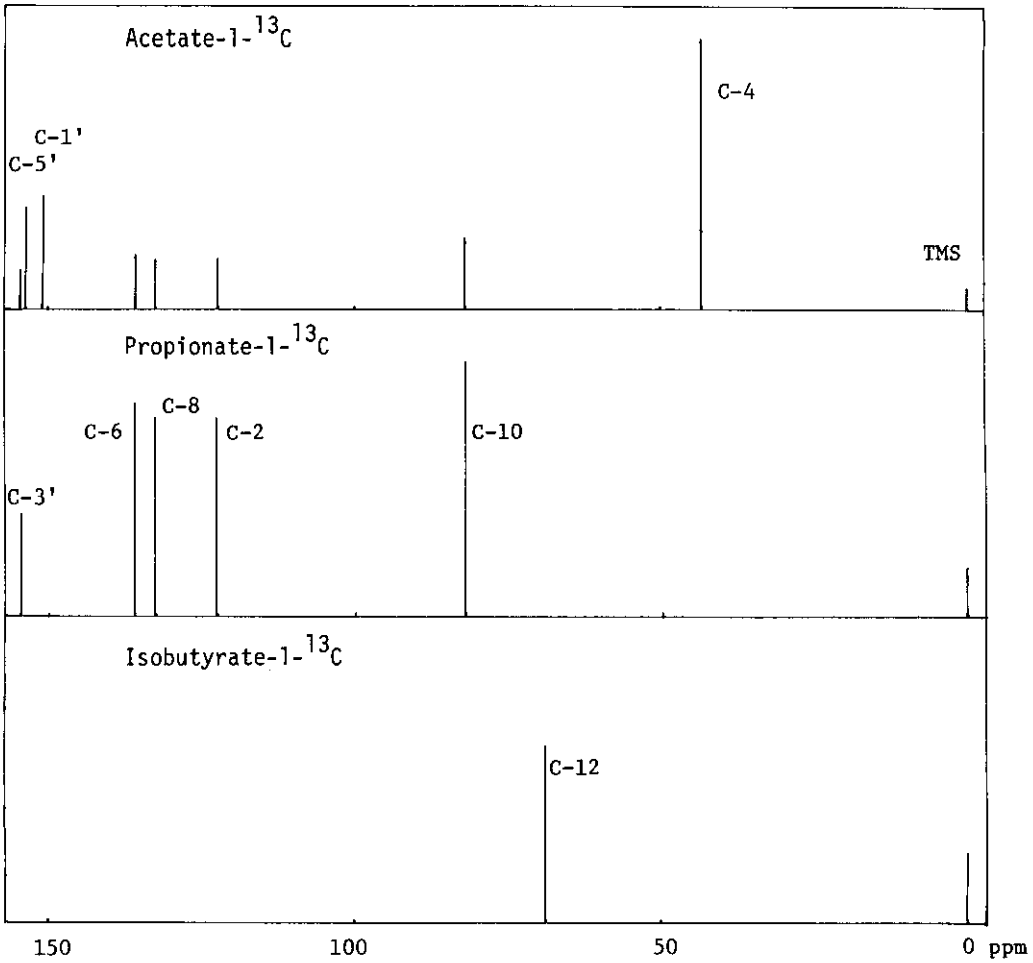
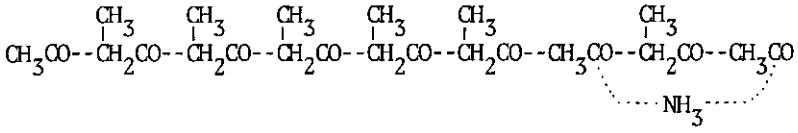
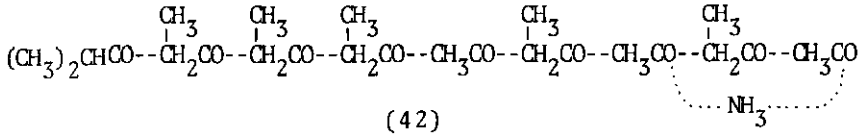


Fig. 11. $^{13}C$-NMR Spectra of $^{13}C$-labeled $PC_3$

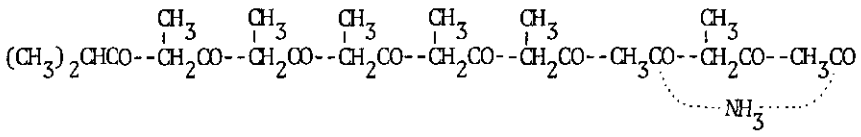


(41)



(42)



(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 $PA_1$ and $PB_1$ inhibit NADH oxidation at very low concentration. Mitsui et al.[20] also observed that both piericidins $A_1$ and $B_1$ 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 $A_1$ to the enzyme is the most potent among them.

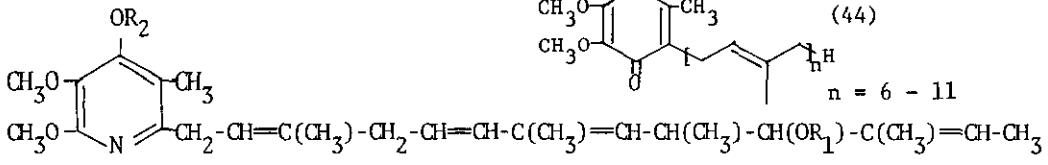
Thus, it has been doubtlessly established that piericidins block the site between NADH dehydrogenase flavoprotein and ubiquinone specifically. Recently, Takahashi[2] reported the respiratory inhibition caused by various piericidins obtained from *S. pactum* as listed in Table 9. In spite of the structural 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 $PA_1$ derivatives (17, 45 - 50) on the mitochondrial respiration were observed to

Table 9. $I_{50}$ ($10^{-7}$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 |



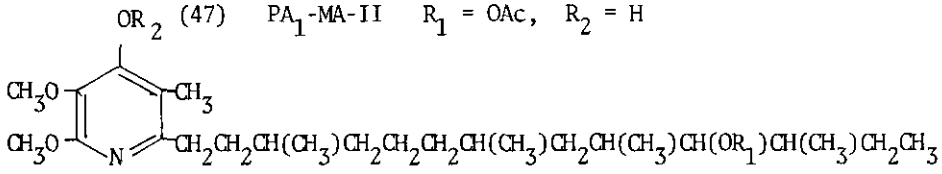




(44)

n = 6 - 11

(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



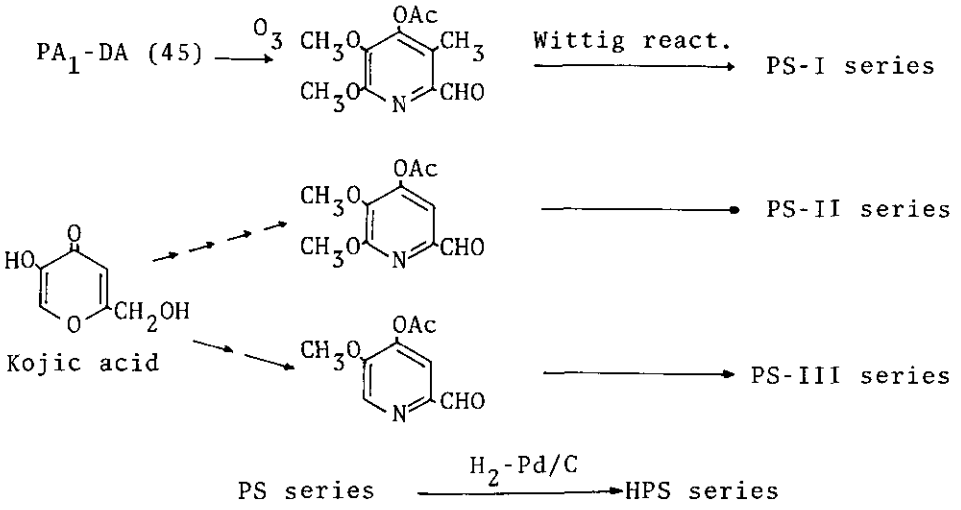

(48) $H_8PA_1$, $R_1$, $R_2$ = H
(17) $H_8PA_1$-DA, $R_1$, $R_2$ = OAc
(49) $H_8PA_1$-MA-I, $R_1$ = H, $R_2$ = OAc
(50) $H_8PA_1$-MA-II, $R_1$ = OAc, $R_2$ = H

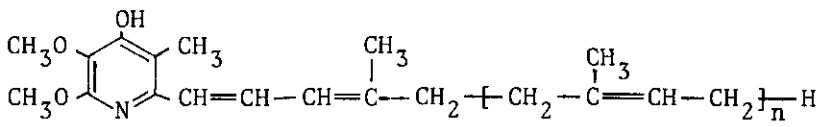
be compared with that of $PA_1$. As shown in Table 10, the result indicates that two functionarities are necessary for exhibiting the activity, namely, unsaturations in the side chain and a free phenolic hydroxyl in the pyridine ring. Yoshida et al.[23] further investigated on the structure-activity 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 |

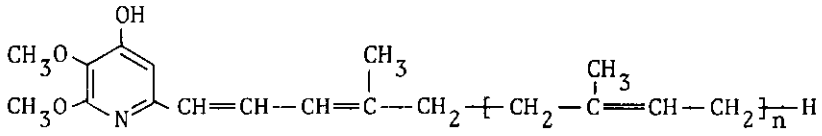
Fig. 12. Preparation of Piericidin Related Compounds.



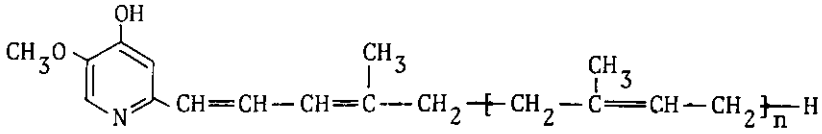

(51) $PS_6$-I; n = 0 (52) $PS_{11}$-I; n = 1

(53) $PS_{16}$-I; n = 2 (54) $PS_{21}$-I; n = 3



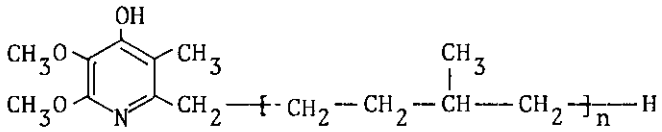
(55) $PS_6$-II; n = 0 (56) $PS_{11}$-II; n = 1

(57) $PS_{16}$-II; n = 2 (58) $PS_{21}$-II; n = 3



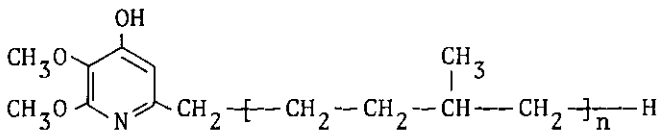
(59) $PS_6$-III; n = 0 (60) $PS_{11}$-III; n = 1

(61) $PS_{16}$-III; n = 2 (62) $PS_{21}$-III; n = 3



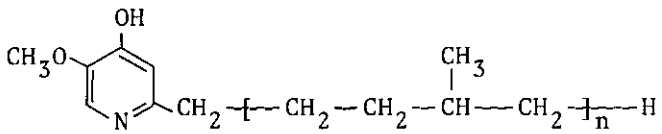
(63) $HPS_6$-I; n = 1 (64) $HPS_{11}$-I; n = 2

(65) $HPS_{16}$-I; n = 3 (66) $HPS_{21}$-I; n = 4



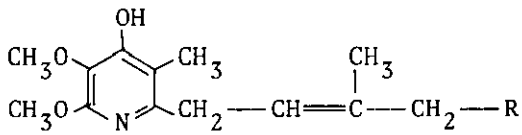
(67) $HPS_6$-II; n = 1 (68) $HPS_{11}$-II; n = 2

(69) $HPS_{16}$-II; n = 3 (70) $HPS_{21}$-II; n = 4



(71) $HPS_6$-III; n = 1 (72) $HPS_{11}$-III; n = 2

(73) $HPS_{16}$-III; n = 3 (74) $HPS_{16}$-III; n = 4



(75)

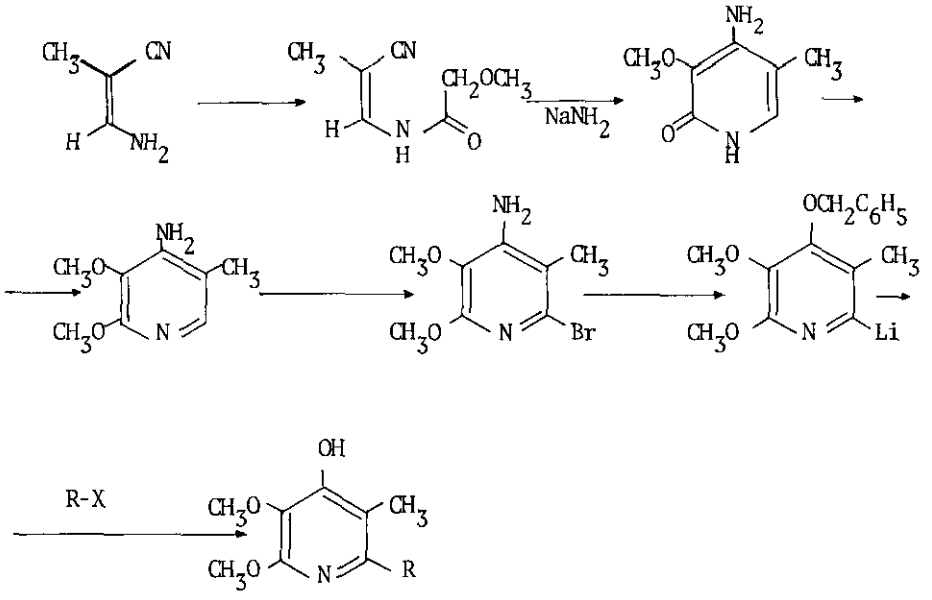
synthetic analogues (51 - 74), which were prepared by the method illustrated in Fig. 12. The result, as listed in Table 11, suggests that the conjugated diene of piericidins does not contribute to the inhibition activity, because the effect of 5,8-dihydro $PA_1$ ($H_2PA_1$) is as strong as of $PA_1$. 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, Takahashi[2] 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 "ubicidins", totally synthetic products, which were synthesized by Schmidtchen et al.[10] through the procedure as shown in Fig. 13. All "ubicidins" 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 simples* Butler). Concerning other pericidins of *S.pactum*, Takahashi[2] 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. $LD_{50}$ (μg/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*

| | Dry film method, % mortality in 48 hr | | | | |
|---|---|---|---|---|---|
| Piericidin | 1 | 10 | 100 | 500 | 1000 μg/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**

| | Topical application, % mortality in 24 hr | | | | |
|---|---|---|---|---|---|
| Piericidin | 1.65 | 6.6 | 26.3 | 52.6 | μg/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, $PA_1$ is the most toxic among them. Although PC series is less toxic than

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* 30 insects/tube were used in each concentration.

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

$PA_1$, 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*

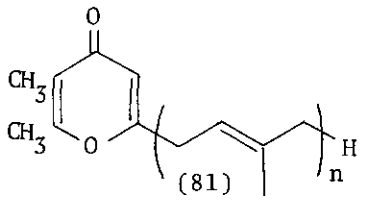
Antimicrobial activities of piericidins $A_1$ and $C_{1-4}$ against bacteria and fungi were tested according to the usual manner. The result listed in Table 16 indicates that all of them are very active against some fungi, *Trycophytons* and *Cryptococcus neoformans* at 0.2 - 5 μg/ml, while they are inactive against other fungi and bacteria below 20 μg/ml concentration.

Table 16. Antimicrobial Activity of Piericidins

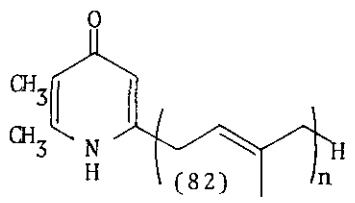
| Test organisms | Minimum inhibitory concentration μg/ml | | | | |
|---|---|---|---|---|---|
| | $PA_1$ | $PC_1$ | $PC_2$ | $PC_3$ | $PC_4$ |
| *Trycophyton mentagrophytes* | 5 | 5 | 5 | 2 | 1 |
| *T. rubrum* | 5 | 5 | 5 | 2 | 1 |
| *Cryptococcus neoformans* | 0.5 | 1 | 0.2 | 0.5 | 0.5 |
| *Piricularia oryzae* | 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.



(81)



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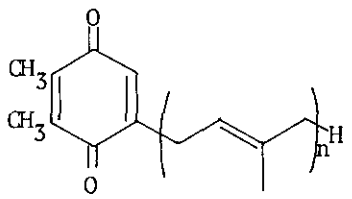

(83) Plastoquinone

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