

Fig. I~IV The CMR Spectra of PA and ^{13}C -Enriched PA

Carbon Assignments†

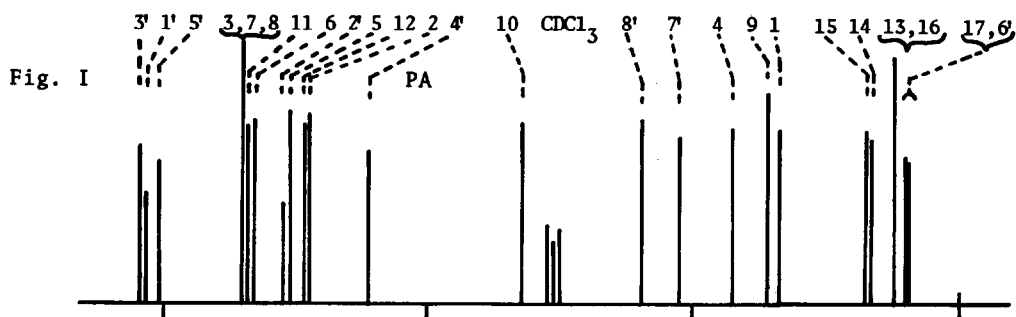


Fig. II

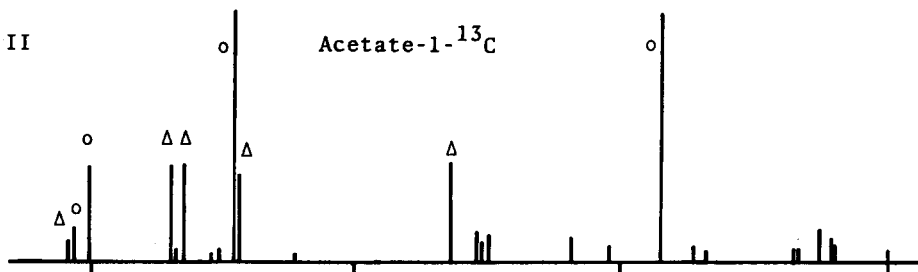


Fig. III

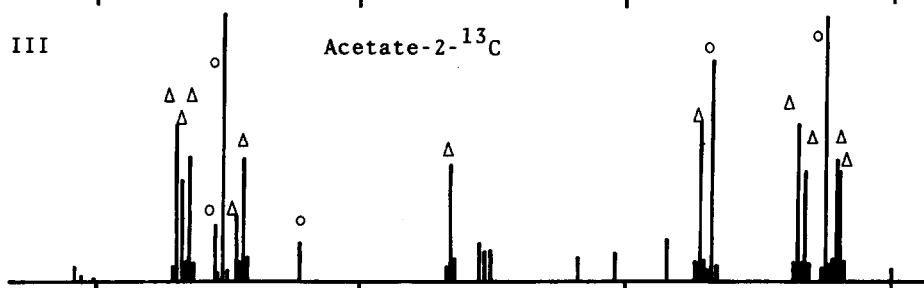
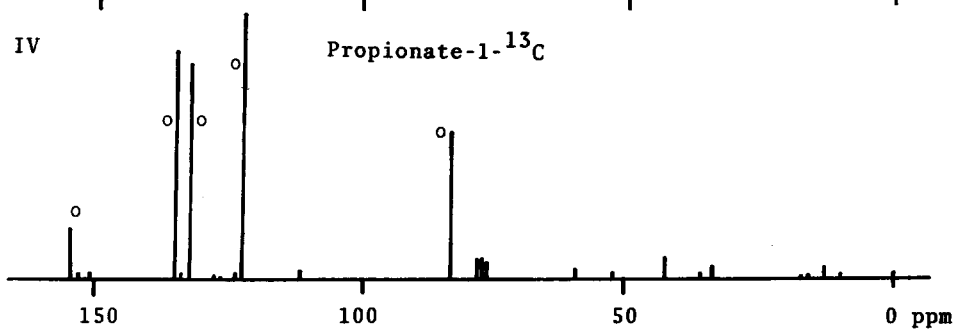


Fig. IV



† Detail discussion will be published in elsewhere.

o Intensified signals by direct incorporation of the isotope.

Δ Intensified signals by randomization of the isotope.

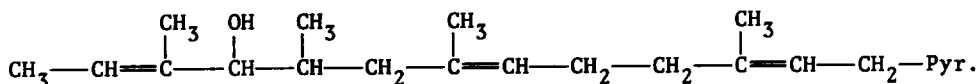
to methylene carbons by single-frequency off-resonance decoupling technique, should be assigned to C_1 and C_6 in Ia or C_1 and C_4 in IIa respectively. As shown in Fig. II, the spectrum of PA obtained by feeding experiment of sodium acetate- $1-^{13}C$ shows four clearly intensified peaks, only the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

Table. Results of Spin Decoupling Experiment on Dihydro-PA

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

* reference 5

requires M^+ 417.2849) with $Ca-NH_3$ in a quantitative yield. In the 100 MHz PMR spectrum of dihydro-PA in CCl_4 , a doublet was observed at the same chemical shift as in that of PA [$\delta 3.35$ ppm (Pyr.- CH_2 -CH=)]. On the basis of spin decoupling experiment summarized in Table, the structure IV is explainable by the consideration that the conjugated system in PA should locate between C_5 and C_8 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.



IV

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 m/e 236 due to a fission between C_4 and C_5 as intense as m/e 222 due to that between C_3 and C_4 , while fissions of double bonds caused very weak fragment-peaks; e.g. m/e 196 and 248. Accordingly, its mass spectral feature is better rationalized by the structure IIa than Ia.

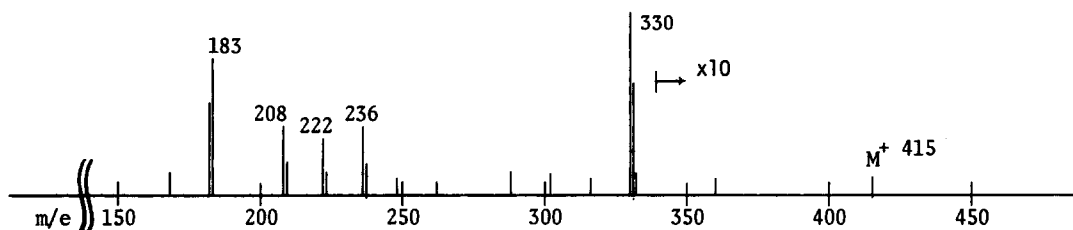
Besides structural evidences, the CMR spectral studies on PA obtained by the feeding of ^{13}C -labeled precursors afforded some interesting and direct informations on the biosynthesis

in the case of sodium propionate- $1-^{13}C$ (Fig. IV), the both signals were not intensified.

Thus, the signal at $\delta 34.4$ ppm should be assigned to C_1 and that at $\delta 43.1$ ppm to C_4 . 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, $C_{25}H_{39}NO_4$

Fig. V The mass spectrum of PA



of ptericidins. 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- ^{13}C , 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- ^{13}C (Fig. III), the isotopes were incorporated into all positions of propionate units with clear ^{13}C - ^{13}C 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 acetyl-CoA in frequent recycling of TCA cycle. These findings support the proposed conversion mechanism³ of acetates; the conversion of succinyl-CoA to propionate via methylmalonyl-CoA in this microbe.

References

1. a) S. Tamura, N. Takahashi, S. Miyamoto, R. Mori, S. Suzuki and J. Nagatsu, Agr. Biol. Chem., **27**, 576 (1963) b) N. Takahashi, A. Suzuki, Y. Kimura, S. Miyamoto, S. Tamura, T. Mitsui and J. Fukami, Agr. Biol. Chem., **32**, 1115 (1968)
2. N. Takahashi, A. Suzuki and S. Tamura, Agr. Biol. Chem., **30**, 1 (1966); N. Takahashi, A. Suzuki and S. Tamura, J. Am. Chem. Soc., **87**, 2066 (1965)
3. Y. Kimura, N. Takahashi and S. Tamura, Agr. Biol. Chem., **33**, 1507 (1969)
4. cf. A. Suzuki, N. Takahashi and S. Tamura, Agr. Biol. Chem., **30**, 18 (1966)
5. H-4, 5 and 8 were observed at very close chemical shifts.