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The Biosynthesis of Pyridomycin. I*

Hiroshi Ogawara, Kenji Maeda, and Hamao Umezawa

ABSTRACT: Biosynthesis of pyridomycin by growing cells of *Streptomyces pyridomyceticus* in a complex medium was studied by observing incorporation of various labeled compounds into this antibiotic. L-Aspartic acid, glycerol, 3-hydroxypicolinic acid, propionic acid, pyruvic acid, L-threonine, and L-isoleucine were incorporated into pyridomycin. L-Lysine, DL-2-aminoadipic acid, L-proline, L-phenylalanine, L-methionine, formic acid, L-serine, and L-alanine were not incorporated into pyridomycin. Degradation studies of pyridomycin according to Figure 1 showed that the radioactivity of L-[^{14}C]aspartic acid and [^{14}C]glycerol was found in both pyridine nuclei of pyridomycin. Sodium pyruvate was also incorporated into 3-hydroxypicolinic acid. These facts indicate that 3-hydroxypicolinic acid is biosynthesized from L-aspartic acid and glycerol or pyruvic acid and the pyridine ring of the C_{11} moiety of pyridomycin is derived from L-aspartic acid and glycerol. This is the first paper reporting the biosynthesis of pyridine nuclei by *Streptomyces*. [^{3}H]3-Hydroxypicolinic acid was introduced into the 3-hydroxypicolinic acid part of pyridomycin.

However, DL-[^{14}C]tryptophan and [^{3}H]3-hydroxyanthranilic acid were not incorporated into pyridomycin. Experiments testing the incorporation of propionic acid and pyruvic acid showed that the C_6 side-chain part of the C_{11} moiety ($C_{11}H_{16}N_2O_3$) containing pyridine was derived from these two precursors. [^{14}C]-Propionic acid was incorporated at high rate and selectively into the terminal C_3 part of this moiety. To the best of our knowledge, this is the first study to observe the participation of propionic acid in the biosynthesis of an amino acid and a peptide. Label from L-threonine was found in the L-threonine moiety of pyridomycin, and that of L-isoleucine was detected almost exclusively in the α-keto-β-methylvaleric acid portion of pyridomycin. From these results, the biosynthetic pathway of pyridomycin was illustrated in Figure 2.

Pyridomycin is an antimycobacterial antibiotic (Maeda *et al.*, 1953) produced by *Streptomyces pyridomyceticus* (Okami *et al.*, 1954). The structure has been recently determined by X-ray analysis (Koyama *et al.*, 1967) and chemical degradation (Ogawara *et al.*, 1968). Pyridomycin, a 12-membered ring compound, is hydrolyzed to α-keto-β-methylvaleric acid, 3-hydroxypicolinic acid, L-threonine, and 4-amino-3-hydroxy-2-methyl-5-(3-pyridyl)pentanoic acid which is further degraded to α-formylpropionic acid and 3-pyridylacetaldehyde as shown in Figure 1. These hydrolysis and degradation products are easily obtained and it is possible to study the biosynthetic route of this antibiotic.

Concerning the biosynthesis of the pyridine ring by higher plants and microorganisms, tryptophan-3-hydroxyanthranilic acid pathway, aspartate–glycerol pathway, aspartate–pyruvate pathway, and mevalonate pathway have been reported (Leete, 1965). Although *Neurospora* and mammals utilize tryptophan as a source of niacin, *Escherichia coli* and *Bacillus subtilis* synthesize the pyridine ring from aspartic acid (Mattoon, 1963; Ogasawara *et al.*, 1967). In higher plants, for example, *Nicotiana rustica*, glyceraldehyde is incorporated into the pyridine ring of nicotine (Fleeker and Byerrum, 1967).

With these considerations in mind, experiments were performed to elucidate the biosynthetic route of the py-

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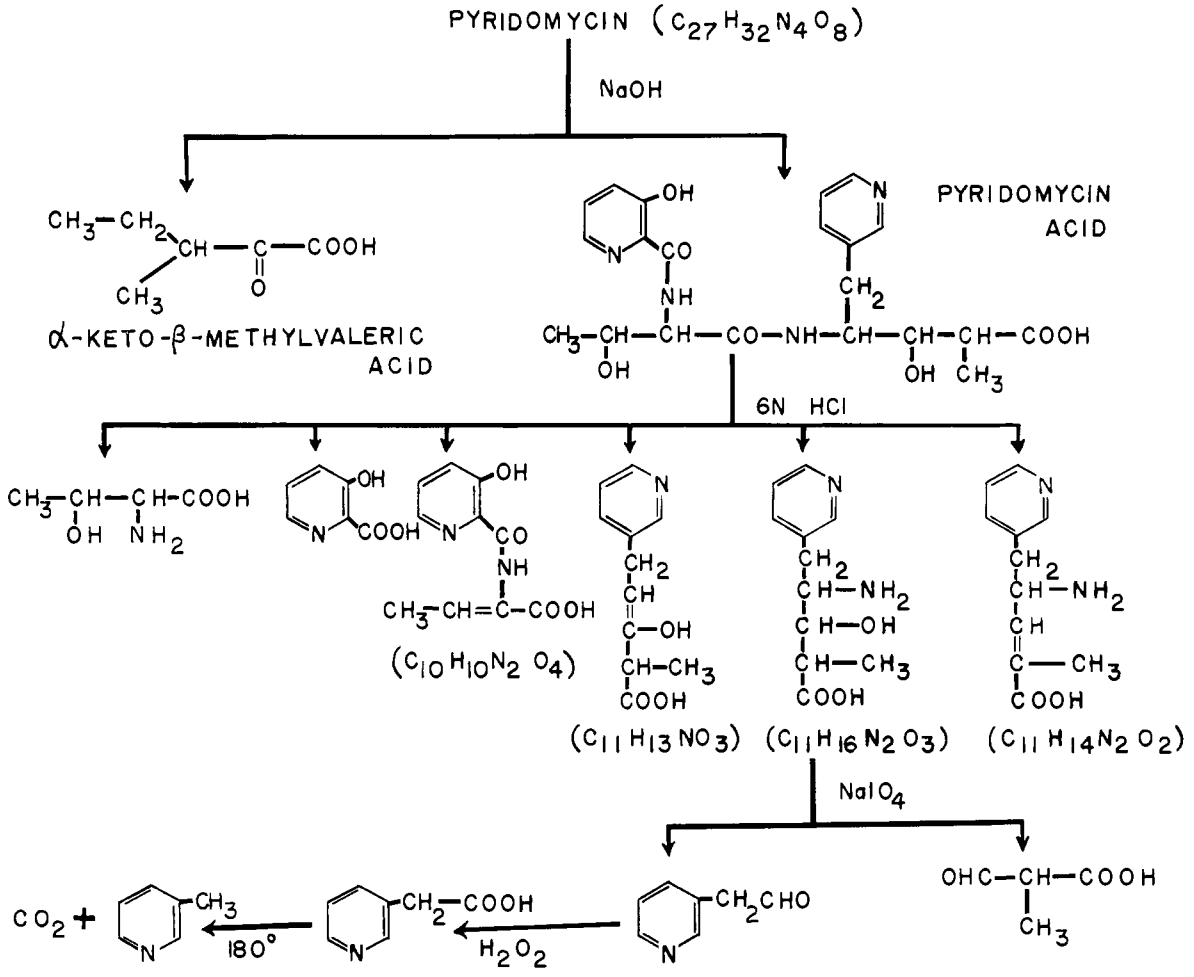
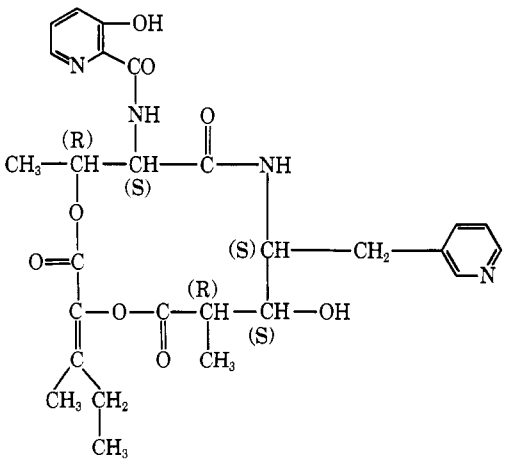


FIGURE 1: The degradation procedure of pyridomycin.

ridine rings of pyridomycin by *S. pyridomyceticus*. The results described in this paper showed that an aspartate–glycerol and/or aspartate–pyruvate pathway was the biosynthetic route of 3-hydroxypicolinic acid and an aspartate–glycerol pathway was the biosynthetic route of the pyridine ring of the C_{11} moiety of pyridomycin in this organism.



pyridomycin

The structure of the C_{11} compound ($C_{11}H_{16}N_2O_3$, Figure 1) also aroused the authors' interest in its biosynthesis. It can be divided into three parts: pyridine ring, alanine, and C_3-terminal part. Incorporation studies showed that the C_3-terminal part was derived from propionic acid and the alanine part was derived from pyruvic acid, but not from alanine. This is the first report describing the participation of propionic acid in the biosynthesis of an amino acid.

Experimental Procedures

Materials. L-[U-^{14}C]Threonine, L-[U-^{14}C]serine, DL-[1-^{14}C]glutamic acid, [^{14}C]sodium formate, and L-[U-^{14}C]lysine were obtained from The Dai-ichi Chemicals Co. (Tokyo, Japan) and L-[U-^{14}C]isoleucine, DL-[*bz*-U-^{14}C]tryptophan, DL-[1-^{14}C]2-aminoadipic acid, L-[U-^{14}C]proline, [2-^{14}C]sodium propionate, [2-^{14}C]sodium pyruvate, L-[U-^{14}C]aspartic acid, L-[U-^{14}C]methionine, [1-^{14}C]glycerol, L-[U-^{14}C]phenylalanine, and L-[U-^{14}C]alanine were purchased from The Radiochemical Centre (Amersham, Buckinghamshire, England). [U-^{3}H]3-Hydroxypicolinic acid (mp 215–216°) and [U-^{3}H]3-hydroxyanthranilic acid (mp 243° dec) were prepared from nonradioactive compounds by tritiation.

TABLE I: Ultraviolet Absorption of Degradation Products of Pyridomycin.

Compound	Solvent	λ_{max} (mμ)	$E^{1\%}_{1\,cm}$
α-Keto-β-methylvaleric acid 2,4-dinitrophenylhydrazone	Ethanol	372	795
Pyridomycin acid	0.08 N HCl	306	312
3-Hydroxypicolinic acid	H_2O	304	642
$C_{10}H_{10}N_2O_4 \cdot HCl \cdot 2H_2O$	H_2O	318	310
$C_{11}H_{13}NO_3 \cdot HCl$	H_2O	262	127
$C_{11}H_{16}N_2O_3 \cdot 2HCl \cdot H_2O$	H_2O	262	154
$C_{11}H_{14}N_2O_2 \cdot 2HCl$	H_2O	262	167

Each of the products thus obtained gave one spot when subjected to thin-layer chromatography, and identities were confirmed by mixture melting point determinations and infrared absorption spectra. The soybean meal (Prorich) which was used as the nitrogen source in the fermentation medium was purchased from The Ajinomoto Co. (Kawasaki, Japan) and had the following amino acid composition:[1] lysine, 3.22% (w/w); histidine, 1.216%; arginine, 3.688%; aspartic acid, 6.185%; threonine, 2.007%; serine, 3.329%; glutamic acid, 9.360%; proline, 3.427%; glycine, 2.130%; alanine, 2.097%; cystine, 0.907%; methionine, 0.685%; valine, 2.676%; isoleucine, 2.742%; leucine, 3.933%; tyrosine, 1.624%; phenylalanine, 2.521%; and tryptophan, 0.702%.

Fermentation Procedure. S. pyridomyceticus was grown on slant agar composed of glucose, 1.0%; sodium asparaginate, 0.1%; asparagine, 0.05%; $CaCO_3$, 0.05%; $MgSO_4 \cdot 7H_2O$, 0.05%; K_2HPO_4, 0.1%; and agar, 1.5%. After 10–20 days of growth at 27°, a seed culture of *S. pyridomyceticus* was prepared in a medium consisting of glucose, 3.0%; soybean meal (Prorich), 1.5%; NaCl, 0.25%; KCl, 0.05%; $MgSO_4 \cdot 7H_2O$, 0.05%; K_2HPO_4, 0.3%; $Na_2HPO_4 \cdot 12H_2O$, 0.3%; and silicone (Shin Etsu), 0.02%. The pH was adjusted to 6.4 with 1 N hydrochloric acid prior to sterilization. The cultures were incubated at 27° for 40–48 hr on a reciprocative shaker. A fermentation medium which had the same composition as that of seed culture was inoculated at a rate of 2% (v/v) with the 40–48-hr seed culture. Each 500-ml Sakaguchi flask contained 100 ml of medium and was incubated at 27° on a reciprocative shaker (120 rpm, 8-cm stroke). After 8- or 24-hr incubation, 0.1 ml of water solution containing 5–10 μCi of ^{14}C or ^{3}H compounds was added. Fermentation beers were harvested at 48 hr. All isotope incorporation experiments were carried out under these conditions.

Isolation of Pyridomycin. Fermentation beers were extracted with two 100-ml portions of butyl acetate. The combined extract was concentrated to dryness under reduced pressure. This residue was then dissolved in 2 ml of a benzene and methanol mixture (30:1, v/v) and the solution was put on a column of Mallinckrodt silicic acid (silic AR, Lot PLR, 100 mesh, 2 × 20 cm). The column was eluted with benzene–methanol (30:1, v/v), and the eluate was cut into 5-ml fractions. One fraction which inhibited growth of *Mycobacterium* 607 yielded pure pyridomycin after evaporation. The following criteria were used to characterize pyridomycin. (1) Thin-layer chromatography: Pyridomycin was spotted on Eastman chromatogram sheet (type K301R2), and developed with 1-butanol–acetic acid–water (3:1:1). Pyridomycin, which showed an R_F value of 0.25, was detected by ultraviolet light absorption, by spraying the dried sheet with 3% $FeCl_3$, and by the inhibition activity against *Mycobacterium* 607. (2) Spectrophotometry: Pyridomycin showed a maximum absorption at 305 mμ with $E^{1\%}_{1\,cm}$ 177 in ethanol. Optical measurements were performed using the Hitachi Model EP-2 recording spectrophotometer. The elemental analysis of a sample of recrystallized pyridomycin gave the following. *Anal.* Calcd for $C_{27}H_{32}N_4O_8$: C, 59.99; H, 5.97; N, 10.37; O, 23.67; mol wt, 540.56. Found: C, 59.55; H, 6.14; N, 10.13; O, 23.50; mol wt (mass spectrum, M^+), 540. Pyridomycin was quantitatively determined by the disk-plate method (de Beer and Sherwood, 1945) using *Mycobacterium* 607 (Gottlieb *et al.*, 1951) as a test organism. Only about 10–30% of the pyridomycin in the medium could actually be isolated and all isotope incorporation values were normalized based on the amount of pyridomycin as determined by the disk-plate assay.

Degradation of Pyridomycin. Pyridomycin was degraded according to the procedure illustrated in Figure 1. A mixture of radioactive pyridomycin isolated from 100 ml of the medium and 10 mg of cold pyridomycin was hydrolyzed in 2 ml of dioxane and 0.5 ml of 1 N sodium hydroxide overnight at room temperature. The hydrolysate was lyophilized and dissolved in 3 ml of water, acidified with 1 ml of 1 N hydrochloric acid, and extracted with five 4-ml portions of ether. From the extract, α-keto-β-methylvaleric acid was obtained. α-Keto-β-methylvaleric acid thus obtained was quantitatively determined by the optical density of its 2,4-dinitrophenylhydrazone. It showed a maximum absorption at 372 mμ with $E^{1\%}_{1\,cm}$ 795 in ethanol, and was assayed for radioactivity after being dissolved in methanol. From the water layer, pyridomycin acid was obtained. Pyridomycin acid was further hydrolyzed with 1 ml of constant-boiling hydrochloric acid at 105° for 16 hr in a sealed tube. The hydrolysate was adsorbed on a column of Dowex 50W-X8 (200–400 mesh, H^+ form, 2 × 20 cm) and the column was eluted initially with 100 ml of 1 N hydrochloric acid, and then with a linear gradient (1 N to 4 N hydrochloric acid, each 500 ml). The eluate was cut in 10-ml fractions and detected by absorption at 253.7 mμ (Uvicord, type 4701A, LKB-Produkter) and by reactions with ferric chloride and ninhydrin on thin-layer sheets. Each fraction was concentrated, dried under reduced pressure for 24 hr, and dissolved in water. The radioactivity and the concentration were measured. L-Threonine was determined by the ninhydrin reaction (Yemm and Cocking, 1955).

[1] The data of The Ajinomoto Co.

TABLE II: Incorporation of Various Substrates into Pyridomycin by Cultures of *S. pyridomyceticus*.

Compound	Sp Act. of Substrate (mCi/mmole)	Time of Addn (hr)	Amount Added (cpm)	Sp Act. of Pyridomycin (cpm/mmole)	% Incorp into Pyridomycin
L-[U-^{14}C]Isoleucine	8.7	24	6.50×10^6	2.65×10^7	1.85
L-[U-^{14}C]Threonine	3.5	24	7.98×10^6	4.00×10^7	2.32
L-[U-^{14}C]Aspartic acid	143.0	8	1.72×10^7	1.27×10^7	0.38
		24	5.28×10^6	*a*	0
[1-^{14}C]Glycerol	4.7	8	1.43×10^7	3.96×10^7	0.95
		24	8.77×10^6	8.28×10^4	0.008
[U-^{3}H]3-Hydroxypicolinic acid	13.0	24	1.42×10^{7b}	6.06×10^{7c}	1.18
[2-^{14}C]Sodium propionate	9.9	8	1.04×10^7	3.68×10^8	24.6
		24	1.20×10^7	5.99×10^6	0.31
[2-^{14}C]Sodium pyruvate	10.0	8	1.20×10^7	2.55×10^7	0.83
		24	1.11×10^7	3.91×10^5	0.02

[a] Within the error. [b] Disintegrations per minute. [c] Disintegrations per minute per millimole.

Other degradation products were determined quantitatively by their optical densities (Table I).

An aqueous solution of $C_{11}H_{16}N_2O_3$ in 1 ml of 0.1 M sodium periodate was allowed to stand overnight at room temperature. The solution was acidified with 0.5 ml of 1 N hydrochloric acid and extracted with ether five times, then made alkaline with 1 ml of 1 N sodium hydroxide and extracted with ether five times. Both extracts were concentrated, dissolved in methanol, and assayed for radioactivity.

3-Pyridylacetaldehyde obtained by the periodate oxidation was dissolved in a mixture of 3% aqueous hydrogen peroxide solution and 1 N sodium hydroxide (each 1 ml) and was allowed to stand at 65° for 20 min. After the reaction mixture was dried under vacuum, 3-pyridylacetic acid thus obtained was heated at 180° for 1 hr under nitrogen. β-Picoline was obtained from the residue by ether extraction and carbon dioxide was collected as the salt of (*p*-diisobutylcresoxyethoxyethyl)-dimethylbenzylammonium hydroxide (Hyamine 10X hydroxide). Details of the degradation studies and the structure determination of pyridomycin were published elsewhere (Ogawara *et al.*, 1968).

Radioactivity Measurement. The radioactivity of all ^{14}C and ^{3}H compounds was determined by Beckman liquid scintillation counter LS-200B. Test solutions of 1 ml each were added to 5 ml of scintillation fluid which was made from 3 g of 2,5-diphenyloxazole, 50 g of naphthalene, and 500 g of dioxane. In the cases of β-picoline and Hyamine 10X carbonate, 2-ml test solutions were added to 10 ml of scintillation fluid which was made from 2 g of 2,5-diphenyloxazole, 0.2 g of 1,4-bis[2-(5-phenyloxazole)]benzene, and 1000 ml of toluene. The figures listed in Tables II and III were calculated from the average of two 10-min counts. The radioactivity of C_{11} compounds was the average of those of three C_{11} compounds. The efficiency of the counter for ^{14}C was approximately 88% and for ^{3}H was 50%. In the case of ^{3}H, the detected counts were corrected. The percentage incorporation of radioactive compounds into pyridomycin was calculated from the amount of isotope added, the specific activity of pyridomycin isolated, and the amount of pyridomycin produced in the medium.

Results

Fermentation. The time course of pyridomycin synthesis and concentrations of reducing sugar and amino nitrogen in shaking cultures of *S. pyridomyceticus* was tested. Pyridomycin was produced after 24-hr fermentation and the maximum potency was reached at 48 hr.

Derivation of α-Keto-β-methylvaleric Acid Moiety of Pyridomycin from L-Isoleucine. Tracer studies using L-[U-^{14}C]isoleucine (Table II) indicated that this amino acid was readily incorporated into pyridomycin. The location of label in pyridomycin formed from L-[U-^{14}C]-isoleucine was determined by the degradation procedure in which α-keto-β-methylvaleric acid and pyridomycin acid portions of pyridomycin were separated and assayed for radioactivity (Table III). It was shown that 85.2% of this radioactivity was located in the α-keto-β-methylvaleric acid moiety and 11.1% was present in the pyridomycin acid part. This result indicates that the α-keto-β-methylvaleric acid moiety of pyridomycin is derived from L-isoleucine. The low incorporation rate into pyridomycin (such as 1.85% as shown in Table II) is considered to be due to a large amount of nonradioactive isoleucine in the fermentation medium.

Derivation of L-Threonine Moiety of Pyridomycin from L-Threonine. The result shown in Table II indicated that radioactivity was incorporated from L-[U-^{14}C]threonine into pyridomycin. This radioactive pyridomycin (specific activity 4.00×10^7 cpm/mmole) was degraded chemically, separated into α-keto-β-methylvaleric acid, threonine, 3-hydroxypicolinic acid, 3-hydroxypicolinyl-L-threonine, and three C_{11} compounds (Figure 1), and

TABLE III: Distribution of Radioactivity in Pyridomycin Synthesized from Different Precursors.

Source of Radioactivity	Time of Addn (hr)	Distribution(%) of Radioactivity in Pyridomycin: α-Keto-β-methylvaleric Acid	L-Threonine	3-Hydroxypicolinic Acid	C_{11} Compound
L-[U-^{14}C]Isoleucine	24	85.2	*a*	*a*	*a*
L-[U-^{14}C]Threonine	24	25.0	68.7	*b*	*b*
L-[U-^{14}C]Aspartic acid	8	11.8	11.8	15.8	60.0
[1-^{14}C]Glycerol	8	*b*	*b*	19.5	80.3
	24	*b*	89.8	10.1	*b*
[U-^{3}H]3-Hydroxypicolinic acid	24	*b*	*b*	100.0	*b*
[2-^{14}C]Sodium propionate	8	*b*	0.1	1.5	98.2
	24	*b*	*b*	*b*	94.0
[2-^{14}C]Sodium pyruvate	8	53.8	20.6	11.5	13.0

[a] Not determined. [b] Within the error.

assayed for radioactivity and concentration. The specific activity of the L-threonine portion was 68.7% of the specific activity of the pyridomycin, and 25.0% of the specific activity of pyridomycin was recovered from α-keto-β-methylvaleric acid (Table III). 3-Hydroxypicolinic acid and three C_{11} compounds showed practically no radioactivity. The radioactivity in α-keto-β-methylvaleric acid can be explained by the known pathway from L-threonine to L-isoleucine as follows: L-threonine → α-ketobutyric acid → α-keto-β-methylvaleric acid → L-isoleucine. In support of this assumption, L-threonine is known to be effectively incorporated into the L-isoleucine part of actinomycin (Katz, 1967).

Biosynthesis of Two Pyridine Nuclei of Pyridomycin. Incorporation experiment with L-[U-^{14}C]aspartic acid (Table II) showed that the radioactivity was not incorporated into pyridomycin when it was added to 24-hr culture medium, but when it was added to 8-hr culture medium, the radioactivity was effectively incorporated into pyridomycin, which had specific activity of 1.27×10^7 cpm/mmole. Chemical degradation of this radioactive pyridomycin gave α-keto-β-methylvaleric acid, threonine, 3-hydroxypicolinic acid, 3-hydroxypicolinyl-L-threonine, and three C_{11} compounds (Figure 1). Radioactivity in pyridomycin was distributed in the following ratio (Table III): α-keto-β-methylvaleric acid, 11.8%; threonine, 11.8%; 3-hydroxypicolinic acid, 15.8%; and C_{11} compounds, 60.0%. Further degradation of a C_{11} compound ($C_{11}H_{16}N_2O_3$) by periodate oxidation gave aldehyde acid and aldehyde pyridine. Radioactivity was exclusively located in the latter compound. These results indicated that both nuclei of 3-hydroxypicolinic acid and C_{11} compound were derived from L-aspartic acid. Pyridine nuclei in pyridomycin seem to be synthesized at the early stage of fermentation. This conclusion was also supported by the experiment using [1-^{14}C]glycerol, which showed that very little radioactivity was found in pyridomycin when the labeled compound was added to 24-hr culture medium, but when the labeled compound was added to 8-hr culture flask, radioactivity of [1-^{14}C]glycerol was effectively incorporated into pyridomycin. This pyridomycin, of specific activity 3.96×10^7 cpm/mmole, was degraded and separated. No radioactivity was detected in α-keto-β-methylvaleric acid and L-threonine thus obtained, but 19.5% of the label was found in 3-hydroxypicolinic acid and 80.3% was detected in C_{11} compounds (Table III). By further degradation of a C_{11} compound ($C_{11}H_{16}N_2O_3$) by periodate oxidation, it was found that almost all the radioactivity (95%) was located in aldehyde pyridine. Tracer studies with [2-^{14}C]sodium pyruvate (Table III) verified that sodium pyruvate was also incorporated into 3-hydroxypicolinic acid and C_{11} compounds. However, in the case of C_{11} compound, radioactivity was only located at the γ-carbon of the side chain, which was proved by periodate oxidation followed by hydrogen peroxide oxidation and decarboxylation as described in the next section. The evidence presented above indicates that the pyridine nucleus of 3-hydroxypicolinic acid is biosynthesized from L-aspartic acid and a compound derived from glycerol and pyruvic acid, and the pyridine nucleus of C_{11} compound is derived from L-aspartic acid and glycerol.

The radioactivity of [U-^{3}H]3-hydroxypicolinic acid was effectively incorporated into pyridomycin. The label in pyridomycin was exclusively located in 3-hydroxypicolinic acid. In the cases of L-[U-^{14}C]lysine, DL-[1-^{14}C]2-aminoadipic acid, L-[U-^{14}C]proline, DL-[*bz*-U-^{14}C]tryptophan, DL-[1-^{14}C]glutamic acid, L-[U-^{14}C]-phenylalanine, and [U-^{3}H]3-hydroxyanthranilic acid, no radioactivity entered into pyridomycin.

Biosynthesis of the Side Chain of C_{11} Compound. The result of an experiment in which the labeled compound was added to 24-hr culture medium showed that radioactivity from [2-^{14}C]sodium propionate was incorporated into pyridomycin. Chemical degradation of the latter gave radioactive C_{11} compounds, and periodate oxidation showed that all the radioactivity was present

in the aldehyde acid. Neither L-[U-^{14}C]methionine nor [^{14}C]sodium formate was incorporated into pyridomycin. This indicates that the carbon skelton of sodium propionate is used for the biosynthesis of the $CH(CH_3)COOH$ of C_{11} compound. This fact was firmly supported by the data presented below, which showed that, when the labeled sodium propionate was added to the 8-hr culture flask, 24.6% of the added radioactivity was found in pyridomycin and the result of degradation studies demonstrated that almost all the radioactivity was located in aldehyde acid (Figure 1).

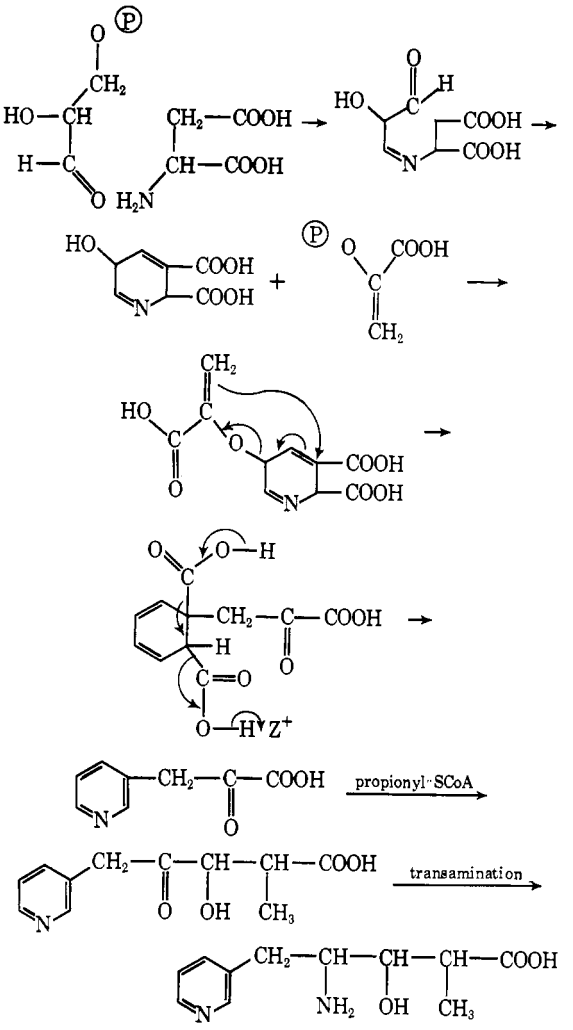
Tracer studies with [2-^{14}C]sodium pyruvate showed that the radioactivity was incorporated into pyridomycin. This pyridomycin was degraded chemically, separated, and assayed for radioactivity. In this case, considerable randomization occurred, but of the radioactivity in pyridomycin, 13.0% was recovered in C_{11} compounds, which were further degraded to aldehyde acid and aldehyde pyridine. The aldehyde acid was not radioactive. Radioactive aldehyde pyridine was oxidized with hydrogen peroxide and decarboxylated at 180° to obtain β-picoline and carbon dioxide. Only the latter compound was radioactive. Therefore, it was clarified that radioactivity from [2-^{14}C]sodium pyruvate was introduced into the γ-carbon of the side chain of C_{11} compound. From these results, it was concluded that the side chain of the C_{11} compound was biosynthesized from sodium propionate and sodium pyruvate. The radioactivity from L-[U-^{14}C]serine, [^{14}C]sodium formate, L-[U-^{14}C]methionine, and L-[U-^{14}C]alanine was not incorporated into pyridomycin.

Discussion

The pyridomycin structure containing the moiety of 3-hydroxypicolinyl-L-threonine is closely related to those of etamycin (Sheehan *et al.*, 1958) and mikamycin (Watanabe, 1960). In addition, it contains α-keto-β-methylvaleric acid and 4-amino-3-hydroxy-2-methyl-5-(3-pyridyl)pentanoic acid, and possesses one 12-membered ring containing two ester functions. Pyridine nuclei are contained in several natural products from *Streptomyces*, but there have been no studies to elucidate their biosynthetic pathways (Leete, 1965).

The results reported in this paper demonstrate that L-[U-^{14}C]aspartic acid and [1-^{14}C]glycerol are effectively incorporated into the two pyridine nuclei of pyridomycin. Low incorporation of [1-^{14}C]glycerol can be accounted for by the reason that it must be converted into glyceraldehyde 3-phosphate for the synthesis of the pyridine ring and also because it is used as a carbon source for the growth of the organism. Although randomization occurred in the case of sodium pyruvate, it was also incorporated into 3-hydroxypicolinic acid. Thus, 3-hydroxypicolinic acid was shown to be biosynthesized from aspartic acid and glycerol or pyruvic acid. It is possible that glycerol and pyruvic acid are converted into a compound which is used for synthesis of 3-hydroxypicolinic acid. The pyridine ring of the C_{11} moiety is biosynthesized from aspartic acid and glycerol. This is the first report concerning the biosynthesis of pyridine nuclei by *Streptomyces*. Although [U-

SCHEME I



^{3}H]3-hydroxypicolinic acid is readily incorporated into pyridomycin, neither DL-[*bz*-U-^{14}C]tryptophan nor [U-^{3}H]3-hydroxyanthranilic acid is incorporated. This suggests that 3-hydroxypicolinic acid, which is biosynthesized from aspartic acid and glycerol, participates directly in formation of pyridomycin.

The data described above clarify that the C_{11} compound in pyridomycin is biosynthesized from aspartic acid, glycerol, propionic acid, and pyruvic acid. Fleeker and Byerrum (1967) have suggested a possible pathway from L-aspartic acid and glyceraldehyde 3-phosphate to 2,3-dicarboxy-5-hydroxydihydropyridine and Leete (1963) has suggested a modus of decarboxylation of dihydropyridine-3-carboxylic acid. If we assume similar types of biosynthetic reactions of pyruvate and 2,3-dicarboxy-5-hydroxydihydropyridine as the reactions of shikimate and phosphoenolpyruvate to form chorismate, then the pathway (Scheme I) to C_{11} compound can be speculated. The participation of propionic acid is commonly found in the biosynthesis of macrolide antibiotics (Corcoran and Chick, 1966). In this respect, it is very interesting that propionic acid

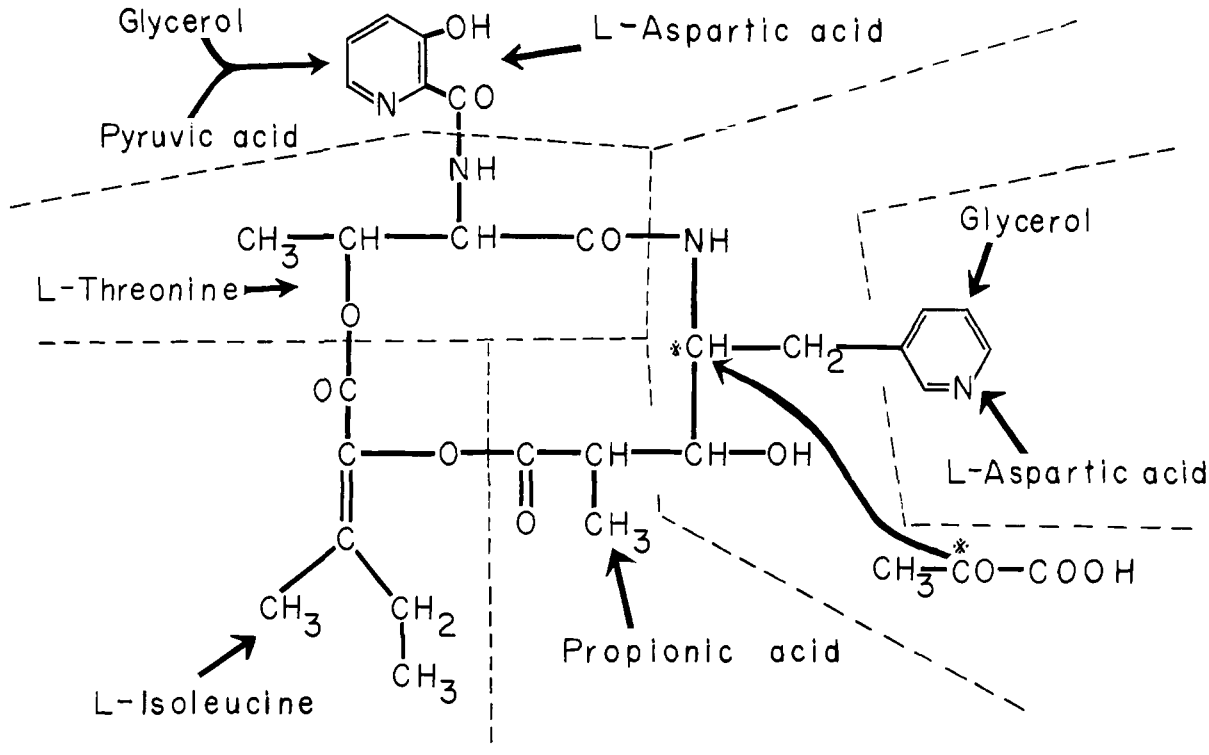


FIGURE 2: The biosynthetic pathway of pyridomycin.

participates also in the biosynthesis of an amino acid ($C_{11}H_{16}N_2O_3$).

The results of the study on the biosynthetic pathway of pyridomycin can be summarized as in Figure 2.

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