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BIOSYNTHESIS OF PIERICIDINS A AND B

Nobutaka Takahashi, Yasuo Kimura and Saburo Tamura

Department of Agricultural Chemistry, The University of Tokyo,

Tokyo, Japan

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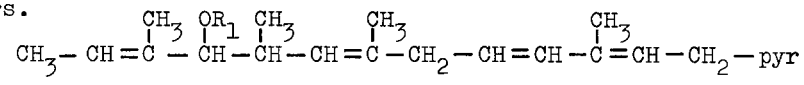
Piericidins A(PA) and B(PB) are natural insecticidal substances isolated from mycellia of Streptomyces mobaraensis (1), and their structures have been elucidated as Ia and Ib, respectively (2,3). The novel structures prompted us to investigate the biosynthesis of these compounds using ^{14}C-labelled precursors.

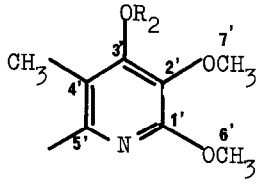


Ia: $R_1 = R_2 = H$

Ib: $R_1 = CH_3$, $R_2 = H$

Ic: $R_1 = R_2 = COCH_3$

pyr=



The microorganism was cultured in the "C_4 medium" described previously (1) to get a seed culture, which was transfered into the medium composed of 2% glucose, 0.5% peptone, 0.2% K_2HPO_4 and 0.2% NaCl. After 48 hr, the precursors shown in Table 1 were added to the medium and incubated for an additional 48 hr. Labelled PA was isolated from mycellia according to the procedure mentioned earlier (1).

PA diacetate (Ic) as well as octahydro-PA diacetate (II) was subjected to the degradations as illustrated in Fig. I. The radioactivities of PA and degradation products were measured by a liquid scintillation counter in toluene and that of CO_2 was determined as $BaCO_3$ in toluene containing Cab-O-Sil-M5.

Incorporation ratios of the precursors into PA are shown in Table I. The incorporation of propionate was higher than that of acetate, but that of mevalonate was significantly low. These data suggest that biosynthesis

TABLE I

Incorporation Ratios of ^{14}C-compounds into PA

Acetate-1-^{14}C	0.9%	DL-Mevalonic acid lactone-2-^{14}C	0.02%
Acetate-2-^{14}C	1.2%	DL-Methionine (methyl-^{14}C)	7.4%
Formate-^{14}C	0.00%	L-Aspartic acid-U-^{14}C	0.5%
Propionate-1-^{14}C	4.5%		

of PA proceeds in the similar course to that of macrolides, characteristic metabolites of Streptomyces species.

Specific activities in the degradation products are summarized in Table II. Five propionate units were incorporated into PA, two units each being found in III and IV. The radioactivity of acetaldehyde obtained from PA labelled by propionate was negligible. The S-methyl group in methionine was incorporated exclusively into two methoxyl attatched to the pyridine ring. These evidences

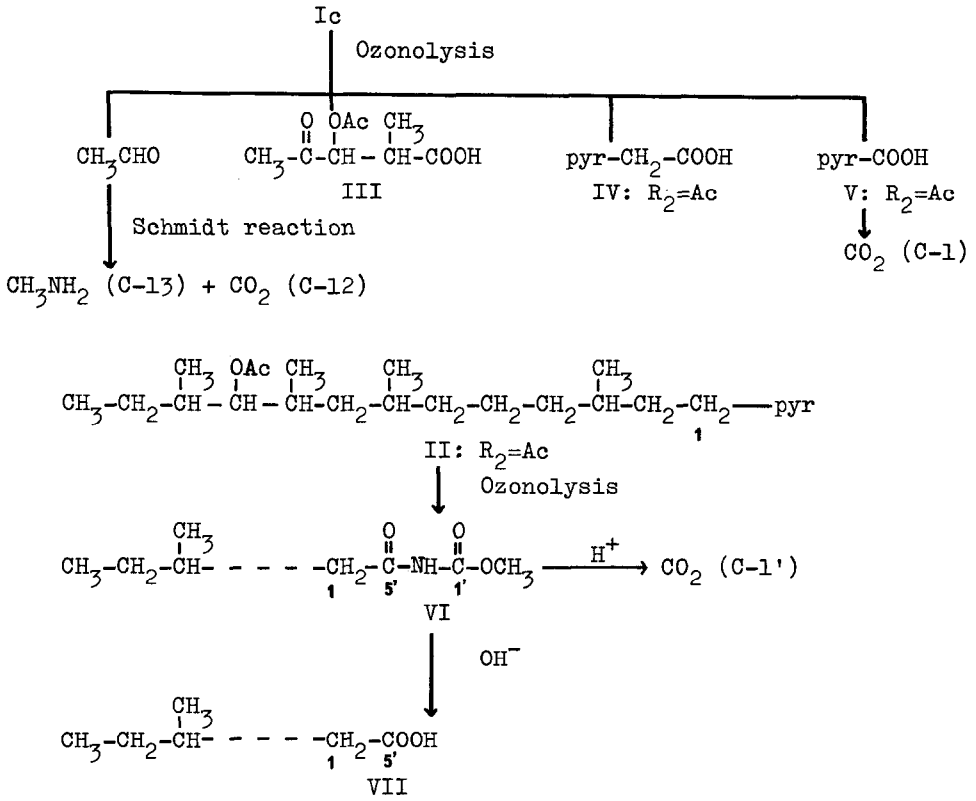


FIG. I

TABLE II. Percentage Distribution of Radioactivity in Degradation Products of PA

	Acetate-1-^{14}C		Acetate-2-^{14}C		Propionate-1-^{14}C		Methionine-^{14}Methyl	
I	100%	(100%)**	100%	(100%)	100%	(100%)	100%	(100%)
III	6.4	(0.0)	20.7	(0.0)	39.2	(40.0)	-	
IV	48.5	(50.0)	39.6	(50.0)	39.9	(40.0)	98.7	(100.0)
V	47.0	(50.0)	35.0	(50.0)	18.8	(20.0)	-	
$BaCO_3$ (C-1)	-		12.3	(25.0)	-		-	
CH_3CHO	18.7	(25.0)	12.3	(25.0)	0.9	(0.0)	-	
CH_3NH_2 (C-13)	0.6	(0.0)	11.7	(25.0)	-		-	
$BaCO_3$ (C-12)	18.1	(25.0)	0.6	(0.0)	-		-	
VI	96.5	(100.0)	-		79.8	(80.0)	-	
$BaCO_3$ (C-1')	20.4	(25.0)	-		-		-	
VII	75.5	(75.0)	-		82.9	(80.0)	-	
CH_3I (C-6',7')*	-		-		-		47.7	(50.0)

indicate that the methyl groups at C-3, 7, 9, 11 and 4' must be derived from C-3 methyl of propionate.

In the molecules of PAs obtained from acetate-1-^{14}C and 2-^{14}C, extensive randomizations of isotopes were observed, the radioactivities being found even in the parts having their origins in propionates. This may be explained by the conversion of acetate into methylmalonate *via* succinate (4,5) as in the case of methymicin biosynthesis (6). In spite of these randomizations, the regularity in the incorporation into probable acetate units was recognized. Thus specific activities of C-1(CO_2 from V) and C-13(methylamine from acetaldehyde) of PA labelled by acetate-2-^{14}C and those of C-1'(CO_2 from VI) and C-12(CO_2 from acetaldehyde) of PA labelled by acetate-1-^{14}C were the same in each case.

These evidences suggest that in the biosynthesis of PA a long C_{23}-chain was formed at first from four acetate and five propionate units and then a nitrogen atom was incorporated at the terminal part of the chain to form the pyridine ring. This demonstrates a novel type of biosynthesis for pyridine rings.

PB was shown to be produced from PA at the later stage of the fermentation. Labelled PA was added to the medium pre-cultivated for 4 days, and mycellia was harvested after 2 weeks. PB thus isolated contained 9.5% of isotopes. When

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* Methyl iodide was obtained from IV by Zeisel reaction.

** Theoretical percentage distribution

labelled methionine was similarly incubated together with non-labelled PA, 2.7% of the isotopes of the methionine was incorporated into the PB molecule. This indicates that PB was biosynthetically derived from PA by the methylation of C-10 hydroxyl of PA with methionine.

In conclusion, biosynthesis of PA and PB is illustrated in Fig. II.

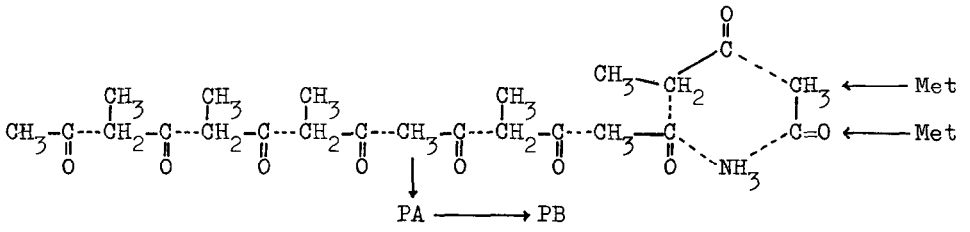

FIG. II

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