NIACIN

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Chapter III

Niacin

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1. Nomenclature

The name niacin is often used for two compounds, namely pyridine-3-carboxylic acid and pyridine-3-carboxylic acid amide. This confusion occurs because there is no consistency in the empirical nomenclature used in nutrition literature. Harris[1] lists niacin and niacinamide as the terms preferred in the United States, and nicotinic acid and nicotinic acid amide in the British literature. A convenient solution to the problem would be to use the term niacin to refer generically to the two compounds of nutritional significance as anti-pellagra factor and adopt the names nicotinic acid and nicotinamide to refer specifically to each. Nicotinamide has been adopted by the Commission for the Reform of Nomenclature in Biological Chemistry of the International Union of Pure and Applied Chemistry, and nicotinic acid is already in current use in most British, United States and other scientific literature in English.

Other names which apply to both compounds and refer to their nutritional role, but are now seldom used, are vitamin PP, PP factor, pellagra preventive factor, and pellagramine.

2. History

Nicotinic acid was isolated for the first time from natural materials by Suzuki *et al.*[2] in Japan and Funk[3] in Europe, during their investigations of the factor present in rice bran responsible for its curative and preventive action against polyneuritis. At the time, these investigators did not recognize the nutritional essentiality of the compound. The structure of nicotinic acid had been known for around 40 years as a laboratory isolate from the oxidation products of nicotine.

The next step of great significance was the discovery of Warburg and his group[4] that the amide of nicotinic acid was part of coenzyme II (TPN, NADP) and functionally essential for the hydrogen-transporting role of this coenzyme, shortly followed by a parallel finding by Von Euler et al.[5] in coenzyme I (DPN, NAD).

Its previous discovery in yeast, as well as its presence in heart muscle extracts, emphasized the metabolic significance of nicotinamide. The emergence of nicotinic acid or nicotinamide as a nutritional essential came from the demonstration by Elvehjem et al.[6] that black tongue in dogs could be cured and prevented by its addition to the deficient diet. Simultaneously, Fouts et al.[7], Smith et al.[8] and Spies et al.[9] reported the successful treatment of pellagra in human subjects. Its universal occurrence in living tissue and its fundamental role in cellular respiration are now well established[10].
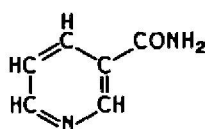
3. Chemistry, determination of structure and properties

(a) Nicotinic acid

Nicotinic acid and nicotinamide are pyridine-3-carboxylic acid and its amide respectively; their structural formulas are:
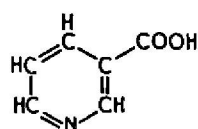
Nicotinic acid   Nicotinamide

The isolation of nicotinic acid among the products of chromic acid oxidation of nicotine[11,12] lead to the determination of its structure. The basic pyridinic ring structure was determined by the formation of free pyridine upon distilling the compound with calcium hydroxide and removal of CO_2. The presence of the —COOH group was demonstrated through the preparation of specific derivatives including salts of copper and silver, esters and the acyl chloride. The basic character of its nitrogen was determined by the formation of the hydrobromide, the hydrochloride or quarternary ammonium derivatives.

The actual location of the —COOH group at carbon 3 was established by Skraup and Cobenzl[13] by physical and chemical criteria. Through a series of steps 3-phenylpyridine is prepared from p-naphthylamine and glycerine, and upon oxidation it yields nicotinic acid. Skraup and Vortmann[14], also prepared nicotinic acid from synthetic dipyridyl of known m-structure. The three position isomers have very different melting points: picolinic acid (o-, or 1,2) around 136°; nicotinic acid (m- or 1,3-) around 236°; and isonicotinic acid[15] (p- or 1,4-) around 319°.

Nicotinic acid has a molecular weight of 123.11. From water or alcohol it crystallizes in white, odorless needles with a tart taste. It melts[15] at 236.6° (234–237° as cited in reference[10]) and sublimes without decomposition. In aqueous solution it has an absorption maximum in the ultraviolet region at 261.5 mμ. The molecular extinction coefficient at this point is pH-dependent, decreasing with increase in pH from 1.28 to 13.0. By two methods involving successive approximations K_a, the thermodynamic acid dissociation constant, was found to be $1.12 \cdot 10^{-5}$ and $1.23 \cdot 10^{-5}$, while K_b, the basic dissociation constant was $3.55 \cdot 10^{-11}$ by both methods[16] (22°). At 25°, Ostwald[17] found the acid dissociation constant to be $1.4 \cdot 10^{-5}$, that is between propionic and butyric acids. Nicotinic acid is not in amphoteric ion form in solution[16].

Nicotinic acid is very stable in air and non-hygroscopic and in water solution resists autoclaving at 120° for 20 min without decomposing. It is also stable in acid and alkaline solutions even with heat. Slobodin and Goldman[18] give the following solubilities in grams per 100 ml for nicotinic acid at 0°, 15°, 38°, 61°, 78° and 100°: in distilled water, 0.86, 1.3, 2.47, 4.06, 6.0, 9.76; in 96% alcohol 0.57, 0.92, 2.1, 4.2, 7.06, —. Nicotinic acid hydrochloride, and to a greater extent, sodium nicotinate, are more soluble in water. Nicotinic acid is also soluble in propylene glycol and insoluble in ether.

Its nitrogen readily becomes pentavalent, forming quaternary ammonium salts. Some derivatives of known properties, useful for qualitative identification are: the hydrochloride, white crystals melting at 272° with decomposition; the nitrate, m.p. 184–185°; the picrate, yellow rhombic prisms (from absolute alcohol) melting at 221–222° to a red oil[19]; the p-toluidine, m.p. 150° and the anilide[20], m.p. 85°. Alkyl iodides readily react with it to form water-soluble compounds. The carboxylic group of nicotinic acid behaves typically, forming salts with alkalies, alkaline earth hydroxides or heavy metals; the latter salts are quite insoluble and their preparation is useful to separate nicotinic acid from mixtures.

Ester derivatives may be prepared using regular procedures such as heating the acid with an alcohol in the presence of hydrogen ions or starting with the nicotinyl chloride and an alcohol. The preparation of the amide derivative (nicotinamide) is described below.

(b) Nicotinamide

Nicotinamide has a molecular weight of 122.12 and occurs as odorless, bitter-tasting white crystals, which melt[10] at 129–131°. It distills at 150–160° under a vacuum of $5 \cdot 10^{-4}$ mm Hg and is very soluble in water (1 g in about 1 ml) and alcohol (1 g in about 1.5 ml of ethanol). It also dissolves in butanol, amyl alcohol, ethylene glycol, acetone and chloroform, and is slightly soluble in benzene and ethyl ether. It is stable in dry form at temperatures below 50°.

In water solution it may be heated in the autoclave for 20 min at 120° with no destruction, but in acid or alkaline medium it hydrolyzes to nicotinic acid[15,21].

The crystalline forms of nicotinamide have been described by several investigators. Wright and King[22] give data on the morphological, optical and structural characteristics of the compound. When crystallized from ethylene glycol it forms monoclinic units of prismatic habit, predominant form [110] and from water, acetone, benzene, glycerol and aqueous ethylene glycol it gives lath-shaped monoclinic units, predominant form [010].

Jellinek and Wayne[23] reported the ultraviolet absorption for nicotinamide at pH values of 5.7 to 1.06 with a $3 \cdot 10^{-4}$ M solution giving a maximum at 261.5 mμ, that is, the same as for nicotinic acid. From this work, a pK value of 10.6 was calculated for the H^+RCONH_2 form and 13.5 for the $H^+RCONH_3^+$ form. They found the thermodynamic dissociation constants of $2.24 \cdot 10^{-11}$ for the ring nitrogen and $3.16 \cdot 10^{-14}$ for the amide group.

The chemical reactions of nicotinamide at the ring nitrogen site are typical, combining with alkyl halides and acids to form quaternary ammonium compounds. Knox and Grossman[24] have reported that ring carbon 6 is the reactive position of the quaternary nicotinamide ring, as evidenced by studies in which oxidation of N^1-methylnicotinamide chloride by a quinine-oxidizing enzyme resulted in 1-methyl-3-carboxylamide-6-pyridone.

4. Isolation

(a) Nicotinic acid

The technique for the isolation of nicotinic acid depends on the starting material. In most cases, a preliminary hydrolysis is required either with acids or alkalies. The extractions are more complete if the material is rendered free of lipids, a necessary step when working with animal products. The free acid is extracted from the hydrolysate with organic solvents such as hot alcohol. It may then be separated as such from the organic solvent extract or in the form of an ester or as the copper salt; the free acid can be recovered from the copper salt by H_2S treatment. Purification is carried out by crystallization from concentrated water or alcohol solutions. Nye et al.[25] extracted nicotinic acid from the mycelium of *Neurospora* with acetone. Subsequent purification steps included the formation of the barium salt, acidification with H_2SO_4 and adsorption of the free nicotinic acid on charcoal. Elution was accomplished with 4% aqueous aniline and the final purification step involves recrystallization from a 1:4 mixture of acetic acid and benzene. Leifer et al.[26] have applied paper chromatography with *n*-butanol saturated with ammonia to separate nicotinic acid from contaminating materials.

(b) Nicotinamide

Nicotinamide can be isolated from natural materials by water extraction, followed by partial hydrolysis with 0.1 N H_2SO_4 to liberate it from the combined form in which it exists. The amide is then extracted with butanol or chloroform. The chloroform solution may be subjected to fractional distillation at $150°-160°$ under $5 \cdot 10^{-4}$ mm Hg. It can be recrystallized from chloroform, benzene or ethylene glycol. Methods have been described by Warburg and Christian and by Elvehjem and coworkers to isolate the compound from red blood cells and liver tissue[21].

The separation of nicotinic acid from its amide can be accomplished by extraction of a water solution with ether, chloroform or benzene. Nicotinamide dissolves in the organic solvents while nicotinic acid remains in the aqueous phase. The two compounds can also be separated by paper chromatography[26,28].

5. Synthesis[21,29]

(a) Nicotinic acid

(i) From quinoline

The stability of the pyridinic ring surpasses that of benzene[30], therefore, oxidation results in destruction of the benzene ring.
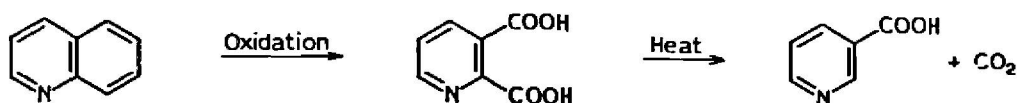
Oxidation may be accomplished by heating at $300°$ with sulfuric acid, nitric acid with mercuric oxide as catalyst, or by treatment with hydrogen peroxide. Selenium oxide has also been used as catalyst. Nicotinic acid in high yield is usually recovered as the copper salt[21].

(ii) From p-substituted pyridines

The same stability principle applies to the historical synthesis of nicotinic acid from nicotine which, for example, upon oxidation with permanganate[31], chromic acid[11] or fuming nitric acid[12], loses four carbons and the N of the 5-membered ring.


Similar reaction mechanisms are the bases for the synthesis of nicotinic acid from 3-methyl pyridine, 3-ethyl pyridine, 3-phenyl pyridine, and 3,3'-

dipyridyl. Appropriate oxidation is carried out with phosphoric–nitric acid mixture and vanadium pentoxide with small amounts of ferric oxide, or even electrochemically[21].

(iii) From pyridine

Treatment of pyridine with fuming sulfuric acid yields pyridine-3-sulfonic acid which is converted to 3-cyanopyridine by distillation of its sodium salt with potassium cyanide[32]. Better yields are claimed by initial bromination of pyridine in the 3-position and subsequent treatment with cuprous cyanide[33]. The 3-cyanopyridine is then hydrolyzed to nicotinic acid.
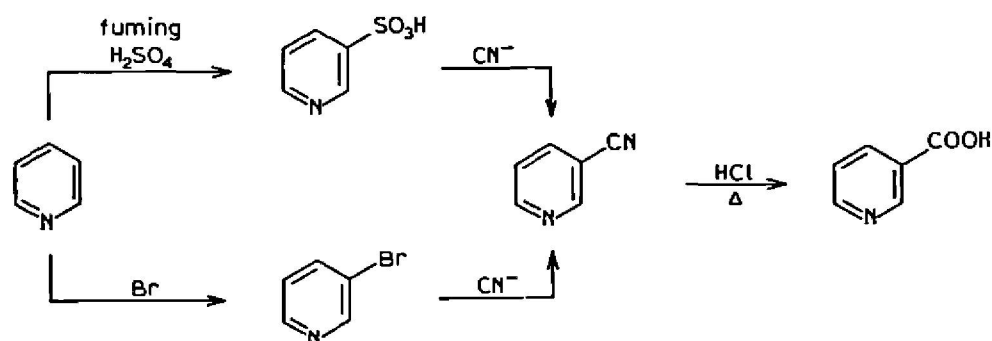
(iv) Labeled nicotinic acid

By halogen–metal interconversion, Murray et al.[34] prepared nicotinic acid labeled with ^{13}C (82% yield) or ^{14}C (62% yield) on the carboxylic radical. Using n-butyl lithium and 3-bromopyridine, 3-pyridyl lithium was prepared and carbonation of this compound was carried out with $^{13}CO_2$ or $^{14}CO_2$ generated from the respective labeled barium carbonates with acid.
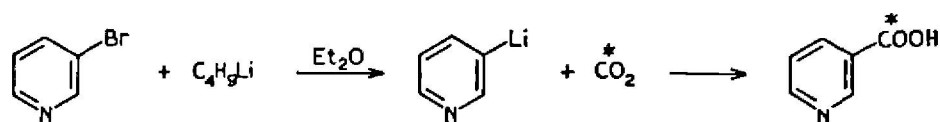
The ^{14}C-labeled material had a specific activity of around 8 $\mu C/mg$. Deuterization of nicotinic acid has been effected by direct exchange with deutero-sulfuric acid, with 83% yield, of a product containing approximately 55% of hydrogen replaced by deuterium[35].
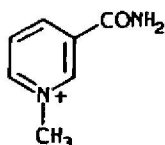
(b) Nicotinamide

This compound can be synthesized by passing ammonia gas into nicotinic acid or by treating it with molten urea[36,37] at 230°. The methyl or ethyl esters of nicotinic acid also undergo amidation upon reaction with ammonia in aqueous or alcoholic media[38].

6. Properties and synthesis of some related compounds of biochemical importance[15,21,39]

N[1]-methylnicotinamide has a molecular weight of 172.5 and crystallizes from alcohol in white rosettes or prismatic bars, m.p. 233–234°. It is soluble in water and ethanol and can be prepared by treating nicotinamide with methyl (or other alkyl) iodides which are then converted to the chloride. The picrate melts at 189.5°.
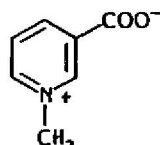
6-Pyridone of N[1]-methylnicotinamide forms white crystals from acetone with a molecular weight of 152.15; it melts at 212–214° and is soluble in water and ethanol. It may be prepared from coumalic acid by ring closure with methyl amine. It is the sole product of the enzymatic oxidation of N[1]-methylnicotinamide with a quinine-oxidizing enzyme, and is also obtained from N[1]-methylnicotinamide or the N[1]-methylbetaine of nicotinic acid by oxidation with alkaline ferricyanide and subsequent treatment with $SOCl_2$ and NH_3.
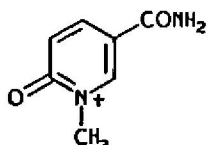

N[1]-methylnicotinamide　　　6-Pyridone of N[1]-methylnicotinamide　　　Trigonelline

Trigonelline has a molecular weight of 137.13; it crystallizes from alcohol in hygroscopic prisms with one molecule of water, becomes anhydrous at 100° and melts at 218° with decomposition. It is very soluble in water, soluble in alcohol and slightly soluble in ether or chloroform. It is prepared in the laboratory from nicotinic acid by heating with CH_3I and treatment with silver oxide.
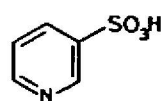
Nicotinuric acid has a molecular weight of 180.15, melts at 240–242° and crystallizes from dilute hydrochloric acid in white crystals which are very soluble in water and ethanol.
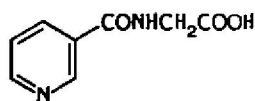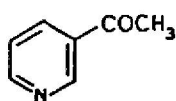
Nicotinuric acid　　　　3-Acetyl-pyridine　　　　Pyridine-3-sulfonic acid

3-Acetyl pyridine. The free base has a molecular weight of 121, is liquid at

room temperature, melting at 13–14° and boiling at 90–92° under 5 mm Hg. The hydrochloride (molecular weight 157.5) forms a white crystalline powder melting at 176–177.5°, and is soluble in water and ethanol. It may be prepared by condensation of the ethyl esters of nicotinic and acetic acids in the presence of sodium ethoxide followed by hydrolysis of the condensation product to 3-acetyl-pyridine.

Pyridine-3-sulfonic acid is a crystalline compound with a molecular weight of 159.11; m.p.[40] 357°; its ammonium salt melts at 243°. It is prepared by sulfonation of pyridine with fuming sulfuric acid at temperatures above 200°. The reaction at 225° with mercuric sulfate as catalyst yields only pyridine-3-sulfonic acid.

6-Amino nicotinic acid has a molecular weight of 138.12 and crystallizes with two molecules of water from dilute acetic acid. It decomposes above 300° and is slightly soluble in most solvents. The potassium salt is freely soluble in water as is the hydrochloride. The picrate crystallizes in yellow needles which melt at 248°. This compound is prepared by treating 6-chloronicotinic acid with ammonia.
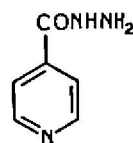
Nicotinic acid hydrazide has a molecular weight of 137.14, crystallizes from dilute alcohol or from benzene in white needles. It melts at 158–159°, is very soluble in water and alcohol, and only slightly soluble in benzene. It is synthesized by treatment of ethyl nicotinate with hydrazine hydrate.
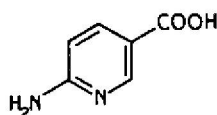
6-Amino nicotinic acid   Nicotinic acid hydrazide   Isonicotinic acid hydrazide
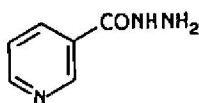
Isonicotinic acid hydrazide with a molecular weight of 137.14 forms white crystals, melting at 171°. It is soluble in water (14% at 25°) and alcohol (2% at 25°). It is slightly soluble in chloroform and insoluble in ether or benzene. It may be heated in aqueous solution at 120° for 30 min with no apparent destruction and is prepared by condensation of ethyl isonicotinate with hydrazine.

7. Methods of determination

The methods for the determination of nicotinic acid and nicotinamide can be classified as: (*a*) chemical, (*b*) bacteriological, and (*c*) biological.

(a) Chemical methods[10,29,41-44]

(i) Cyanogen bromide method

This is based on the reaction of derivatives of pyridine with cyanogen bromide in the presence of an aromatic amine giving specific colors. Amines commonly used are 2-naphthylamine-1-sulfonic acid, *p*-aminoacetophenone, *N*-methyl-aminophenol sulphate, aniline and *p*-naphthylamine. The reaction gives a yellowish-green color which is measured photometrically. The color is soluble in amyl alcohol which can be used to extract it from water solutions, but is not stable unless a phosphate buffer of pH 6.1 is used. This method is not specific for nicotinic acid since other pyridine derivatives and derivatives of nicotinic acid such as trigonelline, nicotinuric acid and nicotine give a similar color.

(ii) 2,4-Dinitrochlorobenzene method

This compound reacts only with free nicotinic acid or its amide. The dry material is fused with 2,4-dinitrochlorobenzene and the reaction product is dissolved in ethyl alcohol. Potassium hydroxide is then added to develop the color which is measured colorimetrically.

This method also lacks specificity since many other pyridine derivatives give the same color test. For example, in the determination of the nicotinic acid content of several plant and animal materials, especially cereal grains, the intensity of the color reactions is usually in excess of the amounts of nicotinic acid actually present, as determined by other methods.

Both nicotinic acid and its amide can be determined by the above methods. If the determination of either one alone is desired, the two compounds can be separated by extracting the amide derivative with ether from an aqueous solution.

(iii) Chromatographic separation of nicotinic acid and related compounds[27]

A mixture of nicotinic acid, nicotinamide, trigonelline and tryptophan can be separated chromatographically, using as solvent a 5:1 mixture of *n*-butanol and concentrated hydrochloric acid, saturated with water. Tryptophan is revealed by ninhydrin, while nicotinic acid, nicotinamide and trigonelline are detected with phosphomolybdic acid reagent and the color developed with stannous chloride, which reduces the phosphomolybdic acid to give a blue spot. The R_F values for the butanol–HCl solvent are: nicotinic acid, 0.37; nicotinamide, 0.28; trigonelline, 0.38 and tryptophan, 0.62.

(b) Biological methods

Several experimental animals have been used for the biological determination

of nicotinic acid and derivatives. These are: chicks[45,46], growing niacin-depleted rats[47], dogs[6,48,49], and pigs[50,51].

The animal assays offer little opportunity for the quantitative determination of nicotinic acid because of interfering factors, mainly the fact that tryptophan is metabolized partly into nicotinic acid in most aninals. Biological tests offer possibilities, however, in assessing the over-all pellagra preventive potency of the foods tested. Nicotinic acid-free rations for chicks are available for the assay of the vitamin using this experimental animal and the growth response is satisfactory for biological testing. Nicotinic acid-depleted rats also respond satisfactorily to niacin feeding. The cat does not convert tryptophan into nicotinic acid, offering special opportunities for the assay of nicotinic acid, as well as the study of its metabolism.

(c) Bacteriological methods[52–56]

Niacin is required by several micro-organisms for growth and this fact is useful in determining nicotinic acid by microbiological methods. The organism commonly used is *Lactobacillus arabinosus*, which responds almost linearly to varying concentrations of nicotinic acid. The concentration is estimated either by measuring the turbidity of the media after 18 h of incubation or by titrating the lactic acid produced after 72 h of incubation at 38°. The yeast *Torulopsis utilis* has been used particularly for differentiations between related compounds such as nicotinic acid, nicotinamide and trigonelline.

(d) Hydrolysis of the samples[57–59]

For the successful application of all chemical and bacteriological methods for the determination of nicotinic acid, it is necessary to hydrolyze the sample under study, with either an acid or an alkaline reagent. Some materials of vegetable origin, particularly cereal grains, contain nicotinic acid in a form which is not biologically available to the organism. This so called "bound" form of nicotinic acid[47,60] is present in the seed coat of corn and other cereal grains. In order to liberate nicotinic acid from its bound form, it is necessary to hydrolyze the samples with dilute H_2SO_4 or, as advocated by several workers, with NaOH. The pellagragenic effect of corn has been attributed to the "bound" form of nicotinic acid in this cereal grain, which, as mentioned above, is not available to the organism.

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