The Chemical Conversion of Nicotinic Acid and Nicotinamide to Derivatives of *N*-Methyl-2-pyridone by Methylation and Oxidation

By W. I. M. HOLMAN and C. WIEGAND, *Medical Research Council, Department of Experimental Medicine, University of Cambridge, and Farbenfabriken Bayer, Wuppertal-Elberfeld*

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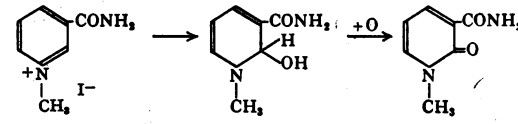
The recent work of Knox (1946) and of Knox & Grossman (1946, 1947) has shown that *N*-methylnicotinamide chloride can be oxidized by an enzyme present in rabbit liver to the amide of *N*-methyl-2-pyridone-5-carboxylic acid. These workers have also reported the isolation of the amide of *N*-methyl-2-pyridone-5-carboxylic acid from human urine after the ingestion of 0·6–0·9 g. of nicotinamide per day. It is already well known that nicotinamide is methylated in the human body and that *N*-methylnicotinamide is normally excreted in human urine. The results of Knox & Grossman (1946, 1947) therefore indicate that part of the *N*-methylnicotinamide formed from nicotinamide in the human body may be oxidized by liver enzymes to *N*-methyl-2-pyridone-5-carboxylic acid amide.

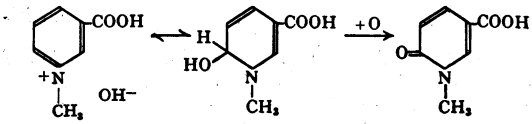
The amide of *N*-methyl-2-pyridone-5-carboxylic acid has not previously been described, but the acid itself was prepared and described by von Pechmann & Welsh (1884), who synthesized it by two methods, viz. by the action of methylamine on the methyl ester of coumalic acid, with subsequent saponification, and by evaporating 2-pyridone-5-carboxylic acid to dryness with potassium hydroxide (2 mol.) and methylating the salt with methyl iodide in methanol. Meyer (1905) prepared the acid by methylating 2-pyridone-5-carboxylic acid with methyl iodide and aqueous alkali.

Neither the method of preparation from coumalic acid and methylamine, which involves the building up of the entire ring structure, nor the method of preparation by methylation of 2-pyridone-5-carboxylic acid, explains how the *N*-methylnicotinamide is converted into the pyridone derivatives *in vitro* or *in vivo*. It seemed, therefore, of interest to see if *N*-methyl-2-pyridone-5-carboxylic acid amide can be formed from *N*-methylnicotinamide by the application of the method of Decker (1892, 1893) for the conversion of pyridinium compounds to 2-pyridones by means of alkaline ferricyanide. *N*-Methylnicotinamide was prepared by methylation of nicotinamide, and was oxidized with alkaline ferricyanide. By extraction of the reaction mixture with chloroform a compound of m.p. 216–216·5°* was isolated, which had the same elementary composition as the amide of *N*-methyl-2-pyridone-5-carboxylic acid, but which showed ultraviolet absorption properties entirely different from those reported by Knox & Grossman (1947), and which fluoresced strongly in ultraviolet light. In order to check the identity of this compound it was saponified. An acid was obtained with m.p. 183–183·5°. This acid was *N*-methyl-2-pyridone-3-carboxylic acid, for which Späth & Koller (1923) reported a melting point of 184°, and not *N*-methyl-2-pyridone-5-carboxylic acid, which has a widely different melting point (237–238° according to von Pechmann & Welsh (1884); 238–239° according to Meyer (1905)). The following series of reactions therefore occurred:



The product of the oxidation of *N*-methylnicotinamide was the amide of *N*-methyl-2-pyridone-3-carboxylic acid and not that of *N*-methyl-2-pyridone-5-carboxylic acid.

In a search of the literature a patent (D.R.P. no. 522060) of Räth (1932) was found in which it was stated that *N*-methyl-2-pyridone-5-carboxylic acid may be prepared from nicotinic acid by methylation with methyl sulphate followed by oxidation. Nicotinic acid was therefore methylated by a method similar to that used by Winterstein & Weinhagen (1917), and the product was oxidized with alkaline ferricyanide. A good yield of *N*-methyl-2-pyridone-5-carboxylic acid (m.p. 238–238·5°) was obtained from the reaction mixture. The following reactions occurred:

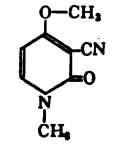


* Unless otherwise stated, the melting points given in this paper are uncorrected.

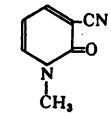
It seems that, *in vitro*, the methylation and oxidation of nicotinic acid yields *N*-methyl-2-pyridone-5-carboxylic acid, while the methylation and oxidation of nicotinamide gives the amide of *N*-methyl-2-pyridone-3-carboxylic acid. There is no evidence to suggest that mixtures of both isomers are formed in either of these reactions, since, if both amides were formed by the oxidation of *N*-methylnicotinamide, they would be extracted by chloroform* and would give, after saponification, a mixture of acids with an ill defined melting point; if both acids were formed by the methylation and oxidation of nicotinic acid, they would be precipitated by hydrochloric acid and would also give a mixture with an incorrect melting point. It was thought possible that the course of the reactions might be influenced by the nature of the methylating agent used. To test this point nicotinic acid was methylated with methyl iodide and the product oxidized, and nicotinamide was methylated with methyl sulphate and the *N*-methyl compound was oxidized. The same results were obtained as in the previous experiments, viz. nicotinic acid gave *N*-methyl-2-pyridone-5-carboxylic acid, and nicotinamide gave *N*-methyl-2-pyridone-3-carboxylic acid amide. The methylating agent, therefore, had no influence on the course of either of the reactions.

amide by a different series of reactions in the animal body from that *in vitro*, or that *N*-methyl-2-pyridone-5-carboxylic acid is first formed from nicotinic acid and is subsequently converted to the amide.

The amide of *N*-methyl-2-pyridone-3-carboxylic acid, which is formed by the oxidation of *N*-methylnicotinamide *in vitro*, but not in the animal body, has a similar structure to two compounds of interest in plant biochemistry, viz. ricinine and ricinidine. It is conceivable that plants may be capable of producing these substances from *N*-methylpyridinium compounds by oxidation.



Ricinine



Ricinidine

The amide of *N*-methyl-2-pyridone-5-carboxylic acid was prepared by converting the acid to the acid chloride with thionyl chloride and by decomposing the acid chloride with aqueous ammonia; it was also prepared from the ethyl ester of the acid. The ultraviolet absorption of the amide prepared by these methods (see Fig. 1) was identical with that reported

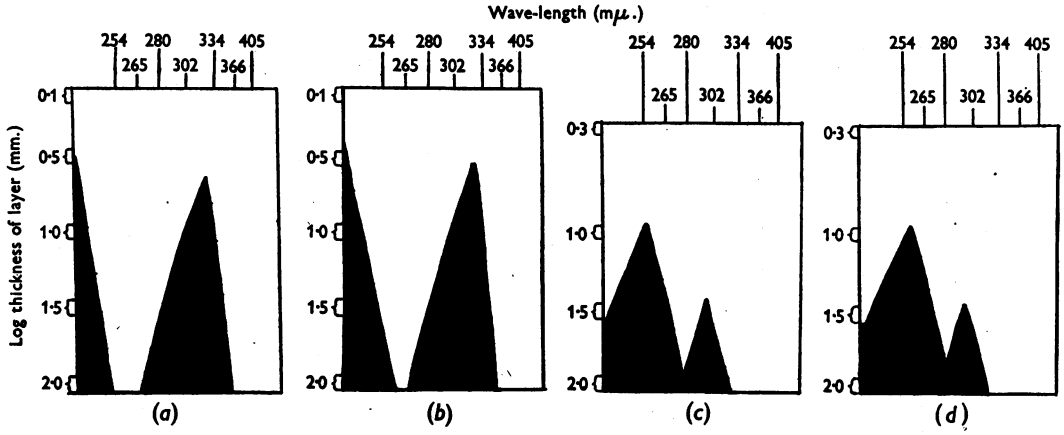


Fig. 1. Approximate diagrammatic reproductions of ultraviolet absorption photographs. (*a*) *N*-Methyl-2-pyridone-3-carboxylic acid, 0·005 % in water. (*b*) *N*-Methyl-2-pyridone-3-carboxylic acid amide, 0·005 % in water. (*c*) *N*-Methyl-2-pyridone-5-carboxylic acid, 0·001 % in water. (*d*) *N*-Methyl-2-pyridone-5-carboxylic acid amide, 0·001 % in water.

The results of the above experiments indicate either that the amide of *N*-methyl-2-pyridone-5-carboxylic acid is produced from *N*-methylnicotinamide by a different series of reactions in the animal body from that *in vitro*, or that *N*-methyl-2-pyridone-5-carboxylic acid is first formed from nicotinic acid and is subsequently converted to the amide.

* See later sections for the preparation of the amide of *N*-methyl-2-pyridone-5-carboxylic acid. Both the 2:3- and the 2:5-amides are slightly soluble in chloroform and are reasonably stable in the alkaline ferricyanide solution. Neither of the isomeric acids could be isolated from the mother liquor after extraction with chloroform.

The amide of *N*-methyl-2-pyridone-5-carboxylic acid, which is formed by the oxidation of *N*-methylnicotinamide *in vitro*, but not in the animal body, has a similar structure to two compounds of interest in plant biochemistry, viz. ricinine and ricinidine. It is conceivable that plants may be capable of producing these substances from *N*-methylpyridinium compounds by oxidation.

The amide of *N*-methyl-2-pyridone-5-carboxylic acid was prepared by converting the acid to the acid chloride with thionyl chloride and by decomposing the acid chloride with aqueous ammonia; it was also prepared from the ethyl ester of the acid. The ultraviolet absorption of the amide prepared by these methods (see Fig. 1) was identical with that reported by Knox & Grossman (1947). Its elementary composition agreed closely with the theoretical values. The melting points of four samples prepared by different methods (201–204°; 204–206·5°; 203–205°; 202–203° (205·5–206·5° corrected)) were definitely lower than that of 212–214°, which was found by Knox & Grossman (1947) for *N*-methyl-2-pyridone-5-carboxylic acid amide prepared enzymically from *N*-methylnicotinamide chloride. Further

efforts to test the purity of our specimens of the amide showed that the acid (m.p. 236–237°) could be recovered by saponification with 2·5N-sodium hydroxide, and that no change in the melting point resulted after repeated recrystallizations from various solvents, after extraction with chloroform or ether, or after previous heating of the substance to its melting point.

It is interesting to observe (Fig. 1) that there is a wide difference in ultraviolet absorption between the amide of *N*-methyl-2-pyridone-3-carboxylic acid and that of *N*-methyl-2-pyridone-5-carboxylic acid. The former compound shows strong blue fluorescence in ultraviolet light, the latter has no fluorescence. Knox & Grossman (1947) stated that *N*-methyl-2-pyridone-5-carboxylic acid amide fluoresces slightly under the conditions used in the estimation of *N*-methylnicotinamide in urine, but their preparations, which were not white in colour, may have contained traces of impurities.

The present results suggest that, in the estimation of aneurin in urine by the thiochrome method, the alkaline ferricyanide used to oxidize aneurin to thiochrome may also convert a part, at least, of the *N*-methylnicotinamide present to the fluorescent *N*-methyl-2-pyridone-3-carboxylic acid amide, which would then be extracted by *iso*butanol together with the thiochrome. The error, which Najjar & Ketron (1944) found to be introduced into the estimation of aneurin in urine by the presence of *N*-methylnicotinamide, may be attributable to a difference in the intensity of fluorescence of *N*-methyl-2-pyridone-3-carboxylic acid amide compared with that of the product of the reaction of alkali alone on *N*-methylnicotinamide, and/or to partial destruction of the latter substance by alkaline ferricyanide and incomplete formation of the former. Najjar & Ketron (1944) suggested that *N*-methylnicotinamide was converted to a pyridone by alkali and ferricyanide, but they did not isolate and identify the compound.

EXPERIMENTAL

N-*Methylnicotinamide iodide*

Nicotinamide (25 g.) was dissolved in methanol (50 ml.) and the solution boiled gently under reflux for 8 hr. with 43·7 g. methyl iodide (1·5 mol.). After removal of methanol and of the excess of methyl iodide by filtration, *N*-methylnicotinamide iodide remained as a pale yellow crystalline mass (54·3 g.) of m.p. 203–204° (cf. m.p. 204° reported by Karrer, Schwarzenbach, Benz & Solmssen, 1936).

N-*Methyl-2-pyridone-3-carboxylic acid amide*

N-Methylnicotinamide iodide (20 g.) was dissolved in water (120 ml.) in a 1 l. flask. The flask was cooled in ice. To the contents were added slowly, with constant stirring, a solution of 53 g. $K_3Fe(CN)_6$ in 170 ml. of water, and, simultaneously, a solution of 20 g. KOH in 40 ml. water. The mixture was stirred for 1 hr. The precipitated $K_4Fe(CN)_6$ was filtered off and *N*-methyl-2-pyridone-3-carboxylic acid amide was isolated from the filtrate by extracting repeatedly with chloroform. After evaporation of the combined chloroform extracts to dryness, the pale brown residue (2 g.) was purified by crystallization from methanol. The compound crystallized in the form of white needles with a faint greenish fluorescence, m.p. 216–216·5°. Späth & Koller (1923) reported a m.p. of 216° for this compound. (Found: C, 55·6; H, 5·64; N, 18·4. Calc. for $C_7H_8O_2N_2$: C, 55·2; H, 5·26; N, 18·4%.) Both the 2:3- and the 2:5-amides tend to leave a residue which contains N and C. Care must therefore be taken in the determination of N and C to ensure complete combustion.

N-*Methyl-2-pyridone-3-carboxylic acid*

N-Methyl-2-pyridone-3-carboxylic acid amide (0·5 g.) was saponified by boiling gently under reflux for 3 hr. with 2·5 ml. of 2·5N-NaOH. The solution was acidified with 2N-HCl and the precipitated *N*-methyl-2-pyridone-3-carboxylic acid was filtered off. After crystallization from water the acid was obtained as thin, pure-white needles of m.p. 183–183·5°. Späth & Koller (1923), who prepared this compound by another method, found m.p. 184°.

N-*Methyl-2-pyridone-5-carboxylic acid*

Nicotinic acid (82 g.) was covered with methanol (200 ml.), and the mixture boiled gently under reflux with 90 g. methyl sulphate. After about 0·25 hr. the nicotinic acid dissolved. After heating for 2 hr. the methanol and the excess of methyl sulphate were removed *in vacuo*. The viscous brown liquid which remained was transferred to a 3 l. flask with 200 ml. water. The flask was cooled in ice, and to the contents were added, drop by drop, with constant stirring, a solution of 440 g. $K_3Fe(CN)_6$ in 750 ml. water, and, simultaneously, a solution of 194 g. KOH in 500 ml. water. The resulting mixture was stirred for 1 hr. and was filtered to remove precipitated $K_4Fe(CN)_6$. The filtrate was acidified with 6N-HCl. The precipitated *N*-methyl-2-pyridone-5-carboxylic acid was filtered off and washed on the filter four times with cold water (yield 60 g.). It was dissolved in hot water and the solution boiled with charcoal. After crystallization from water, white needles (30 g.) were obtained with m.p. 238–238·5°. This melting point agrees well with the values of 237–238° reported by von Pechmann & Welsh (1884), and of 238–239° found by Meyer (1905).

N-*Methyl-2-pyridone-5-carboxylic acid ethyl ester*

N-Methyl-2-pyridone-5-carboxylic acid (10 g.) was esterified by heating for 6 hr. with absolute ethanol (100 ml.), through which a stream of dry HCl was bubbled continuously. The ethanol and HCl were removed by evaporation *in vacuo*. The dry residue was ground in a mortar with solid Na_2CO_3 and the ester (m.p. 72·5–74°) was extracted with benzene. It was purified by crystallization from a mixture of benzene (1 vol.) and ligroin (3 vol.); m.p. 72–73·5° (cf. m.p. 74° reported by Räth & Schiffmann, 1931).

N-*methyl-2-pyridone-5-carboxylic acid amide*

(*a*) *N*-Methyl-2-pyridone-5-carboxylic acid (10 g.) was boiled gently under reflux for 1–2 hr. with 50 ml. thionyl chloride. The excess of thionyl chloride was evaporated

in vacuo. The greenish yellow crystalline residue was ground in a mortar, and was slowly added to 200 ml. conc. NH_3, with cooling. The mixture was stirred continuously for 3 hr. *N*-Methyl-2-pyridone-5-carboxylic acid amide was precipitated as a white crystalline mass, which was filtered off (yield 5 g., m.p. 201–203·5°), and purified by crystallization from absolute ethanol (pure-white needles of m.p. 201–204°). (Found: C, 54·8; H, 5·32; N, 18·2; amide N, 9·0. Calc. for $C_7H_8O_2N_2$: C, 55·2; H, 5·26; N, 18·4; amide N, 9·2%.)

(*b*) The same procedure was used as in (*a*), except that 22·5 g. of *N*-methyl-2-pyridone-5-carboxylic acid and correspondingly greater amounts of the other reagents were taken, and the time of heating with thionyl chloride was somewhat shorter (1 hr.). The yield of the crude product was 15 g. After two recrystallizations from absolute ethanol 8·5 g. of white needles of m.p. 204–206·5° were obtained.

(*c*) The procedure was the same as in (*a*), except that the time of heating with thionyl chloride was reduced to 20 min. After crystallizing once from absolute ethanol, white needles of m.p. 202–203·5° were obtained. The product, however, contained a trace of an impurity, presumably unchanged acid, which melted at 233·5°. After treatment with a solution of $NaHCO_3$ and crystallization from absolute ethanol, no trace of the high-melting impurity remained and the melting point was sharp (202–203°, uncorrected; 205·5–206·5°, corrected). After saponification for 7 hr. with 2·5N-NaOH, *N*-methyl-2-pyridone-5-carboxylic acid was recovered (m.p. 236–237°).

(*d*) *N*-Methyl-2-pyridone-5-carboxylic acid ethyl ester (5 g.) was heated for 8 hr. at 200° under a pressure of 63 kg./sq.cm. with 20 ml. methanol saturated with NH_3. After removal of methanol and NH_3 by evaporation on a steam bath, the residue was extracted once with benzene to remove unchanged ester. The amide of *N*-methyl-2-pyridone-5-carboxylic acid was isolated from the benzene-insoluble residue by crystallization from absolute ethanol. It was purified by a second crystallization from absolute ethanol; almost white crystals of m.p. 203–205° were obtained.

SUMMARY

1. Methods are described for the preparation of the amide of *N*-methyl-2-pyridone-3-carboxylic acid by the methylation and oxidation of nicotinamide, and for the preparation of *N*-methyl-2-pyridone-5-carboxylic acid by the methylation and oxidation of nicotinic acid. Methods are also given for the conversion of the former compound to the corresponding acid, and of the latter compound to the corresponding amide.

2. The properties of the two isomeric acids and amides are compared. The course of the reactions which occur in the formation of these compounds *in vitro* is discussed in relation to the biological formation of the amide of *N*-methyl-2-pyridone-5-carboxylic acid from nicotinamide in the human body.

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Absorption of 3-Methylglucose from the Small Intestine of the Rat and the Cat

By P. N. CAMPBELL and H. DAVSON
Departments of Biochemistry and Physiology, University College, London

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On the basis of the phosphorylation theory (Verzár & McDougall, 1936) certain sugars are absorbed from the intestine by an active process, one stage of which involves the phosphorylation of the sugars. Sugars so absorbed are thought to leave the gut more rapidly than those depending only on simple diffusion, and it is customary to judge whether or not a sugar is actively absorbed by comparing its rate of absorption with that of glucose. On this criterion, galactose and glucose are classed as being actively absorbed whilst the pentoses, such as xylose, are thought to enter by simple diffusion only. In the course of work on the synthetic 3-methylglucose, it became of interest to determine whether it was actively absorbed from the intestine. Two approaches were made: first, the rate of absorption from the