Kirk-Othmer Encyclopedia of Chemical Technology.

PYRIDOXINE (B_6)

Pyridoxine [65-23-6] (vitamin B_6) is the recommended IUPAC-IUB designation for a group of closely related, biologically interconvertible 3-hydroxy-2-methylpyridines that exhibit the biological activities of pyridoxine in rats (1). This group includes pyridoxine [65-23-6] 1, pyridoxal [66-72-8] 1, and pyridoxamine [85-87-0] 1 and their respective 5′-phosphates 1. In common usage, though, the term vitamin B_6 most often refers to pyridoxine hydrochloride [58-56-0] 1, the most important commercial form (Fig. 1). Contrary to previous proposals and explanations, pyridoxine is not to be used as a general name for this group of vitamers. Pyridoxine is the official name of the specific substance 5-hydroxy-6-methyl-3,4-bis(hydroxymethyl)pyridine 1 which usually comprises only a fraction of the total amount of vitamin B_6 in natural sources. The older term pyridoxol is seldom used.

Vitamin B_6 is widely distributed in small amounts in the tissues and fluids of nearly all living substances. Bound to enzymes as the active form, pyridoxal phosphate (2), it acts as a coenzyme for a number of biochemical conversions of amino acids important to general cellular energy supply, to growth, and to specific organ functions. Deficiency in animals leads to a variety of symptoms ranging from mild (dermatitis, loss of appetite, irritability, and muscular weakness) to serious (anemia, weight loss, and nerve degradation leading to convulsive seizures) and even death. Vitamin B_6 requirements depend on protein intake and other factors. Meats, cereals, beans, and nuts are rich sources. Bioavailability and stability vary with the food sources and methods of preparation and storage. As a preventive measure, pyridoxine hydrochloride, the most stable form, is used in food, feed, and nutrient supplements. Pyridoxine is also used therapeutically for treatment of deficiency conditions and other diseases. No natural sources are rich enough, nor is biosynthetic understanding sufficiently advanced, to allow cost-effective production from biological sources. All commercially available pyridoxine hydrochloride is manufactured by chemical processes practiced at fine chemical scale.

Vitamin B_6 was found in the 1940s during the unraveling of the mysteries of the nitrogen-base B vitamin complex. A diet-induced pellagra-like condition in rats was shown to be cured by a water-soluble base, later differentiated from the known vitamins B_1 and B_2 and named vitamin B_6 by Gyorgy in 1934. Several research groups undertook isolation and characterization of this substance and five different laboratories announced this achievement in 1938. The chemical structure of pyridoxine, the form first isolated, was correctly identified and reported along with two independent syntheses in 1939 (2, 3). Snell showed microorganisms that could not use pyridoxine were satisfied with the natural vitamin B_6 extracts. This led to the discovery and synthesis of the vitamers pyridoxal 1 and pyridoxamine 1 in 1944. Subsequent clinical and biochemical investigations have shown the vitamin B_6 complex has therapeutic and prophylactic uses for treatment of a number of diseases. Commercial production of pyridoxine hydrochloride began in the 1940s. Production rose rapidly to more than 3000 metric tons per year worldwide.

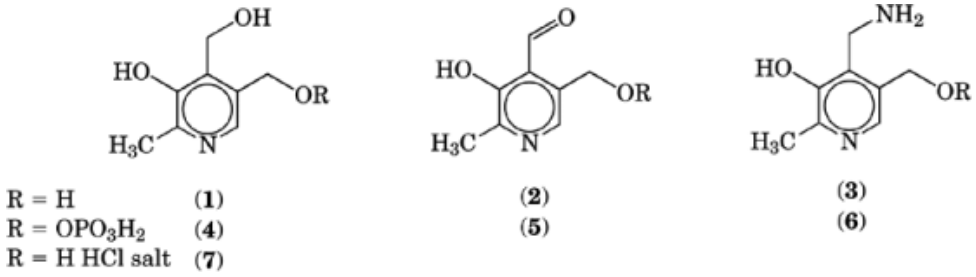

Fig. 1. Forms of vitamin B_6.

Table 1. Properties of Pyridoxine, Pyridoxamine, Pyridoxal, and Derivatives

Substance	Structure number	CAS Registry Number	Formula	Mol wt	Form	Mp in water, °C	Solubility, g/100 mL	p*K* values
pyridoxine	1	[65-23-6]	$C_8H_{11}NO_3$	169.18	needles	160	soluble	5.0, 9.0
pyridoxine HCl	1	[58-56-0]	$C_8H_{12}ClNO_3$	205.64	platelets	204–206 dec	22	
pyridoxine-5′-PO_4	1	[447-05-2]	$C_8H_{12}NO_6P$	249.16	needles	212–213 dec	soluble	5.0, 9.4
pyridoxamine	1	[85-87-0]	$C_8H_{12}N_2O_2$	168.20	crystals	193	soluble	3.4, 8.1, 10.5
pyridoxamine di-HCl		[524-36-7]	$C_8H_{14}Cl_2N_2O_2$	241.12	platelets	226–227 dec	50	
pyridoxamine-5′-PO_4	1	[529-96-4]	$C_8H_{13}N_2O_5P$	248.18			soluble	3.5, 5.8, 8.6, 10.8
pyridoxal	1	[66-72-8]	$C_8H_9NO_3$	167.16			50	4.2, 8.7, 13
pyridoxal HCl		[65-22-5]	$C_8H_9NO_3$	203.63	rhombic	173 dec	50	
pyridoxal-5′-PO_4	1	[54-47-7]	$C_8H_{10}NO_6P$	247.14			soluble	3.5, 8.4

1. Chemical and Physical Properties

1.1. Physical and Chemical Characterization

Ultraviolet absorptions of the B_6 vitamers vary with pH and the substituent at position 4. As hydroxypyridines, the B_6 vitamers show strong fluorescence (excitations at 310–330 nm, emissions at 365–425 nm, depending on structure and pH) which are useful in their detection (4, 5). Ir and ^1H, ^{13}C, and ^{15}N nmr show the expected effects of N-protonation, hydrogen bonding, and exchange of the acidic phenolic proton (6–8). In mass spectrometry (ms), normal electron impact ionization is sufficient to observe the molecular ion and an intense peak for a quinone–methide fragment from loss of water at the 4-hydroxymethyl position (9). Thermospray hplc/ms at picogram sample levels showed particularly strong molecular ions for pyridoxine and derivatives (10).

1.2. Reactions and Stability

The B_6 vitamers show weakly basic and acidic properties (4) and undergo reactions expected of their functionalities (11, 12). Acidity constants are reported in Table 1. Pyridoxine gives the characteristic reactions of phenols such as color tests, ether formation, diazo coupling, etc. Ketalization or acetalization of pyridoxine gives the thermodynamically more stable six-membered ring products involving the 3- and 4-positions. Esterification gives first mainly the 4,5-diester, then the triester with excess acylating agent. Pyridoxal and pyridoxamine are phosphorylated on the 5′-position with reagents such as phosphorus pentoxide in phosphoric acid or metaphosphoric acid. Pyridoxine requires prior protection of substituents at positions 3- and 4- as a ketal to allow selectivity. In biological systems, glycosylation occurs at the 5′-hydroxyl.

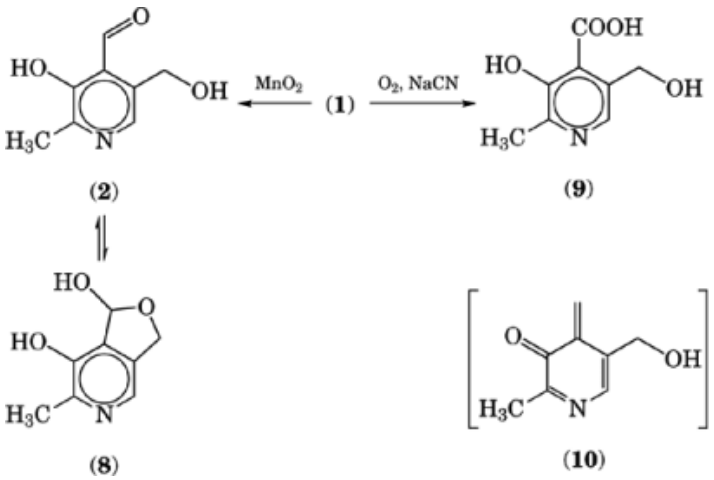

Fig. 2. Reactions of pyridoxal (**2**).

The B_6 vitamers are labile. Pyridoxine is the most stable and pyridoxal the least. The reactions of the substituents at the 4-carbon dominate. On mild oxidation, pyridoxine is converted to pyridoxal 1, which is in equilibrium with its hemiketal [17281-92-4] 2 (13). Air oxidation gives pyridoxic acid [82-82-6] 2, an intensely blue fluorescent substance of value for quantitation (4). Heating pyridoxine with ammonia converts it to pyridoxamine 1 and with alcohols to the 4-ethers (13). Pyridoxine, pyridoxal, and pyridoxamine are relatively stable in acid when cold or heated at 100°C for short periods. Prolonged heating of pyridoxine in hydrochloric acid gives the 4,5-bis-chloromethyl analogue, the 4-chloromethyl group of which is preferentially hydrolyzed. Heating pyridoxine in neutral solution in the absence of good nucleophiles gives dimeric products (14). Some of these reactions are readily rationalized by an intermediate quinone–methide 2 (Fig. 2).

Pyridoxal and its 5′-phosphate undergo typical aldehyde reactions, such as aldol condensations, bisulfite addition, etc (4, 11, 12). Both form imines, assisted by the electronegative 2-methylpyridine ring and the acidic 3-hydroxyl. Extensive kinetic and thermodynamic data on imine formation in model systems are available (4). The epsilon-amino group of a lysine unit is usually involved in the active sites of enzymes but other groups can bind pyridoxal during thermal processing and storage, reducing bioavailability (4, 15). Hydroxylamines and hydrazine derivatives, such as isoniazid, react and cause enzyme inhibition (4). Imine formation with pyridoxal is used as a tool for selective modification of proteins in biochemical studies (4).

Reactions of imines and their tautomers are the basis of the important biochemistry of pyridoxal 5′-phosphate 1, the coenzyme form for over 100 enzymatic reactions. Reaction with an epsilon-amino group of a lysyl residue and ionic interaction of the 5′-phosphate binds pyridoxal to the enzyme in the resting state. Displacement of lysine by a substrate amino group initiates a sequence of facile equilibrations of the aldimine, ketimine, and enamine forms, along with elimination and addition reactions which rationalize the various conversions and products. Known reactions involving the alpha-, beta-, and gamma-carbons of the amino acid and their substituents include decarboxylations, transaminations, racemizations, aldol and retroaldol reactions, eliminations, and hydration reactions (4) (Fig. 3). Many of these transformations have been modeled *in vitro* in chemical studies. Some of this chemistry can cause covalent bonding of a substrate to other reactive residues in the active site, resulting in irreversible "suicide inactivation" of the enzyme. This is a mechanism of action of some naturally occurring toxins and a protocol in rational drug design (4).

Pyridoxine hydrochloride is stable if kept dry or in acidic solution. Its aqueous solutions may be heated for 30 min at 120°C without decomposition (16). Losses have been reported for mixtures of the vitamers

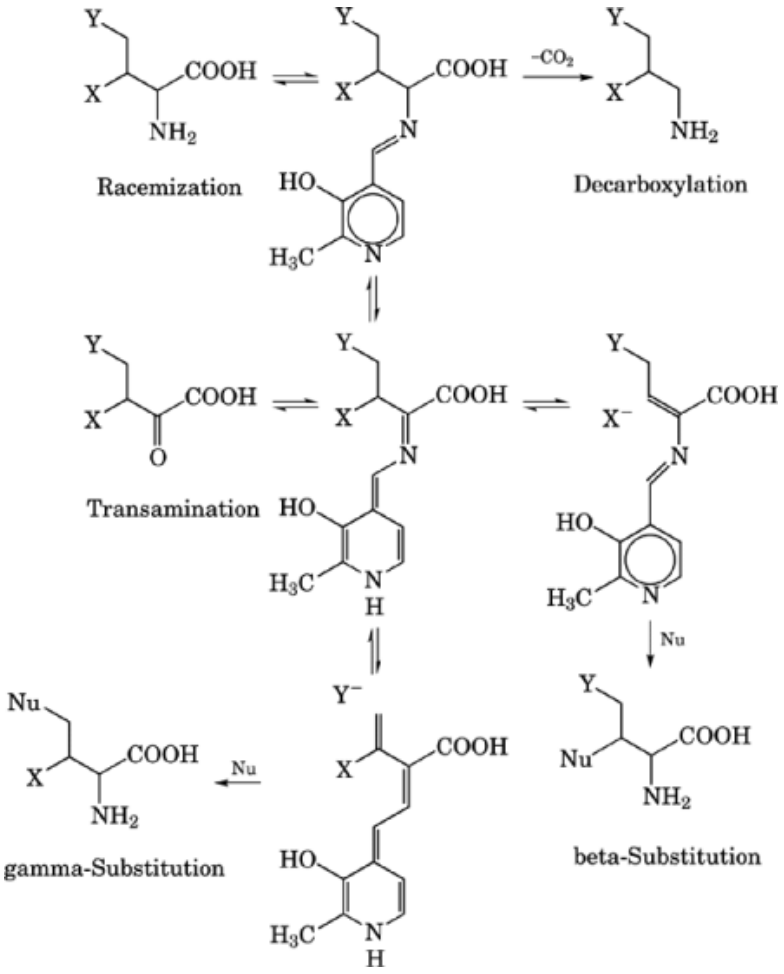

Fig. 3. Reactions of imines and their tautomers.

stored in solution at room temperature or below unless they are kept acidic (17). Decomposition in foods on processing and storage depends mainly on pH, moisture, and temperature. First-order kinetics are often followed and stabilities can be estimated using Arrhenius calculations (4, 18). All B_6 vitamers are susceptible to decomposition by near-uv light to give reddish brown materials, especially in solution at neutral or higher pH. Pyridoxal and pyridoxamine decompose most rapidly, even in acid (18, 19). Gamma irradiation of food can also cause losses (20). Hydroxyl radical formed from ascorbate gives the 6-hydroxyl derivative of pyridoxine. This process may occur during the storage of fruits and vegetables containing high ascorbate levels (21, 22).

1.3. Salts and Derivatives

Generally the B_6 vitamers are high melting crystalline solids that are very soluble in water and insoluble in most other solvents. Properties of the common forms are listed in Table 1. The only commercially important form of vitamin B_6 is pyridoxine hydrochloride 1. This odorless crystalline solid is composed of colorless platelets melting at 204–206°C (with decomposition). In bulk, it appears white and has a density of ~0.4 kg/L. It is

Table 2. Average Vitamin B_6 Contents of Raw Foods, mg / 100 g[a]

Food	Vitamin B_6	Food	Vitamin B_6
beef liver	0.84	brown rice	0.55
beef	0.33	white rice	0.17
chicken	0.33–0.68	whole wheat flour	0.34
pork	0.35	white flour	0.06
fish	0.17–0.43	beans	0.56–0.81
eggs	0.11	peanuts	0.40
whole milk	0.04	corn	0.25
bananas	0.51	potatoes	0.25
raisins	0.24	vegetables, green	0.15–0.28
other fruits	0.02–0.07	tomatoes	0.10

[a]By microbial assay.

very soluble in water (ca 0.22 kg/L at 20°C), soluble in propylene glycol, slightly soluble in acetone and alcohol (ca 0.014 kg/L), and insoluble in most lipophilic solvents. A 10% water solution shows a pH of 3.2. Both the hydrochloride and corresponding free base sublime without decomposition (16).

2. Occurrence

Vitamin B_6 occurs in the tissues and fluids of virtually all living organisms, attesting to its essential role in amino acid biochemistry. Pyridoxal phosphate bound to protein is the main form in animals. Pyridoxine, pyridoxamine, and their phosphates are the usual forms in plants (23, 24). Significant percentages in plants occur as pyridoxine glycosides, which are substantially less bioavailable (23, 25, 26). Nearly all foods in a typical human diet contain detectable amounts of the B_6 vitamers. Relatively rich sources are edible yeast, meats (especially salmon and calf liver), whole grain cereals, legumes, and nuts (qv). Cheeses with extensive mold growth are also good sources. Broccoli, cauliflower, spinach, corn, potatoes, bananas, and raisins are good sources, but vegetables and fruits in general are not (24) (Table 2).

As vitamin B_6 is mainly located in the germ and aleurone layer in cereal grains; polishing for the production of flour removes a substantial portion. White bread is therefore a poor source unless fortified. Some nonedible yeasts contain up to 38 mg/100 g dry weight vitamin B_6, the highest level of the natural sources (4, 27). As a rule, these amounts are too low for cost-effective isolation.

3. Biochemical and Physiological Functions

Because vitamin B_6 is widely distributed in nature, severe deficiency symptoms are seldom observed in humans with adequate diets. However, there is little doubt vitamin B_6 is essential in human and animal nutrition. As enzyme cofactors, pyridoxal 5′-phosphate and pyridoxamine 5′-phosphate are required for important biotransformations of amino acids (4). Decarboxylations of amino acids give rise to a number of important amines, among them the vasodilator histamine, the vasoconstrictor and neurotransmitter serotonin, and the major neurotransmitters gamma-aminobutyric acid, epinephrine, norepinephrine, tyramine, and dopamine. Transamination interconverts specific pairs of amino acids and their corresponding ketoacids and amino groups and aldehydes, key functions in amino acid biosynthesis and catabolism. Racemization provides D-amino acids, constituents of the cell walls of bacteria. Other important transformations requiring pyridoxal include bioconversions of tryptophan (involved in the biosynthesis of the vitamin niacin), serine transhydroxymethylation (a key step in one carbon metabolism leading to nucleic acid synthesis), glycogen phosphorylation

Table 3. U.S. RDA for Vitamin B_6

Age	RDA, mg
infants and children <4 yr	0.7
adults and children >4 yr	2.0
pregnant or lactating women	2.5

(in liver and muscle-maintaining glucose supplies), biosynthesis of delta-aminolevulinic acid (a precursor to heme synthesis), and key steps in the metabolism of sulfur-containing amino acids. Other roles of pyridoxal and its phosphate include binding to hemoglobin (affecting oxygen binding affinity), effects on the conversion of linoleic acid to arachidonic acid, possible effects on cholesterol and fatty acid metabolism, and modulation of steroid action. As a result, vitamin B_6 levels affect gluconeogenisis, nervous system activity, red cell formation and metabolism, immune function, and hormone functions (4, 23).

A large variety of biochemical lesions are found in deficiency (23). Depressed antibody, nucleic acid, and protein synthesis are characteristic. Consequences of vitamin B_6 deficiency in humans and many animals include scaling dermatitis (especially around the eyes, nose, and mouth), anemia, loss of appetite, poor growth or weight loss, muscular weakness, central nervous system changes such as irritability or depression, kidney stones, and abnormalities seen by electroencephalography. In severe cases, nerve degradation leading to convulsive seizures and death can occur. Possible vitamin B_6 deficiency must be assessed; estimation of dietary intake is not sufficient. An older method measures the level of pyridoxal-dependent metabolite xanthurenic acid in the urine after ingestion of a large dose of tryptophan. Improved methods measure pyridoxal phosphate in plasma or pyridoxic acid excretion in urine by sensitive chromatographic procedures (23).

Humans cannot biosynthesize vitamin B_6 and must obtain it from dietary sources. Ruminant animals derive some benefit from microfloral supply in the stomach. All animals interconvert the six major forms 1. Thus requirements of the enzyme cofactor forms can be maintained by ingestion of pyridoxine only. The bioavailability of vitamin B_6 in foods is still an active research area with some unresolved issues (4, 15, 23). Reported levels of availability vary widely. Earlier studies suggest processing, storage, and preparation could lead to losses of up to 80% of vitamin B_6, depending on conditions. Irreversible reductive binding to lysyl residues is reported to give inactivity or even antivitamin effects (15, 28). One study estimates bioavailability of vitamin B_6 from foods representing the average North American human diet at ca 70–80% (29).

In humans, vitamin B_6 in phosphorylated forms is mostly unavailable until hydrolyzed, then passively absorbed in the small intestine. The free vitamins are interconverted and supplied to the circulation by the liver, then enter cells by simple diffusion and are phosphorylated. A typical adult pool of pyridoxal is estimated to be about $\geq$25 mg, most of it bound to enzymes in muscle (23). Small amounts of all forms are excreted in the urine. Most is oxidized in the liver to the main urinary metabolite 4-pyridoxic acid 2, the corresponding diacid, and ring-cleaved fragments (4, 30).

Human requirements for vitamin B_6 parallel protein metabolism, with increased intake of protein requiring higher intake of the vitamin. As little as 0.010–0.015 mg of vitamin B_6 per gram of protein prevents deficiency signs with adequate protein intake. Slightly higher intake maintains acceptable metabolite excretion and plasma vitamin levels. The Recommended Daily Allowance (RDA) for vitamin B_6 assumes consumption of twice the recommended amount of protein (Table 3) (31). Allowances for pregnant and lactating women reflect increased protein needs. Low levels in breast milk place infants at risk for deficiency (4, 23).

The typical U.S. daily diet contains 1.1–3.6 mg of vitamin B_6, most coming from meats and vegetables. Poor diets may provide less than half of these amounts and less than the RDA. Some populations require higher amounts: persons with high protein intakes, pregnant and lactating women, users of oral contraceptives,

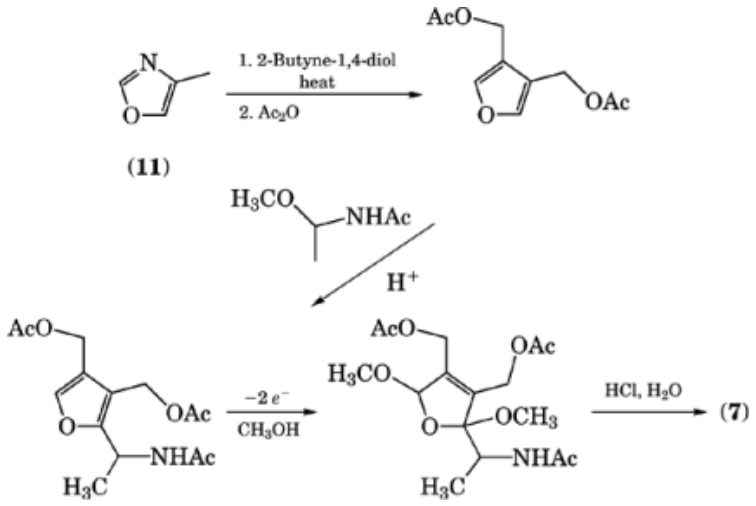

Fig. 4. Furan route to pyridoxine.

alcoholics, users of drugs which interfere with vitamin B_6 function, and those afflicted with some diseases. Several reviews have examined the relationship of vitamin B_6 and specific diseases in more detail (4, 23).

4. Manufacture

Pyridoxal 1 can be prepared by oxidation of pyridoxine 1 (13). Pyridoxamine 1 can be made by reduction of pyridoxal oxime (32). Only pyridoxine 1, the most stable of the three, is manufactured. The earliest syntheses, by degradation of isoquinoline rings, are only of historical interest. The first syntheses to be applied on a large scale relied on classical condensation reactions. Many variations were explored during the 1940s and 1950s and some formed the basis of early production. Such routes required numerous steps giving low overall yields, in part due to difficulties of obtaining the correct oxidation levels of the 4- and 5-substituents. These methods have been reviewed (11, 12). In the mid-1950s, electrooxidation of furan derivatives was shown to provide direct precursors to hydroxypyridines (33). In later improvements, cycloaddition of readily available 2-butyne-1,4-diol to 4-methyloxazole [693-93-6] 4 followed by amidoalkylation conveniently provided the furans (34, 35). The overall yields of these linear sequences are modest (Fig. 4).

The current paradigm for B_6 syntheses came from the first report in 1957 of a synthesis of pyridines by cycloaddition reactions of oxazoles (36) (Fig. 5). This was adapted for production of pyridoxine shortly thereafter. Intensive research by Ajinomoto, BASF, Daiichi, Merck, Roche, Takeda, and other companies has resulted in numerous publications and patents describing variations. These routes are convergent, shorter, and of reasonably high throughput.

Substitution on the azadiene and alkene affect reactivity and only certain pairs react efficiently. Electrophilic alkenes and oxazoles bearing donor groups generally allow faster reactions at lower temperatures (37, 38). Steric effects play a lesser role. Earlier variations with 4-methyloxazole 4 and maleic acid derivatives require the presence of oxidants such as hydrogen peroxide or nitroarenes to aromatize the cycloadducts in order to obtain good yields (11). Subsequent costly reduction steps are also needed to correct the oxidation level of the product side chains. One advantage is the use of leaving groups on one of the cycloaddition partners to facilitate aromatization without loss of the product 3-hydroxyl group. These superfluous groups can be added at the 5-position of the oxazoles or on the alkene.

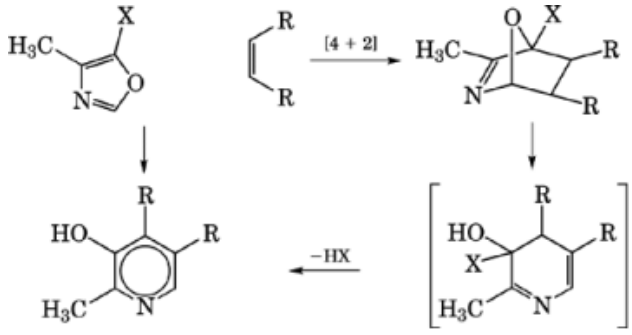

Fig. 5. Synthesis of pyridines by cycloaddition reactions of oxazoles.

Oxazoles preferred in practice bear 5-ethoxy- or 5-cyanosubstituents. Other alkoxy (39–42), trimethylsilyloxy (43), alkythio (44), amino (45), and carboalkoxy (46) substituents are reported. Processes using 5-ethoxyoxazoles 6, 6, and 6 have been published (38, 47–60). Extra carbons carried by the Takeda, Chinese, and Ajinomoto intermediates are lost as waste by decarboxylation. A process with 5-cyano-4-methyloxazole 6 has been disclosed by Roche (59). Reactions of these azadienes with butenediol derivatives, the usual alkene partner, take place at higher temperatures (115–180°C) and require longer reaction times (10–20 h) than those with maleate partners. The ethoxy compound 6 reacts under milder conditions and the adduct is isolable. Mild acid converts it to the product and ethanol. With the higher temperatures required of the cyano compound [1003-52-7] 6, the intermediate cycloadduct is converted directly to the product by elimination of waste hydrogen cyanide. Often the reactions are run with neat liquid reagents having an excess of alkene as solvent. Polar solvents such as sulfolane and *N*-methyl-pyrrolidone are claimed to be superior for reactions of the ethoxy compound with butenediol (53). Organic acids, phenols, maleic acid derivatives, and inorganic bases are suggested as catalysts (51, 52, 54, 59, 61, 62) (Fig. 6).

As the alkene partners, (*Z*)-2-butene-1,4-diol acetals, ketals, esters or ethers (11), and a polymer-bound acetal (63) are described. Butenediol may be used if polar solvents are employed (54). Current manufacturers generally use the cyclic acetal [5417-35-6] 7 of butenediol with isobutyraldehyde, a liquid of conveniently high boiling point that readily allows the product to be deprotected with no waste (Fig. 7). Butenediol is readily and cheaply available from the reaction of acetylene and formaldehyde to 2-butyne-1,4-diol, then catalytic hydrogenation. In an unusual variant of this cycloaddition approach, a leaving group on the alkene facilitates aromatization. Cyclic sulfone [41409-84-1] 7 paired with 4-methyloxazole 4 effectively gives the pyridine nucleus (64, 65) taking advantage of the increased reactivity of an electrophilic alkene while maintaining the correct oxidation level of the side chains. Electrophilic dihydrofuran [332-77-4] 7 reacts efficiently with excess ethoxymethyloxazole 6, also at lower temperatures (66).

Ethoxyoxazole 6 is constructed from *N*-formyl alanine esters (67) and ethoxyoxazole acid 6 from *N*-oxalyl alanine esters (55–58). Alanine is readily available from China by chlorination of propionic acid, then ammonation. Both ethoxyoxazole 6 and the acid 6 are derived from maleic anhydride by *N*-formyl aspartate esters (52, 68). Cyanooxazole 6 is derived from ketene by ethyl chloroacetoacetate and formamide (69) or, alternatively, from diketene (70). All routes require dehydration of amides as a critical step. Traditionally these dehydrations have been accomplished with phosphorus pentoxide, phosphoryl chloride, or phosgene; however, more environmentally acceptable procedures use acetic anhydride (71), cyanuryl chloride (72), or gas-phase catalytic dehydration (73). In some cases, the oxazoles are generated *in situ* in the cycloaddition reactions from *N*-formyl compounds by the isonitriles under dehydrating conditions (45, 74, 75). Other cycloaddition routes include reactions of 1,2,4-triazines with butynediol or enamine derivatives give the pyridoxine nucleus (76, 77), or a cobalt-catalyzed $[2 + 2 + 2]$ cycloaddition of acetylenic ethers with acetonitrile (78, 79) (Fig. 8).

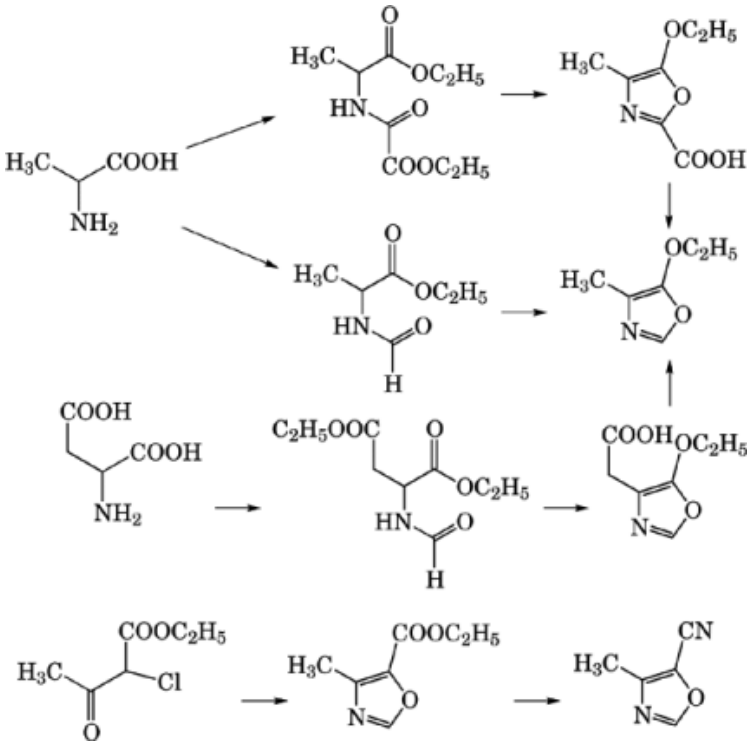

Fig. 6. Process chemistry for oxazoles.

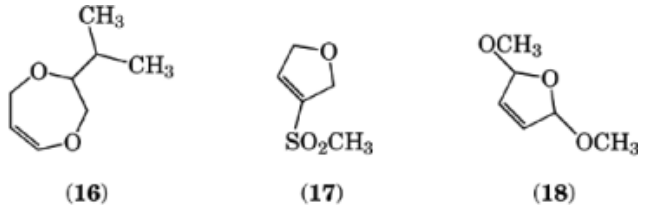

Fig. 7. Dienophiles.

5. Economic Aspects

Significant producers include Daiichi, Hoffmann-La Roche, Takeda, and several factories in mainland China. Takeda and Daiichi practice processes based on alanine by ethoxyoxazole 6, at least one Chinese producer from alanine by oxazole acid 6, and Roche from ketene via cyanooxazole 6. Production is practiced at many hundreds of metric tons annually by batchwise or semicontinuous operation in automated glass and stainless steel equipment typical of fine chemical manufacture. World production in 1995 was estimated at about 3000 MT with sales prices in the United States in the range of $22–$30 per kilogram.

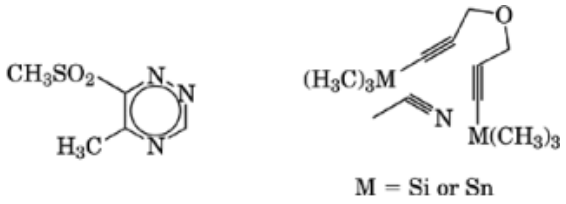

Fig. 8. Other cycloaddition routes.

6. Product Specifications and Testing

Nutrients and diet supplements without claims of therapeutic effects are considered foods, and are thus regulated by the U.S. Food and Drug Administration. These are further subject to specific food regulations. Specifications for pyridoxine hydrochloride 1 for foods are given in the *Food Chemicals Codex* (80) and for pharmaceuticals in the *U.S. Pharmacopeia* (81). General test methods have been summarized (82).

7. Safety and Handling

Pyridoxine hydrochloride is typically packaged in standard 20–25 kg polylined fiber cartons or drums. Smaller packing sizes are also available. Pyridoxine hydrochloride is kept under normal dry storage conditions not to exceed 25°C and protected from light. It is listed in the TSCA inventory and material safety data sheets are available from suppliers. Protection against dust exposure to the eyes, skin, or lungs (IOEL of 2.0 mg/m^3 time weighted average) and fire and dust explosion are indicated. NPFA ratings for health, fire, and reactivity are 1, 2, and 1. Neither national nor international transportation is regulated. The U.S. EPA has not established reportable quantities for environmental releases. The acute LD_{50} (rat oral) of 7750 mg/kg classifies this material as practically nontoxic orally (83), Oral doses of 100–300 mg/d (50–150 times the RDA) have been used in therapeutic studies without adverse effects. Excess amounts are readily cleared by the kidney. More recent studies show that long-term consumption of very large doses can lead to sensory nerve damage. Supplements of more than 200 mg/d should be taken only under medical supervision. Intravenous administration is not associated with toxicity (84).

8. Analytical Methods

Determining vitamin B_6 in foods and tissues is complicated by the number of forms, typical low levels, varying degrees of ionization, lability to heat, light, and base, interconversion, and binding to proteins and glucose. Colorimetric, fluorometric, microbiological, animal, and chromatographic methods are used and have been reviewed in depth (5, 11, 85, 86). Few are fully suitable for simultaneous analysis of all six bioactive forms at the typical low levels. Colorimetric assays involve derivatization, are relatively insensitive, and used only for assay of high potency samples such as premixes. Microbiological assays have previously been the most commonly used, especially for foods. The yeast *Saccharomyces uvarum* (ATCC 9080, also known as *S. carlsbergensis* 4228) responds well to pyridoxine and pyridoxal, less to pyridoxamine, and not at all to the phosphate forms. Hydrolysis is first necessary; by the official AOAC method, the matrix is autoclaved with hydrochloric acid (87). Such methods are slow and overestimate bioavailability. Animal assays in vitamin B_6-deficient chicks or rats are time consuming and expensive but have been useful in determining bioavailability of derivatives and related materials.

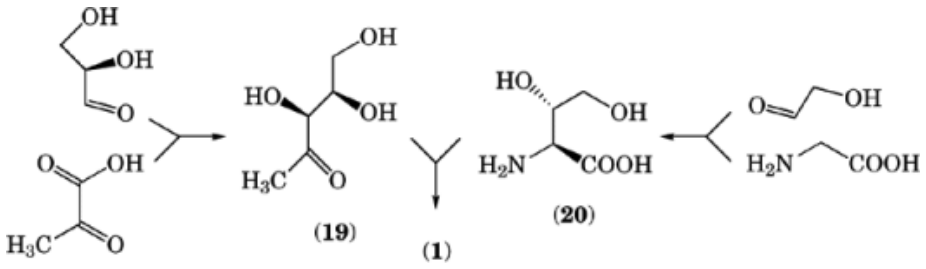

Fig. 9. Biosynthetic pathway to pyridoxine.

Increasingly, chromatographic methods are used for their ease, accuracy, and ability to determine all forms simultaneously. Gas–liquid chromatography is hampered by the polarity of the compounds unless silyl or acyl derivatives are used (5, 88). The comprehensive method of choice generally is high pressure liquid chromatography (hplc) (5). Separations by ion exchange or reverse-phase absorption with ion-pair reagents and isocratic or gradient mobile phases are common. Direct uv detection is less sensitive but useful for premixes and vitamin mixtures. Post-column derivatization and fluorescence detection allows measurement to nanogram levels. High performance capillary zone electrophoresis (cze) coupled with fluorescence detection allows measurement to below picogram levels (89). Gc/ms, hplc/ms, and cze/ms hold promise for measurement and identification of femtomole levels of vitamers and metabolites (90–92). Immunoassay (qv) method are being developed (4).

9. Biosynthesis

Almost all microorganisms synthesize B_6 vitamers intracellularly at levels of 0.05–0.3 mg/g dry weight of cells and excrete at levels of 0.05–0.5 mg/L. Biosynthesis in *E. coli* is known to be controlled by both feedback inhibition and repression. Some organisms can overproduce, for example *B. subtilis*, up to 2.0–5.0 mg/L, *Flavobaterium sp. 238-7* up to 20 mg/L, and yeast *Pichia guilliermondi* up to 25 mg/L (4, 93). The mechanisms of vitamin B_6 biosynthesis are partly understood. Extensive isotopic label feeding studies with *E. coli* mutants show all carbons are derived from glucose and the nitrogen from glycine. Union of the intermediates 1-deoxy-D-xylulose [16709-34-9] 9 and 4-hydroxy-L-threonine [21768-45-6] 9 gives pyridoxine in several steps, only two of which require enzymatic catalysis (Fig. 9) (94). In aerobic bacteria, pyridoxal phosphate is made as needed by the action of oxidase or dehydrogenase enzymes on pyridoxine phosphate or pyridoxamine phosphate (4, 93). The pathways in *Flavobacterium* sp 238-7 (95) and in *Saccharomyces cerevisiae* (96) may differ from that in *E. coli* in the early steps. In microorganisms, some genes and related enzymes for biosynthesis have been identified (94, 97–99). Significant additional progress will be necessary before cost-effective production of vitamin B_6 by bioprocesses is possible.

10. Analogues and Antagonists

Over 250 analogues of the B_6 vitamers have been reported (11, 100). Nearly all have low vitamin B_6 activity and some show antagonism. Among these are the 4-deshydroxy analogue, pyridoxine 4-ethers, and 4-amino-5-hydroxymethyl-2-methylpyrimidine, a biosynthetic precursor to thiamine. Structurally unrelated antagonists include drugs such as isoniazid, cycloserine, and penicillamine, which are known to bind to pyridoxal enzyme active sites (4).

11. Uses

The primary uses of pyridoxine hydrochloride are in multivitamin supplement tablets and for fortification of human food and animal feed, especially for poultry and pigs. Most breakfast cereals and infant formulas in the United States are supplemented. Lesser amounts are used therapeutically to correct deficiencies or to treat specific disorders. Pyridoxine hydrochloride has been used experimentally to treat a variety of conditions with varying degrees of effectiveness (4, 23). Pyridoxine hydrochloride is readily incorporated into premixes and foods.

BIBLIOGRAPHY

"Vitamins, Pyridoxine" in *ECT* 1st ed., Vol. 11, pp. 293–307, by S. A. Harris, G. E. Sita, and P. G. Stecher, Merck & Co.; in *ECT* 2nd ed., Vol. 16, pp. 806–824, by S. A. Harris, E. E. Harris, and R. W. Burg, Merck & Co.; in *ECT* 3rd ed., Vol. 24, pp. 94–107, by D. L. Coffen, Hoffmann-La Roche Inc.

Cited Publications

1. Commission on Biological Nomenclature, *Biochem. J.* **137**, 417 (1974).
2. S. A. Harris and K. Folkers, *J. Am. Chem. Soc.* **61**, 1245 (1939).
3. R. Kuhn and co-workers, *Naturwissenschaften* **27**, 469 (1939).
4. D. Dolphin, R. Poulson, and O. Avramovic, eds., *Coenzymes and Cofactors*, Vols. **1A & 1B**, John Wiley & Sons, Inc., New York, 1986.
5. J. Ubbink, in A. P. De Leenheer, W. Lambert, and H. Nelis, eds., *Modern Chromatographic Analysis of Vitamins*, 2nd ed., Marcel Dekker, New York, 1992, Chapt. 10.
6. W. Korytnyk and R. P. Singh, *J. Am. Chem. Soc.* **85**, 2813 (1963).
7. I. D. Spenser and co-workers, *J. Biol. Chem.* **262**, 7463 (1987).
8. A. Lycka and J. Cizmarik, *Pharmazie* **45**, 371 (1990).
9. W. Korytnyk and co-workers, *J. Am. Chem. Soc.* **88**, 1233 (1966).
10. J. Iida and T. Murata, *Anal. Sci.* **6**, 273 (1990).
11. O. Isler, G. Brubacher, S. Ghisla, and B. Kraeutler, eds., *Vitamine II*, Verlag, New York, 1981, 193–227.
12. J. M. Osbond, *Vitamins and Hormones* **22**, 387 (1964).
13. K. Folkers and co-workers, *J. Am. Chem. Soc.* **73**, 3430 (1951) and cites therein.
14. S. A. Harris, *J. Am. Chem. Soc.* **63**, 3363 (1941).
15. *Nutr. Rev.* **36**, 346 (1978).
16. *The Merck Index*, 11th ed., Merck & Co., Rahway, N.J., 1989, p. 1269.
17. G. S. Shephard and D. Labadarios, *Clin. Chim. Acta* **60**, 307 (1981).
18. B. Saida and J. Warthesen, *J. Agric. Food Chem.* **31**, 876 (1983).
19. C. Y. W. Ang, *J. Assoc. Official Anal. Chem.* **62**, 1170 (1979).
20. A. R. Forester and I. G. Davidson, *Radiat. Phys. Chem.* **36**, 403 (1990).
21. K. Tadera and co-workers, *Agric. Biol. Chem.* **52**, 2359 (1988).
22. M. L. Hu and co-workers, *Chem. Biol. Interact.* **97**, 63 (1995).
23. J. E. Leklem, in L. J. Machlin, ed., *Handbook of the Vitamins*, 2nd ed., Chapt. 6, Marcel Dekker, New York, 1991.
24. M. L. Orr, *Pantothenic Acid, Vitamin B_6 and Vitamin B_{12} in Foods*, Home Economics Report No. 36, U.S. Dept. of Agriculture, Washington, D.C., 1969.
25. J. F. Gregory and D. B. Sartain, *J. Agr. Food Chem.* **39**, 899 (1991).
26. K. Tadera and Y. Naka, *Agr. Biol. Chem.* **55**, 563 (1991).
27. K. D. Lunan and C. A. West, *Arch. Biochem. Biophys.* **101**, 262 (1963).
28. J. Gregory and co-workers, *J. Food Sci.* **51**, 1345 (1986).
29. J. B. Tarr, T. Tamura, and E. L. R. Stokstad, *Am. J. Clin. Nutr.* **34**, 1328 (1981).
30. A. H. Merrill and M. J. Henderson, *Ann. N.Y. Acad. Sci.* **585**, 110 (1990).

31. Committee on Dietary Allowances, Food and Nutrition Board, *Recommended Daily Allowances*, 10th ed., National Academy of Sciences, Washington, D.C., 1980, 142–150.
32. P. Karrer and co-workers, *Helv. Chim. Acta* **32**, 1004 (1948).
33. N. Clauson-Kaas and co-workers, *Acta Chem. Scand.* **9**, 1, 4, 7, 3 (1955).
34. Ger. Pat. 1,935,009 (Jan. 14, 1971), F. Graf and H. Konig (to BASF).
35. Y. Matsumura and co-workers, *Chem. Lett.*, 1121 (1981).
36. M. Y. Karpeiskii and V. L. Flornet'ev, *Russ. Chem. Rev.* **38**, 540 (1969).
37. I. Turchi and M. J. S. Dewar, *Chem. Rev.* **75**, 388 (1975).
38. G. Y. Kondrat'eva and co-workers, *Dokl. Akad. Nauk SSR* **142**, 593 (1962).
39. T. Naito and co-workers, *Chem. Pharm. Bull.* **13**, 873 (1965).
40. Z. I. Itov and co-workers, *Khim. Farm. Zh.* **13**, 59 (1980).
41. E. E. Harris and co-workers, *Tetrahedron* **23**, 943 (1967).
42. Ger. Pat. 3,029,231 (Nov. 3, 1982), H. R. Mueller (to Merck).
43. H. Takagaki and co-workers, *Chem. Lett.*, 183 (1979).
44. Jpn. Pat. 69-23,088 (Oct. 1, 1969), T. Naito, K. Ueno, and T. Miki (to Daiichi Seiyaku Co, Ltd.).
45. Jpn. Pat. 81-128,761 (Oct. 8, 1981) (to Daiichi Seiyaku Co, Ltd.).
46. H. Murakami and M. Iwanami, *Bull. Chem. Soc. Jpn.* **41**, 726 (1968).
47. E. E. Harris and co-workers, *J. Org. Chem.* **27**, 2705 (1962).
48. U.S. Pat. 3,227,721 (May 24, 1965), E. E. Harris, K. Pfister, and R. A. Firestone (to Merck & Co.).
49. Jpn. Pat. 73-30,636 (Sept. 21, 1973), Y. Morita, S. Onishi, and T. Yamagami (to Daiichi Seiyaku Co.).
50. Jpn. Pat. 70-39,259 (Dec. 10, 1970), T. Naito, Y. Morito, and U. Toshihiro (to Daiichi Seiyaku Co.).
51. Jpn. Pat. 72-39,115 (Oct. 3, 1972), T. Miki and T. Matsuo (to Takeda Chemical Industries, Ltd.).
52. Jpn. Pat. 81-99,461 (Aug. 10, 1981) (to Takeda Chemical Industries, Ltd.).
53. T. Miki and T. Matsuo, *Yakugaku Zasshi*, **87**, 323 (1967).
54. U.S. Pat. 3,796,720 (Mar. 12, 1974), H. Miki and H. Saikawa (to Takeda Chemical Industries).
55. J. Maeda and co-workers, *Bull. Chem. Soc. Jpn.* **42**, 1435 (1969).
56. Fr. Pat. 1,533,817 (July 19, 1968) (to Ajinomoto Co.).
57. H. Zhou and co-workers, *Zhongguo Yiyao Gongye Zazhi* **25**, 385 (1994).
58. Chin. Pat. 86-101,512 (July 7, 1986), H. Zhou (to Shanghai Industrial Res. Inst.).
59. U.S. Pat. 3,250,778 (Nov. 11, 1962), W. Kimel and W. Leimgruber (to Hoffmann-La Roche Inc.).
60. M. Dumic and co-workers, *Prax. Vet.* **37**, 81 (1989).
61. D. Szlopmek-Nesteruk and co-workers, *Przem. Chem.* **54**, 238 (1975).
62. U.S. Pat. 3,822,274 (July 2, 1974), E. E. Harris and R. Currie (to Merck & Co.).
63. H. Ritter and R. Sperber, *Macromolecules* **27**, 5919 (1994).
64. W. Boell and H. Koenig, *Ann. Chem.*, 1657 (1979).
65. U.S. Pat. 3,876,649 (Apr. 8, 1975), W. Boell and H. Koenig (to BASF).
66. T. Naito and co-workers, *Tetr. Lett.*, 5767 (1968).
67. P. Karrer and C. Granacher, *Helv. Chem. Acta* **7**, 763 (1924).
68. Jpn. Pat. 68-30,286 (Dec. 26, 1968), T. Kitazawa, T. Matsuyama, K. Kunimitsu, and K. Masuya (to Fujisawa Pharmaceutical Co.).
69. T. Rinderspacher and B. Prijs, *Helv. Chim. Acta* **43**, 1522 (1960).
70. U.S. Pat. 4,026,901 (June 6, 1978), D. L. Coffen (to Hoffmann-La Roche Inc.).
71. U.S. Pat. 4,011,234 (Mar. 8, 1977), H. Hoffmann-Paquotte (to Hoffmann-La Roche Ltd.).
72. Eur. Pat. 612736 (Aug. 31, 1994), W. Bonrath, R. Karge, and H. Pauling (to Hoffmann-La Roche Ltd.).
73. Eur. Pat. 492,233 (July 1, 1992), P. Noesberger (to Hoffmann-La Roche Ltd.).
74. S. Shimada and M. Oki, *Chem. Pharm. Bull.* **32**, 38 (1984).
75. Jpn. Pat. 79-20,493 (July 23, 1979), S. Ohnishi and Y. Morita (to Daiichi Seiyaku Co.).
76. M. Oki and S. Shimada, *Chem. Pharm. Bull.* **35**, 4705 (1987).
77. E. C. Taylor and J. Macor, *J. Org. Chem.* **54**, 1249 (1989).
78. R. E. Geiger and co-workers, *Helv. Chim. Acta* **67**, 1274 (1984).
79. C. A. Parnell and K. P. C. Vollhardt, *Tetr.* **41**, 5791 (1985).

80. Food and Nutrition Board, National Research Council, *Food Chemicals Codex* 3rd ed., National Academy Press, Washington, D.C., 1981, p. 260.
81. United States Pharmacopieal Convention, Inc., *The United States Pharmacopeia XXII* (USP XXII-NF XVII), Rockeville, Md., 1990, p. 1194.
82. H. Y. Aboul-Enein and M. A. Loutfy, in K. Florey, ed., *Analytical Profiles of Drug Substances*, Vol. **13**, Academic Press, New York, 1984, p. 447.
83. *Pyridoxine Hydrochloride*, MSDS 11076, Hoffmann-LaRoche Inc., Nutley, N.J., 1997.
84. M. Cohen and A. Bendich, *Toxicol. Lett.* **34**, 129 (1986).
85. M. M. Polansky, in J. Augustin, B. P. Klein, D. A. Decker, and P. B. Venugopal, eds., *Methods of Vitamin Assay*, 4th ed., Association of Vitamin Chemists, John Wiley & Sons, Inc., New York, 1985, Chapt. 17.
86. J. F. Gregory, *J. Food Comp. Anal.* **1**, 105 (1988).
87. K. Helrich, ed., *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed., Association of Official Analytical Chemists, Inc., Arlington, Va., 1990, p. 961.15.
88. S. P. Colburn and J. D. Mahuren, *J. Biol. Chem.* **262**, 2642 (1987).
89. Y. Kurosu and co-workers, *Bitamin* **66**, 91 (1992).
90. R. D. Smith and co-workers, *Anal. Chem.* **60**, 436 (1988).
91. R. Benyon and co-workers, *J. Chrom.* **581**, 179 (1992).
92. J. Iida and T. Murata, *Anal. Sci.* **6**, 273 (1990).
93. Y. Tani, in E. Vandamme, ed., *Biotechnology of Vitamins, Pigments and Growth Factors*, Elsevier, New York, 1988, Chapt. 13.
94. R. E. Hill, I. D. Spenser, and co-workers, *J. Am. Chem. Soc.* **117**, 1661 (1995).
95. M. Morisaki, *Kurume Igakkai Zasshi* **53**, 649 (1990).
96. K. Tazuya and co-workers, *Biochem. Biophys. Acta*, **1244**, 113 (1995).
97. M. Winkler and co-workers, *J. Bacteriol.* **177**, 2804 (1995) and cites therein.
98. M. Winkler and co-workers, *FEMS Microbiol. Lett.* **135**, 275 (1996).
99. T. Copeland and co-workers, *J. Bacteriol.* **174**, 1544 (1992).
100. W. Korytnik and M. Ikawa, in S. P. Colowick and N. O. Kaplan, eds., *Methods in Enzymology*, Vol. **18A**, Academic Press, New York, 524–566.

DAVID BURDICK
Hoffmann-La Roche Inc.

Related Articles

Vitamins, Survey