BIOSYNTHETIC STUDIES ON PIERICIDIN A AND ITS STRUCTURAL REVISION

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Piericidins A (PA) and B (PB), insecticidal substances as well as potent inhibitors to respiratory chain in mitochondoria, were isolated from mycellia of <u>Streptomyces mobaraensis</u>¹. Of two structures I and II, derived from extensive degradation studies, the former has been selected as the most probable one, on the basis that the proton on C_8 appearing at the highest chemical shift among five olefinic ones in the PMR spectrum of PA has been considered to be included in an isolated double bond². PA has been proved to be constructed from four acetate and five propionate units <u>via</u> polyketide chain III by tracer experiments using ¹⁴C-labeled compounds³. The combination of CMR spectroscopy and feeding experiments of ¹³C-labeled precursors suggests to clarify not only the nature of C₄ and C₆ but also their origins, differentiating two possible structures I and II for PA.



+ Detail discussion will be published in elsewhere.

o Intensified signals by direct incorporation of the isotope.

 Δ Intensified signals by randomization of the isotope.

Fig. I~IV

to methylene carbons by single-frequency off-resonance decoupling technique, should be assigned to C_1 and C_6 in Ia or C_1 and C_4 in IIa respectively. As shown in Fig. II, the spectrum of PA obtained by feeding experiment of sodium acetate-1-¹³C shows four clearly intensified peaks, only the signal at δ 43.1 ppm being included. On the other hand in the spectrum of PA from sodium acetate-2-¹³C (Fig. III), the intensity of the signal at δ 34.4 ppm was enhanced, while

Table. Results of Spin Decoupling Experiment on Dihydro-PA

Position of Protons				Multiplicity
	(ppm)			Change
Irradiated		Observed		
H-1	3.30	H-2	5.32	t→ s
H-5*	2.03	H-6	5.12	t→ s
		,H-10	3.54	d→ s
H-9	1.83	`H-15	0.58	d→ s
H-13	1.59	H-12 /	5.40	q≯s

in the case of sodium propionate-1-¹³C (Fig. IV), the both signals were not intensified. Thus, the signal at δ 34.4 ppm should be assigned to C₁ and that at δ 43.1 ppm to C₄. These evidences clearly indicate that IIa represents the correct structure of PA, excluding the structure Ia.

Another structural evidence for IIa was obtained by the selective hydrogenation of the conjugated system in the side chain of PA. PA was converted to a dihydro derivative (Found: $M^+417.2846$, $C_{25}H_{39}NO_4$

* reference 5

requires M^+ 417.2849) with Ca-NH₃ in a quantitative yield. In the 100 MHz PMR spectrum of dihydro-PA in CCl₄, a doublet was observed at the same chemical shift as in that of PA [δ 3.35 ppm (Pyr.-CH₂-CH=)]. On the basis of spin decoupling experiment summerized in Table, the structure IV is explainable by the consideration that the conjugated system in PA should locate between C₅ and C₈ as in IIa and dihydro-PA was formed by selective 1,4-addition of hydrogens to the conjugated diene system. Since PA has been convertible to PB by a methylation,^{1b} the structure of PB is also revised as IIb.



More evidences to support the position of the conjugated system were obtained by mass spectrometry. The mass spectrum of PA (Fig. V)⁴ exhibited the fragment-peak at $\underline{m/e}$ 236 due to a fission between C_4 and C_5 as intense as $\underline{m/e}$ 222 due to that between C_3 and C_4 , while fissions of double bonds caused very weak fragment-peaks; e.g. $\underline{m/e}$ 196 and 248. Accordingly, its mass spectral feature is better rationalized by the structure IIa than Ia.

Besides structural evidences, the CMR spectral studies on PA obtained by the feeding of 13 C-labeled precursors afforded some interesting and direct informations on the biosynthesis

Fig. V The mass spectrum of PA



of piericidins. It should be noted that conversion of labeled acetates to propionate was clearly observed. As shown in Fig. II, in the feeding of acetate-1-¹³C, the isotopes were incorporated into carbons due to C_1 of propionate units; their abundances were almost half of those of acetate units. In the feeding of acetate-2-¹³C (Fig. III), the isotopes were incorporated into all positions of propionate units with clear ¹³C-¹³C couplings (ca. 30% of the isotopes), and into the C_2 position of acetate units in the highest ratio with less coupling (< 15%) than in the former. Such an incorporation pattern and an observation of $^{13}C-^{13}C$ coupling are well explained by the participation of highly labeled acety1-CoA in frequent recycling of TCA cycle. These findings support the proposed conversion mechanism³ of acetates; the conversion of succiny1-CoA to propionate <u>via</u> methylmalony1-CoA in this microbe.

References

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