

finally acidified with concentrated hydrochloric acid. The crude acid was collected on a filter and recrystallized first from 50% acetic acid and finally from benzene-petroleum ether (b. p. 90–100°). A product resulted which weighed 2.8 g. and melted at 150–152° (uncor.).

That this product is actually 5,6,7,8-tetrahydro-2-naphthoic acid was established by analysis, m. p. and mixed m. p., and by a comparison of the X-ray powder diffraction patterns of the authentic and unknown samples.

The above experiment has been repeated several times with consistent results.

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3-Amidinopyridine (Nicotinamide)

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The failure of Bernthsen¹ to obtain 3-amidinopyridine by the interaction of nicotinonitrile and ammonium chloride, as cited by Krewson and Couch,² makes it desirable to publish details of the preparation of this amidine, accomplished some time ago in these laboratories.

Trypanocidal activity is developed in monoamidines on introduction of a second amidine group,³ and the function of this may be merely to provide a second basic center. Though some of the monoamidines described by Easson and Pyman⁴ fall into this class, the second basic group in these is a weak one. It was thought that a compound such as nicotinamide would possess a second strongly basic center but in fact it titrated as a monoacid base, so that it gave no evidence on the point at issue.

In addition, the work of Fildes⁵ has shown that the antibacterial action of the sulfanil drugs is probably due to competition with an enzymic metabolic process. Hence the synthesis of compounds with a close spatial resemblance to substances vital to, or associated with, bacterial growth, is of particular interest. The relationship between nicotinamide and nicotinamide is sufficiently close to suggest that the latter might have possessed some anti-bacterial properties.

On treating nicotinonitrile⁶ in excess ethyl alcohol with dry hydrogen chloride, a vigorous exothermic reaction occurred, the main reaction product being the hydrochloride of ethyl nicotinate. It was decided, therefore, to use an amount of alcohol only slightly in excess of that required by theory for the production of the corresponding iminoether hydrochloride. Following this procedure, the iminoether base was isolated as an oil which decomposed on attempted distillation at 8 mm. pressure. No further efforts were made to purify this compound, which reacted readily with ammonium chloride to give nicotinamide hydrochloride. The amidine (1 in 4000 aqueous

solution) was not active against *Staphylococcus aureus*; it was also found to be inactive against *T. equiperdum* infection in mice.

Experimental

3-Amidinopyridine.—3-Cyanopyridine (6.0 g.) was dissolved in dry chloroform (50 cc.) to which absolute ethyl alcohol (3.0 g.) had been added. This mixture was saturated at 0° with dry hydrogen chloride and allowed to stand at 0° for sixteen to eighteen hours. The viscous bottom layer which rapidly separated had by this time solidified. The whole was poured into ice-cold 50% sodium hydroxide solution (excess, final reaction alkaline to phenolphthalein), shaken vigorously, and the chloroform extract separated. This was then washed neutral with water, dried (potassium carbonate) and the solvent distilled to leave the crude iminoether base. This was dissolved in 75% ethyl alcohol (20 cc.) containing ammonium chloride (1.2 g.) and heated at 70° for four hours. After filtration (charcoal) a little ammonium chloride was removed after dilution of the liquors with acetone (2–3 vol.). The residual liquors were again diluted with acetone to cloudiness, when they were left to stand at 0° for several hours. 3-Amidinopyridine monohydrochloride separated in long slender colorless needles, m. p. 190°; yield 4 g.

Anal. Calcd. for C₆H₇N₃HCl: N, 26.9; Cl, 21.97. Found: N, 26.1; Cl, 21.6.

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A Medium for Obtaining Maximal Growth Response in Microbiological Assays of Amino Acids

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In microbiological assays of amino acids the data given in support of the proposed media^{1–6} demonstrate that practically no growth was observed when any one of the amino acids essential for the nutrition of the organisms was omitted. When an attempt was made to repeat the work of the above investigators, it was found that substitution of acid-hydrolyzed casein supplemented with cystine and tryptophan produced a greater growth response than when the casein hydrolyzate was replaced by a mixture of eighteen amino acids. The above results indicated that some stimulatory material was absent from the amino acid medium that was necessary for obtaining maximal growth.

The purpose of this paper is to show that maximal growth response with *L. casei* or *L. arabinosus 17-5* can be obtained when certain stimulatory nutrilites are added to a synthetic

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