The first line TB drug pyrazinamide is a phoenix

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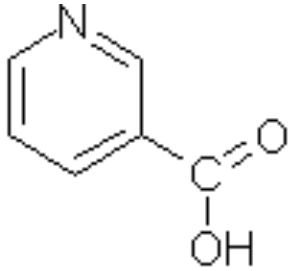
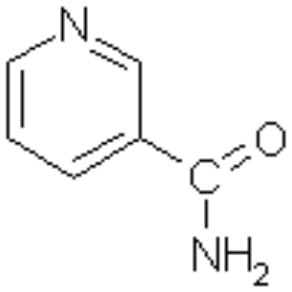
Structure of nicotinamide and related compounds



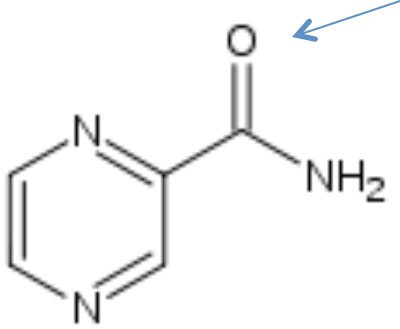
Nicotinic Acid

(Niacin, vitamin B_3, vitamin PP)

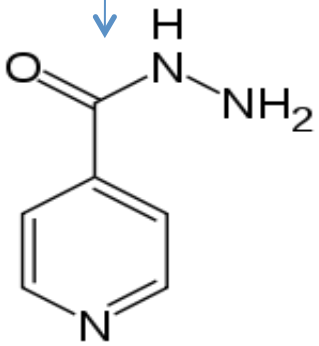


Nicotinamide

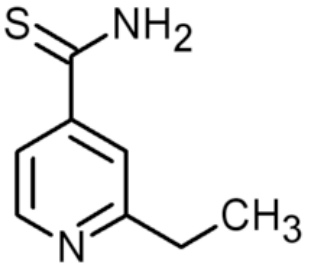
Other names: **pyridine-3-carboxamide**; niacinamide, nicotinic acid amide, Vitamin PP



Pyrazinamide
(pyrazine-2-carboxamide)



Isoniazid (isonicotino-hydrazide)



Ethionamide
(2-ethylpyridine-4-carbothioamide)

Main properties of nicotinic acid (niacin) and nicotinamide

- Niacin cannot be directly converted to nicotinamide
- Both niacin and nicotinamide could be converted to the coenzymes NAD and NADP *in vivo*
- Niacin and nicotinamide are identical in their vitamin activity
- In contrast to niacin, nicotinamide does not reduce cholesterol or cause flushing, although nicotinamide may be toxic to the liver at doses exceeding 3 g/day for adults
- Nicotinamide has demonstrated anti-inflammatory action, especially in patients with inflammatory skin conditions
- Nicotinamide is considered safe as a food additive, and as a component in cosmetics and medication
- This explains the followings:

(Pre)-History of pyrazinamide: the pyridine carboxylic acids

Patented Jan. 23, 1940

UNITED STATES PATENT OFFICE

2,188,244

PYRIDINE CARBOXYLIC ACID MORPHOLIDES

Gilbert Auguste Langlois and Auguste Marius Deloison, Calais, France, assignors to Fabriques de Produits de Chimie Organique de Laire, Issy, Seine, France, a company of France

No Drawing. Application August 17, 1937, Serial No. 159,604. In France October 12, 1936

2 Claims. (Cl. 260—247)

At the present time there exist, among cyclic amides, various amido derivatives of nicotinic acid or, more generally, of the pyridine carboxylic acids, which derivatives have therapeutic properties.

Patented Apr. 25, 1944 2,347,410

UNITED STATES PATENT OFFICE

2,347,410

PYRIDINE CARBOXYLIC ACID

Alfred T. Hawkinson and Arthur A. Elston, Niagara Falls, N. Y., assignors to E. I. du Pont de Nemours & Company, Wilmington, Del., a corporation of Delaware

No Drawing. Application November 27, 1941, Serial No. 420,690

This invention relates to a new and improved process for the manufacture of pyridine carboxylic acids, especially quinolinic and nicotinic acids. More particularly, it relates to an improved method for preparing nicotinic acid from salts, particularly the heavy metal salts, of pyridine monocarboxylic and pyridine dicarboxylic acids.

Pre-history of pyrazinamide I.

- Pyrazinamide was synthesized (ammonolysis of methyl pyrazinoate) by Dalmer and Walter in 1936 (German patent 632257).

Two serendipitous findings in 1945 from treatment with high doses of Vit PP:

- V. Chorine*: High doses (300-500 mg/kg) of nicotinamide or nicotinic acid cure *M. lepraemurium* infection in the rat model and slow down TB infection in guinea pigs; In vitro, the MIC of nicotinamide for *M.tuberculosis* is ~300 µg/ml while nicotinic acid is inactive.
- E.Huant**: in patients with cancer + tuberculosis receiving IM 300 mg + oral 500-1000 mg of nicotinamide, clear-cut improvement of TB disease occurred!

* Vidal Chorine. Activity of nicotinamide against Mycobacteria. Proceedings of Academy of Sciences. Paris 1945; 22:150-151

** Ernest Huant. Activity of very high doses of nicotinamide on tuberculosis lesions. Gazette des Hôpitaux 1945; 16: 259-60.

Pre-history of pyrazinamide II.

1946. H. Pope and D.D. Smith: Comparative production of vitamin-B by human and bovine tubercle bacilli (Am Rev Tub, 1946; 54: 559)
1947. O.D. Bird: High nicotinic acid content – 37 to 61 μg/ml - of tubercle bacilli (Nature 1947; 159: 33)
1948. McKenzie et al.* Out of 2,000-3,000 chemicals screened against the tubercle bacillus on the chorioallantoic membrane of the chick embryo:
- 15% were inhibitory, especially pyridine carboxylic acids.
- These 15% were tested using the mouse model: Nicotinic acid and nicotinamide were the most active at a dose of 0.5 to 0.75% in the diet (800-1000 mg/kg) and as active as 200 mg/kg streptomycin S/C.
- Kushner and coworkers** synthesized 30 derivatives of nicotinamide but they were less active than nicotinamide!
- Drug resistance developed in mice treated with nicotinamide alone but not in combination with streptomycin
- Their belief was that the role of nicotinamide in the treatment of experimentally infected mice is that of a vitamin!
Mouse model: Swiss mice IV infected with 10^{6-7} *Mtb* CFU and treated the day after infection, drug mixed in the diet. Untreated controls died in 3-4 weeks
• McKenzie D, Malone L, Kushner S, Oleson JJ. Subbarow Y: Effect of nicotinic acid amide on experimental tuberculosis of white mice. J Lab Clin Med 1948;33:1249–1253
** Kushner S, Harry Dalalian H, Cassell RT, Sanjurjo JL, Mckenzie D, Subbarow Y. Experimental chemotherapy of tuberculosis. I. Substituted nicotinamides. J Org Chem. 1948; 13:834-836

1952. Discovery of pyrazinamide's antituberculosis activity

Kushner*: pyrazinamide = nicotinamide + nitrogen atom substituted in the ring

- In the mouse**, 0.1% (vs 0.75% for nicotinamide) in the diet or 125 mg/kg is 7 times > nicotinamide
- In the guinea pig***, limited activity with daily doses of 50-75 mg
- In humans****, evaluation on 43 patients between 1949 and 1951 showed it to be clinically active (Reduction of fever and diminution of cough and sputum), at doses of 350 mgx4 on day 1 and to 700 mgx4/per day, i.e., 2800mg/day or 23-46 mg/kg daily for 42 days. Two patients had hepatitis and 10 had joint pain.

Improvement of the mouse model**: "use as indices of activity the survival time of 50% of mice (T_{50}) as compared with 50% of nontreated controls, together with the weight of the mice".

* Kushner et al. Experimental Chemo of TB. Synthesis of PZA and related compounds. J Am Chem Soc.1952; 74: 3617-21.

** Malone L, A. Schurr, H. Lindh, D. McKenzie, J. S. Kiser and J. H. Williams, The effect of pyrazinamide (Aldinamide) on experimental tuberculosis in mice. Am. *Rev.* Tuber 1952; 66: 511-517 .

*** Dessau & al. Pyrazinamide in the experimental tuberculosis of the guinea pig. Am Rev Tuber 1952; 66, 519- 522

**** Yeager RL, Munroe WGC, Dessau FI. Pyrazinamide in the treatment of pulmonary tuberculosis. Am. Rev. Tuber 1952; 66: 523-534

ACTIVATION OF PYRAZINAMIDE AND NICOTINAMIDE IN ACIDIC ENVIRONMENTS IN VITRO[1]

Amer RevTuberc 1954;70:748-754

In summary: Tubercle bacilli of the H37Rv strain are highly susceptible to pyrazinamide *in vitro* in a standard drug-susceptibility test modified only by reducing the pH to 5.0 or 5.5. Nicotinamide is likewise active, although to a lesser degree, when tested in this way. When tested within the "physiologic" ranges of pH, tubercle bacilli of the H37Rv strain are resistant to both pyrazinamide and nicotinamide.

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(with the technical assistance of KURT STERN)

June 23, 1954

In addition mice and guinea pigs infected with *M. bovis* strains do not respond to pyrazinamide treatment.

W. McDermott and R. Tompsett
Amer. Rev. Tuberc. 1954; 70: 748-754

TABLE 3

ACTIVITY OF PYRAZINAMIDE AGAINST H37RV TUBERCLE BACILLI ACCORDING TO ENVIRONMENTAL ACIDITY

(*Inoculum* of standard size; medium, oleic-albumin; *activity* measured by macroscopically demonstrated bacillary growth at three weeks)

pH	Drug, γ per Ml.							
	450	200	100	50	25	12.5	6.25	0
8.0	0	0	0	1+	4+	4+	4+	4+
7.5	1+	2+	3+	4+	4+	4+	4+	4+
7.0	2+	2+	4+	4+	4+	4+	4+	4+
6.5	2+	2+	4+	4+	4+	4+	4+	4+
6.0	0	1+	1+	1+	4+	4+	4+	4+
5.5	0	0	0	0	0	0	0	4+
5.0	0	0	0	0	0	0	0	1+
4.5								0

Conclusion: Even at high concentrations, pyrazinamide is inactive or poorly active at basic and neutral pH.

W. McDermott and R.Tompsett
Amer RevTuberc 1954; 70: 748-754

TABLE 5

ACTIVITY OF STREPTOMYCIN AGAINST H37Rv TUBERCLE BACILLI ACCORDING TO ENVIRONMENTAL ACIDITY

(*Inoculum*, standard size; *medium*, Tween®-albumin; *activity* measured by macroscopically visible bacillary growth at two weeks)

pH	Drug, γ per Ml.								
	10	5	2.5	1.25	0.63	0.32	0.16	0.08	0
8.0	0	0	0	0	0	0	1+	4+	4+
7.5	0	0	0	0	0	0	4+	4+	4+
7.0	0	0	0	1+	3+	4+	4+	4+	4+
6.5	0	0	1+	4+	4+	4+	4+	4+	4+
6.0	0	1+	3+	4+	4+				4+
5.5	0	1+	2+	4+	4+	4+	4+	4+	4+
5.0									0
4.5									0

Conclusion: Even at high concentrations, streptomycin is inactive or poorly active at acid pH. It is the opposite of pyrazinamide

Pyrazinamide-isoniazid in tuberculosis

(W. McDermott et al., Am Rev Tub 1954: 69: 319-333)

- 55 patients with pulmonary tuberculosis

Treatment for 3 months with

- PZA 50 mg/kg (3 g per day), twice 25 mg/kg a day
- INH 5 mg/kg (300 mg per day), twice 2.5 mg/kg a day

Results

- Culture conversion in 90%
- X-ray improvement in 75%
- Hepatitis in 6 patients (10%) , one of which was fatal

Authors' conclusions:

1. "PZA+INH exerts a high degree of antituberculous action in vivo of a sort superior and qualitatively different from that of current antituberculous drugs used either singly or together"
2. "The high incidence of hepatitis during the administration of INH+PZA makes their use together in present dosage an unadvisable procedure in the treatment of tuberculosis"

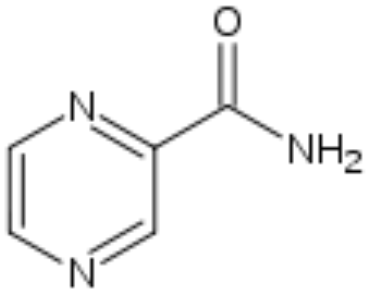
   It was the death penalty for pyrazinamide!

The intracellular activation of pyrazinamide (G.B. Mackaness. Am Rev Tub 1956; 74: 18-28)

Intracellular *M. tuberculosis* present in monocyte cultures								
	After 24h of incubation	After 4 days of incubation						
		µg/ml of pyrazinamide (Z)					H*	Z/H
	0	0	6.25	12.5	25	50	10	12.5/10
Bacilli per monocyte	1.1	4.1	2.9	1.2	1.2	0.9	0.6	0.5
% of infected monocytes	37.0	55	59	36	37	37	27	25.0
Coefficient of increase	-	3.7	2.6	1.1	1.1	0.8	0.6	0.5

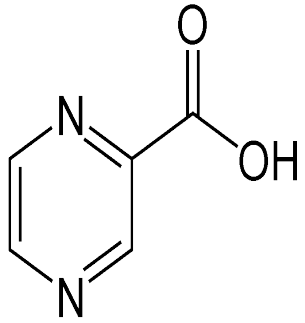
*H= isoniazid

1953 -1956 -1959. K. Konno: the niacin test to identify *M. tuberculosis*
M. tuberculosis produces a great amount of niacin and has a pyrazinamidase/nicotinamidase

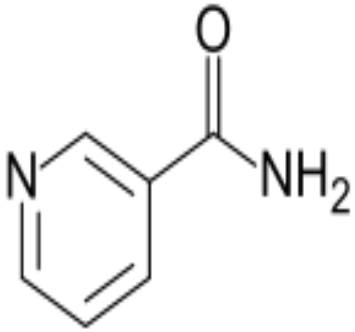


Pyrazinamide
(pyrazine-2-carboxamide)

Pyrazinamidase

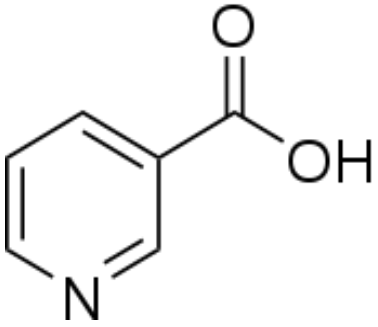


Pyrazinoic acid
(pyrazine-2-carboxylic acid)



Nicotinamide

The same enzyme



Nicotinic acid
Niacin

Nicotinamidase

Pyrazinamide, a phoenix ?

The phoenix depicted in the Aberdeen Bestiary (ca. 12thC)

Pyrazinamide, a phoenix ?

Phoenix depicted in the book of mythological creatures by F.J. Bertuch (1747-1822).

Pyrazinamide, a phoenix ?

The phoenix on top of Kinkaku-ji temple, Kyoto, Japan


Where is or who is the phoenix?

The phoenix properties

(Ancient Greek: Φοῖνιξ, phoínix)

- A phoenix is a mythical bird that is a fire spirit with a colorful plumage and a tail of gold and scarlet.
- It has a 500 to 1000 year life-cycle, then burns fiercely and reduces to ashes, from which a new, young phoenix reborn anew to live again.
- The bird's cry is that of a beautiful song.
- The Phoenix's ability to be reborn from its own ashes implies that it is immortal.

The rebirth of the phoenix pyrazinamide

"It has been argued that streptomycin plus pyrazinamide together form a single bactericidal drug, with streptomycin active in tissues with alkaline pH and pyrazinamide, in those with an acid pH*; pyrazinamide is much cheaper than rifampin"

References:
Mitchison DA. Bacteriological mechanisms in recent controlled chemotherapy studies. Proc. XX Int. Tuberc. Conf 1970, p.316
Fox W. Mitchison DA. Short-course chemotherapy for pulmonary tuberculosis. Am Rev Respir Dis 1975; 111: 325-353

Controlled Clinical Trial of daily 6-month regimens for pulmonary tuberculosis

(EA/BMRC Lancet 1972, 1973, 1974)

Drug regimens	**Total patients with favorable results at 6 mo.**	**Relapses between 6 and 30 months**
6SHR	152	4 (3%)*
6SHZ	153	13 (8%)*
2STH/16TH	133	5 (3%)
S=streptomycin 1 g; H= isoniazid 300 mg daily; R, rifampin 450 (<50 kg b.w.) -600 mg; Z=pyrazinamide 2 g ; T, thiacetazone 100 mg *p=0.05		

Controlled Clinical Trial of daily 6-month and 9-month SHZ regimens for pulmonary tuberculosis in Hong Kong

(Am. Rev. Respir. Dis. 1977; 115: 727-735)

Drug regimens	**Total patients with drug-susceptible strains**	**Relapse rate at 30 months**
6SHZ	167	35 (21%)
9SHZ	179	10 (6%)
S = streptomycin 1 g (< 40 y.o.) - 0.75; H = isoniazid 300 mg daily; Z = pyrazinamide 2 g (≥ 110 lb) - 1.5 g.		

1972. Short-course chemotherapy in pulmonary tuberculosis with rifampin (R) and isoniazid (H) + S or E for 2 months

(British Thoracic Association. Lancet 1975, 1976, 1980)

Treatment duration (months)	**Total patients**	**Relapse after follow-up (in months) of**			
		Up to 18	18 to 33	33 to 54	total
6	182	5 (2.7%)	3 (1.6%)	1 (0,5%)	9 (5%)
9	153	0	0	0	0 *
12	181	0	2	0	2 (1.1%)
18	149	0	0	0	0
Total	665	5	5	1	11 (2%)

*2 patients relapsed 4-5 years after stopping treatment
R , 600 mg (450 if <50 kg); H, 300 mg: E, 25 mg/kg daily; S, 0.75 g IM, six days a week.

Conclusion (1976); “ Treatment with 9 months is acceptable as standard chemotherapy for tuberculosis in Britain”.

The 6-month short-course chemotherapy for tuberculosis with 2RHZE/4RH

(British Thoracic Society. Br J Dis Chest 1984; 78: 330-336)

Treatment		**Total patients**	**Relapses after treatment completion**			
Duration	Regimen		Total (%)	Bact.	Radio. ± Histo.	Month of relapse
6 months	2RHZS/4RH	119	2 (1.7)	1	1	5, 45
	2RHZE/4RH	127	4 (3.1)	3	1	2,4,6,33
9 months	2RHE/7RH	127	2 (1.6)	2	0	6,15

Authors' conclusion: The Research Committee of the British Thoracic Society now recommends the use of either of the two 6-month regimens as an alternative to the currently recommended 9-month regimen.

Conclusion
from the TB viewpoint

- 1952: Birth of *Z*
- 1954: Death of *Z* because of hepatitis when given at high dose (3 g / day) in combination with H
- 1970: Rebirth of *Z* (D. Mitchison)
- 1972-1977: The 9-month regimens with SHZ and SHR
- 1984: The 6-month short-course treatment of TB with R + Z + H + E (versus 18 mo. without R and *Z*)
- Being active only on *M. tuberculosis*, *Z* is “the” TB sterilizing drug … and, when present, somewhat mysteriously, is the critical component for the success of nearly all experimental and clinical drug regimens.