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Author(s)	Suzue, Ryokuero
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Biosynthesis of Pyridine Derivatives

Ryokuero SUZUE*

(Hayaishi Laboratory)

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This article is aimed to show how the hitherto unknown precursors of vitamin B₆, nicotine, ricinine and anabasine are discovered. Though the pathways of their biosyntheses remain unknown, that of nicotinamide-coenzyme is revealed. The mechanism of biosynthesis of the pyridine ring is that the carbonyl group reacts with the amino group in the molecule and so forms the pyridine ring nonenzymatically.

Since there are many biochemically important substances which contain the pyridine ring, the present paper aims to summarize the present status of the biosynthetic mechanism of the pyridine ring.

Many pyridine derivatives appear to enjoy ubiquitous distribution in animals, plants and microorganisms. Pyridine, known as the simplest six-membered heterocyclic compound containing nitrogen, is observed mostly in the distillation products of bone and coal. The formula of pyridine is as follows:

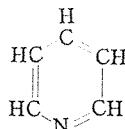


Fig. 1. Pyridine.

Pyridine can be prepared by cyclization of ethylallylamine followed by dehydrogenation.

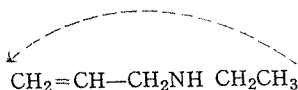


Fig. 2. Ethylallylamine.

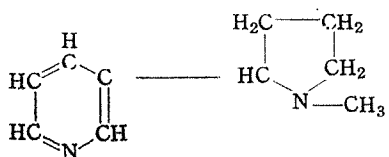
Excepting the considerable slowness in the reaction, pyridine shows chemical behaviors similar to those of benzene ring in halogenation, nitration and sulfonation.

The pyridine ring is found in a specific group of alkaloids. The group includes nicotine, which exists in tobacco leaf in combination with malic and citric acids. Nicotinic acid is obtained by oxidation of nicotine.

Nicotinamide, the amide of nicotinic acid, is biologically important, because it is the active group of pyridine nucleotide coenzymes.

* 鈴江 緑衣郎

Biosynthesis of Pyridine Derivatives



Nicotine (Methyl pyrrolidine-pyridine)

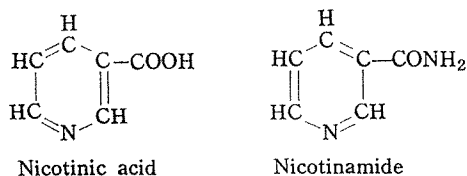


Fig. 3.

Vitamin B₆ is another important group of pyridine derivatives which includes pyridoxin, pyridoxal and pyridoxamine. These vitamins are growth factors for rats as well as for certain bacteria.

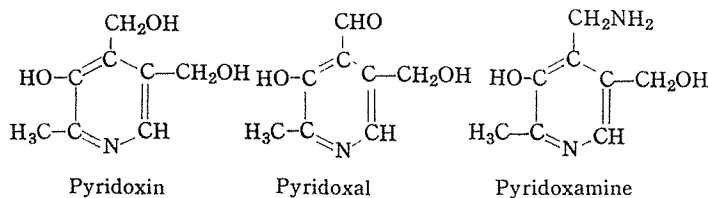


Fig. 4.

Some of the aromatic compounds bearing the hydroxy groups are cloven by microbial enzymes and then the formation of the nucleus of pyridine follows. (See Chapter 5)

Pipecolic acid, baikiaine, quvacine, etc. are all natural six-membered heterocyclic compounds containing a nitrogen atom¹³. Although these compounds have no pyridine nucleus in their molecules, the mechanism of biosynthesis is quite similar to that of the pyridine derivatives.

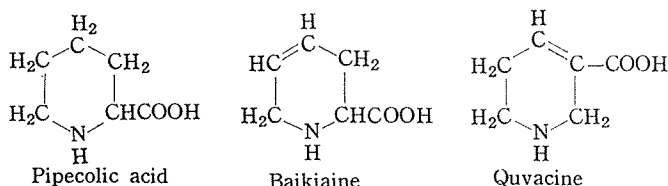


Fig. 5.

1. Mechanism of the Formation of Pyridine Ring

Most of cyclization reactions are due to the reaction of carbonyl group $>C=O$, i.e. aldehyde and ketone. These cyclization reactions are widely used in chemical syntheses

as well as in biosyntheses of the heterocyclic compounds. The main feature that determines the reactivity of the carbonyl group is its polarization, symbolized as $\text{>C}^{\delta+}=\text{O}^{\delta-}$. Thus there are two points of attack: a nucleophilic reagent will attack the electrophilic carbon atom, and an electrophilic reagent will attack the nucleophilic oxygen atom. In many cases, both processes occur.

Aldehydes and some of ketones react with amino compounds. There is a general type of reaction shown by the following equation:



Fig. 6.

In this case, the nucleophilic species, (--N--Y), reacts with the electrophilic carbon atom of the carbonyl group and the stable compound (>C=N--Y) is formed.

Two examples follow:

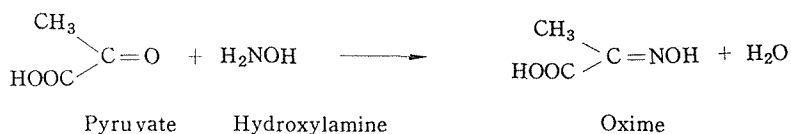


Fig. 7.

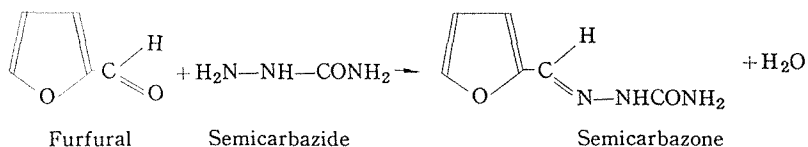


Fig. 8.

A characteristic feature of these reactions is that there is an optimum pH (around the physiological pH); the rate increases on lowering the pH from 7 to about 5, and then the rate drops again as acidity of the solution increases²⁾.

Many cyclization reactions in this manner occur in the biological system. L-glutamic γ -semialdehyde cyclizes to give Δ^1 -pyrroline-5'-carboxylic acid at physiological pH³⁾.

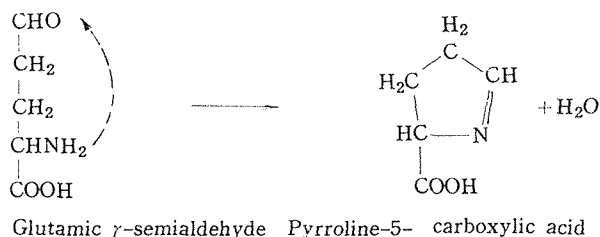


Fig. 9.

Most biosynthetic mechanisms of the pyridine ring seem to be the nonenzymatic reactions of the carbonyl and the amino group in the molecule^{4,5)}.

2. Biosynthesis of Nicotinic acid and its Derivatives

Mammals and *Neurospora* are able to convert tryptophan into nicotinic acid via kynurenine, 3-hydroxyanthranilic acid and quinolinic acid. However, in bacteria the biosynthesis of nicotinic acid is accomplished by a different pathway.

Snell⁶⁾, Stanier⁷⁾, and Yanofsky⁸⁾ reported that several bacteria could utilize neither tryptophan nor any of the intermediates in the tryptophan metabolism as a substitute for nicotinic acid as a growth factor. Ortega and Brown⁹⁾ investigated in an attempt to obtain evidence for the identity of the precursors of nicotinic acid in bacteria. The results suggested that the probable precursors of this vitamin in *E. coli* were a 4-carbon dicarboxylic acid and either glycerol or a compound to which glycerol could be easily converted. The evidence for this conclusion was provided by the finding that both succinic acid-C¹⁴ and glycerol-C¹⁴ were efficiently incorporated into the nicotinic acid molecule. Nicotinic acid, produced from glycerol-1,3-C¹⁴ and non-radioactive succinic acid, appeared to contain C¹⁴ only in the pyridine ring portion of the molecule. Nicotinic acid, produced from succinic acid-1,4-C¹⁴ and non-radioactive glycerol, contained C¹⁴ in the carboxyl group. Nicotinic acid, produced from succinic acid-2,3-C¹⁴ and non-radioactive glycerol, was labeled largely in the pyridine ring. Since Andreoli *et al.*¹⁰⁾ reported that quinolinic acid was a precursor to nicotinamide adenine dinucleotide (NAD) in *E. coli*, succinate and glycerol appeared to be possible precursors to quinolinic acid.

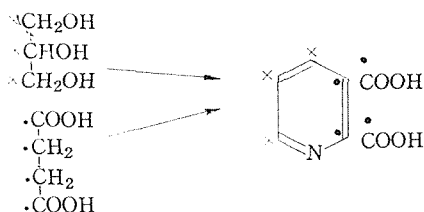


Fig. 10. Biosynthesis of nicotinic acid from glycerol and succinate.

NAD, (DPN), an important coenzyme of general utility, contains the heterocyclic bases, adenine and nicotinamide, and two molecules of D-ribose. The two component nucleotides are joined by a pyrophosphate bridge. There is a related component, which is also widely distributed in the biosphere, and only differs from the above substance by having an additional phosphate residue which is esterified at C-2' of the ribose molecule of adenosine. This substance is known as NADP (TPNH). Reversible reduction of the pyridine ring transforms NAD into the reduced nucleotide NADH⁺ and similarly into NADPH⁺.

In mammals, the biosynthetic pathways of NAD from tryptophan or from nicotinic acid are established as follows:

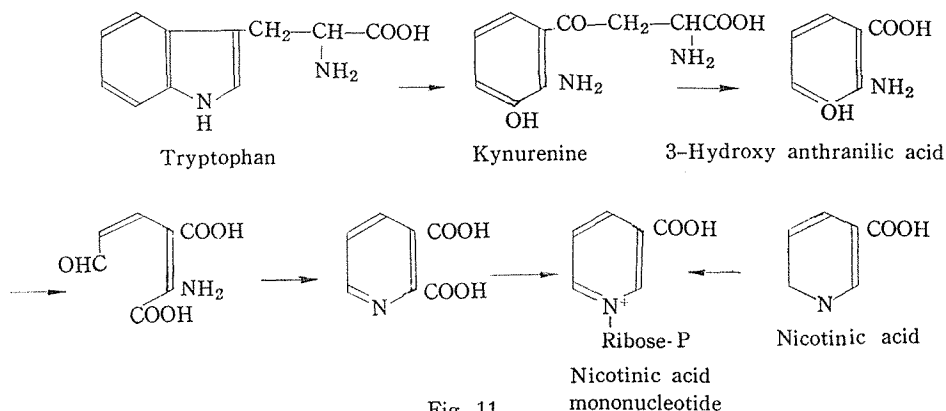


Fig. 11.

Tryptophan pyrrolase, the enzyme that converted tryptophan to formylkynurenine was purified 35-fold from a *Pseudomonas* sp. by Tanaka and Knox¹¹⁾. Recently, Feigelson *et al.*¹²⁾ showed that this enzyme was a heme-containing enzyme and cleared its reaction mechanism.

The formation of the pyridine ring was elucidated by Mehler *et al.*^{13,14)}. 3-Hydroxyanthranilic acid is oxidized to 1-amino-4-formyl butadiene-1:2 dicarboxylate. The aldehyde group and amino group are rearranged, and quinolinic acid is non-enzymatically formed. Three groups of workers have purified 3-hydroxyanthranilic acid oxidase extensively^{15,16,17)}.

In mammals^{19,20)} as well as bacteria¹⁰⁾ and plants^{18,19)} quinolinic acid is shown to be the universal intermediate in the biosynthesis of the pyridine ring compound, though it arises by different pathways in different organisms. Nakamura *et al.*²⁰⁾ found the quinolinate transphosphoribosylase and explained the mechanism of the formation of niacin ribonucleotide through quinolinic acid.

The pathways of the formation of NAD are summarized in the following figure.

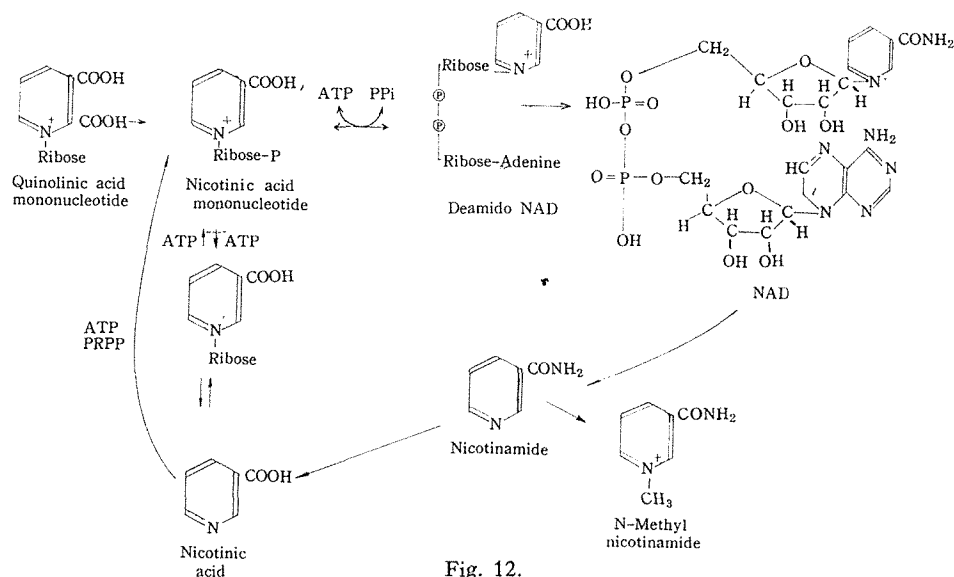


Fig. 12.

Nicotinic acid appears to be the direct precursor to NAD, and nicotinamide is not involved in this reaction. Imsande and Handler²¹⁾ have made a detailed study of the enzymes that convert nicotinic acid and 5-phosphoribosyl-1-pyrophosphate into deamido-NMN and PPI. They found that Mg^{++} was necessary and that ATP stimulated this reaction. They have also investigated the regulatory mechanism of pyridine nucleotide biosynthesis in *E. coli*²²⁾.

3. Biosynthesis of Nicotine, Ricinine and Anabesine

The biosynthetic pathways of nicotine, ricinine and anabesine are not clear yet. In order to elucidate the biosynthetic pathway of nicotine, several tracer experiments *in vivo* have been made with *Nicotiana rustica*²³⁾. Both glycerol-2- C^{14} and aspartic-3- C^{14} were shown to be incorporated into the pyridine ring, whereas propionate-3- C^{14} was not. Glycerol-2- C^{14} was incorporated into the pyridine ring to about the same extent as glycerol-1,3- C^{14} . Partial degradation of the pyridine ring of nicotine from plants which were fed aspartic acid-3- C^{14} , revealed that aspartic acid was not converted directly to the ring, since C^{14} was located in more than a single position in the pyridine ring^{23,24)}.

Ricinine (*N'*-methyl-3-cyano-4-methoxy-2-pyridone), is an alkaloid which is produced in the castor plant, *Ricinus communis L.* It has been shown to be risen from nicotinic acid-7- C^{14} ²⁵⁾. The carbon 14 was located in the cyano group. Studies on the incorporation of succinate, propionate, acetate, glycerol and β -alanine showed the following order of efficiency as precursors of ricinine: succinate, propionate, β -alanine. Acetate and glycerol were incorporated into the pyridine compounds approximately to the same extent. All of the radioactivity in ricinine produced in the presence of succinate-2,3- C^{14} was found in the pyridine ring. The radioactivity in the alkaloid formed in the presence of succinate-1,4- C^{14} was located 75 % in the pyridine ring and 25 % in the cyano group.

When glycerol-2- C^{14} was administered to *Nicotiana glauca* plants, the radioactive anabesine produced, had 38 % of the original radioactivity located in the pyridine ring^{26,27)}. In addition, anabesine derived from glycerol-2- C^{14} was found to have substantial activity at C-2, C-3 and C-5, but only low activity at C-4 and C-6. These results were consistent with the hypothesis that nicotinic acid, the precursor of the pyridine ring of anabesine, was formed from glycerol and succinate or closely related metabolites in *Nicotiana* species.

4. Biosynthesis of Vitamin B₆

This vitamin is a component of the vitamin B complex and cures acrodynia (a pellagra like dermatitis) in rat and deficiency dermatitis in other animals. The structure of vitamin B₆ is 2-methyl-3-hydroxy-4,5-bis(hydroxymethyl) pyridine and this is given the trivial name pyridoxin. Snell and Guirard²⁸⁾ discovered two closely related compounds during their work on the role of pyridoxin in microbial nutrition; these are the corresponding aldehyde, (pyridoxal) and the amine, (pyridoxamine). The main metabolically active form of these compounds is pyridoxal phosphate, which is a

coenzyme for amino acid decarboxylase, transaminase, racemase, etc. Pyridoxamine phosphate can act as the coenzyme for transaminase.

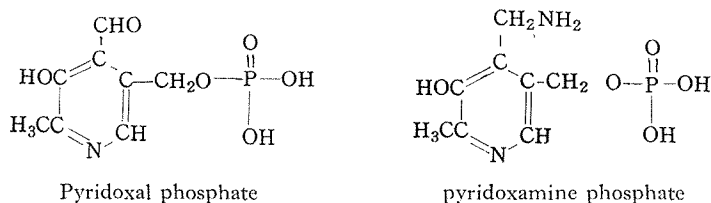


Fig. 13.

Little is known about B₆ synthesis in bacteria. It was considered for some time that D-alanine, which plays the role of pyridoxin in the nutritional requirement of certain *Lactobacilli*, might be a precursor of this vitamin²⁹). But it was later revealed that pyridoxin was concerned with the synthesis of D-alanine which was an essential component of the cell wall of many bacteria^{30,31}). By testing the effects on growth of varying ingredients of the media, Wood and Morris^{32,33}) concluded that serine, glycine and glycolaldehyde were possible precursors in the biosynthesis of B₆ in *E. coli*. However, no isotope experiment has been performed to confirm the hypothesis.

Lunan and West³⁴) isolated the C¹⁴-labeled pyridoxamine from *Candida utilis*. Serine-3-C¹⁴, alanine-1-C¹⁴, acetate-1-C¹⁴ and acetate-2-C¹⁴ gave rise to C¹⁴ labeled pyridoxamine. But the observed extents of incorporation were too low to permit a definitive interpretation of these results in terms of the biosynthesis of vitamin B₆.

The author isolated two strains of B₆ biosynthesizing bacteria from soil. One strain of them belongs to *Klebsiella* and the other belongs to *Flavobacterium*^{35,36}). The production of B₆ by resting cells of these bacteria was increased by the addition of glycerol, and either L-leucine or L-aspartic acid. Maximal synthesis was achieved in the presence of glycerol and leucine or glycerol and aspartic acid. The carbon chains of both glycerol-C¹⁴ and leucine-U-C¹⁴ were efficiently incorporated into newly synthesized pyridoxal by the resting cells, while the radioactivity of leucine-1-C¹⁴ was not. B₆, which was produced by cells incubated with glycerol-2-C¹⁴ and non radioactive aspartic acid, was labeled largely in the pyridine ring. From these results it may be concluded that in the bacteria, B₆ is probably derived from a 3-carbon compound (either glycerol or a compound related metabolically to glycerol), and from a 4-carbon compound which is related metabolically to leucine or aspartic acid (for instance, β-hydroxy-β-methyl-glutaryl CoA), and also is concluded that these compounds will react with NH₄ ion resulting in the formation of the pyridine ring^{37,38,39}).

Biosynthesis of Pyridine Derivatives

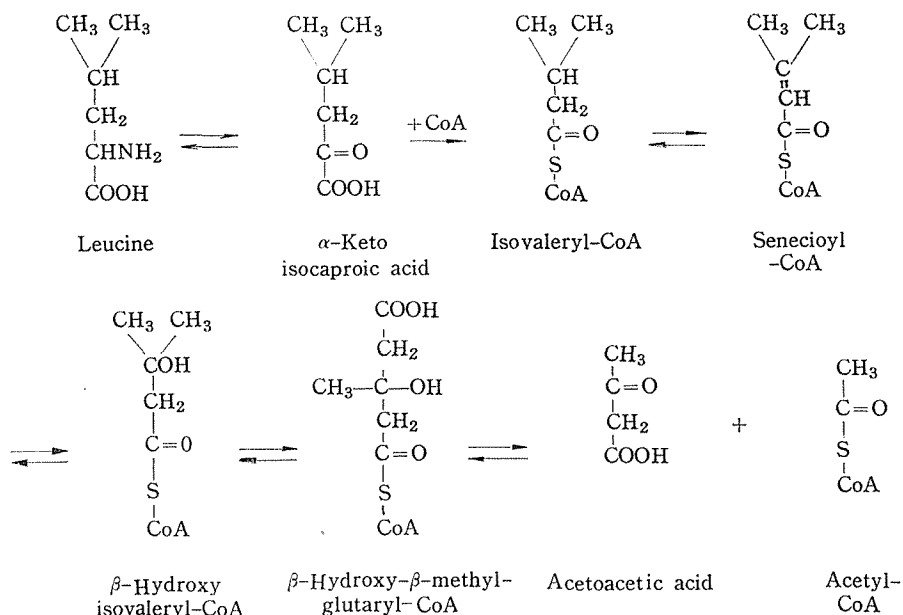


Fig. 14. Metabolic pathway of leucine.

Although pyridoxin is nutritionally active for animals and some microorganisms, it is metabolically inactive as a coenzyme. Enzymatic phosphorylation must occur prior to its utilization. Pyridoxal kinase, the enzyme, which catalyzes the phosphorylation of pyridoxal, has been demonstrated in *Streptococcus faecalis*⁴⁰⁾, *Escherichia coli* and yeast⁴¹⁾, liver^{42,43)}, brain⁴⁴⁾, kidney and muscle^{45,46)}. All the enzymes carry out the same reaction:



5. The Formation of the Nucleus of Pyridine as a Consequence of Oxidation of Benzene⁴⁷⁾

The cell-free extract of *Pseudomonas* oxidizes one mole of protocatechuate (I) with uptake of one mole of oxygen and produces a white, crystalline, dibasic acid, 2,4-luti-dinic acid (IV)⁴⁸⁾. The initial product (II) formed from protocatechuate has a maximum absorption spectrum at 410 $m\mu$. When, however, dilute ammonia is used, this yellow color vanishes within a few minutes and a new compound (IV) is formed with spectrographic and chromatographic properties that are identical with those of 2,4-luti-dinic acid. It is clear that this compound is formed by a nonenzymic reaction between α -hydroxy- γ -carboxy muconic semialdehyde (II), the initial product of ring fission, and the ammonium ion presenting in the enzyme solution^{49,50)}.

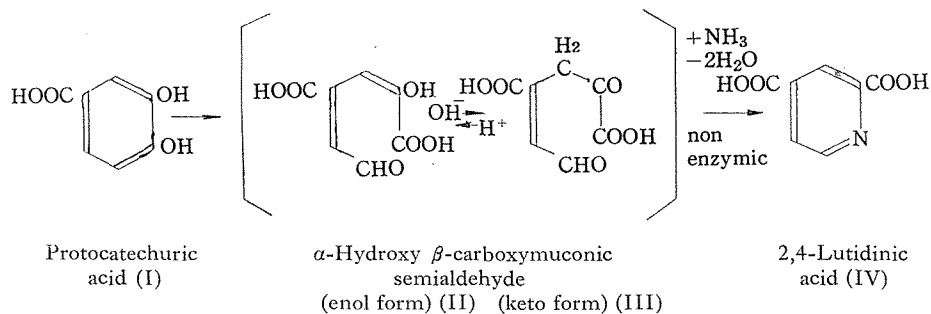


Fig. 15.

The cell-free extract of a strain of gram-negative bacterium oxidizes one mole of catechol (V) with uptake of one mole of oxygen. During the reaction a transient yellow color (VI) is produced, whose peak appears at 373 $m\mu$ in neutral or alkaline solution. α -picolinic acid (VIII) is formed after being stood with α -hydroxymuconic semialdehyde (VI) and ammonium hydroxide for two days at room temperature.

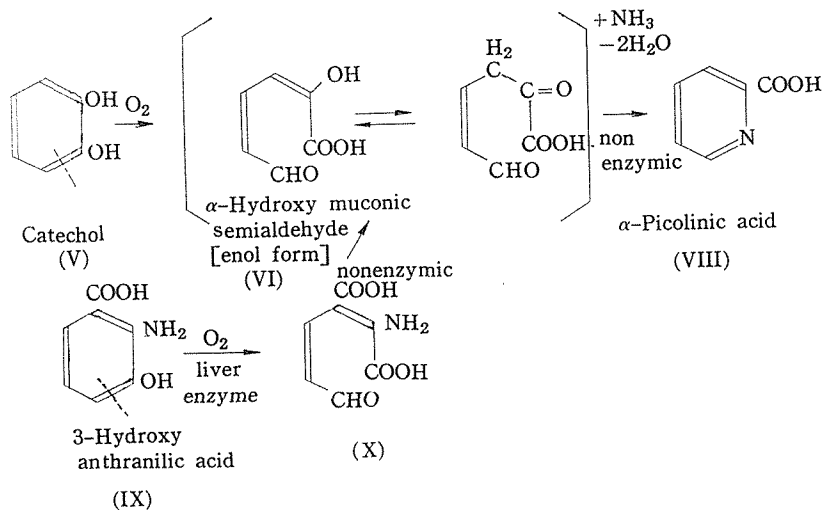


Fig. 16.

Recently Kita and Senoh⁵¹⁾ isolated new pyridine derivatives from *p*-hydroxyphenyl acetate (XI) and identified the products as picolinic-5-acetate (XIV) and picolinic-4-acetate (XVI). The cell-free extract of *Pseudomonas ovali* attacks *p*-hydroxyphenyl acetate and the resulting products (XIII, XV) form pyridine rings in the ammonium-acetate solution after being stood at room temperature for four hours.

Biosynthesis of Pyridine Derivatives

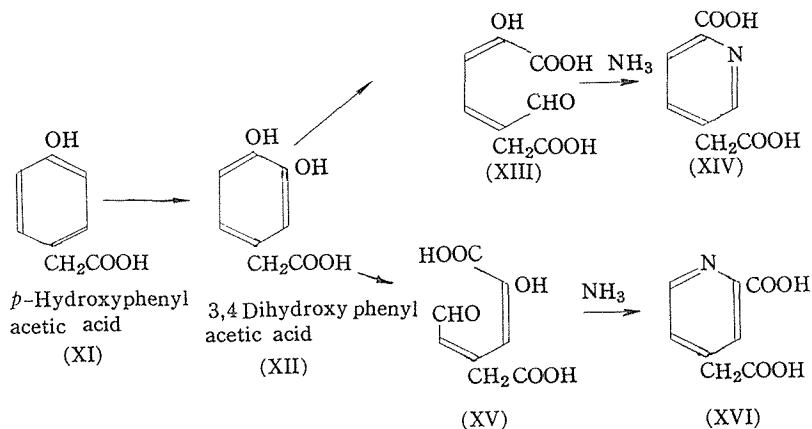


Fig. 17.

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