Chapter 13
MICROBIAL PRODUCTION OF VITAMIN B₆ AND DERIVATIVES

Y. TANI
Research Center for Cell and Tissue Culture, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan

1 INTRODUCTION

In 1934, vitamin B₆ (or pyridoxine) was discovered by György as a rat pellagra preventive factor. Soon after the determination of its chemical nature the total synthesis of the vitamin was accomplished. Moreover, clinical investigations have clarified that the vitamin has therapeutic and prophylactic uses for many diseases. Since microbiological assays pointed towards several forms of the vitamin B₆-active compound the enzymatic conversion of various forms of vitamin B₆ into pyridoxal 5'-phosphate (pyridoxal-P), the active form of the vitamin in vivo, has been intensively investigated. Pyridoxine, pyridoxamine, pyridoxal and their 5'-phosphate esters are now recognized as principal vitamin B₆ compounds (Fig. 1).

Since Gunsalus & Bellamy first reported in 1944 that pyridoxal-P is the coenzyme of L-tyrosine decarboxylase, it has become evident that pyridoxal-P or pyridoxamine 5'-phosphate (pyridoxamine-P) is a coenzyme for various enzyme systems associated with the metabolism of amino acids, amines, saccharides, fatty acids, etc. A large number of vitamin B₆ enzymes has now been found, and the mechanisms of vitamin B₆ dependent reactions have been extensively investigated.

Several biotechnological methods for synthesis of vitamin B₆ compounds have been investigated and are described in this chapter. However, the industrial production is still limited to chemical synthesis processes for pyridoxine and pyridoxal-P.

2 CHEMISTRY AND ASSAY OF VITAMIN B₆

Pyridoxine (3-hydroxy-4,5-dihydroxymethyl-2-methylpyridine) exhibits the properties of a stable hydroxylated weak nitrogen base. Hydrolytic agents such
as mineral acids or aqueous alkali, hot or cold, do not affect the vitamin. With ferric chloride, pyridoxine reacts as a phenolic substance giving a reddish brown coloration. In alkaline solution, pyridoxine on treatment with 2,6-dichloroquinone chlorimide gives an immediate blue color fading to reddish-brown.

Pyridoxine hydrochloride occurs as white platelets, melting point 204-206°C with decomposition. The free base melts at 160°C. The compound is optically inactive. Both base and hydrochloride readily sublime without decomposition. The hydrochloride is freely soluble in water but sparingly in alcohol and acetone. The base is soluble in methanol. Rapid destruction of pyridoxine by light occurs in neutral and alkaline solutions. In 0·1 M hydrochloric acid there is very little destruction.

The tautomeric properties of pyridoxine are well illustrated by the changes in its UV absorption produced by varying the hydrogen ion concentration. The single maximum at 292·5 nm at pH 2 diminishes in intensity at pH 4·5, and concomitantly a new maximum appears at 327·5 nm. This latter band increases in intensity when the pH is changed to 6·75, and the 292·5 nm maximum disappears but a new band appears at 256·0 nm. When the pH is further raised to 10·2, both bands increase in intensity and shift to shorter wavelengths.

The existence of other forms of pyridoxine was recognized as a result of the comparison of microbiological assays on extracts of natural materials with the values based on chemical and animal assays.

When pyridoxine was treated with mild-oxidizing agents, a marked increase in bio-activity towards the micro-organism, *Lactobacillus casei*, was observed. Autoclaving of pyridoxine in the presence of the assay medium or amino acids, greatly increased the activity of pyridoxine towards the test organism, *Streptococcus faecalis* R. The products formed by treating pyridoxine with animating agents and mild-oxidizing agents were, respectively, the amino and aldehyde derivatives of pyridoxine. The compounds are pyridoxamine (2-methyl-3-hydroxy-4-aminomethyl-5-hydroxymethylpyridine) and pyridoxal (2-methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine).

The availability of pyridoxal in synthetic form permitted identification of