THE FLUORESCENT CONDENSATION PRODUCT OF N'-METHYL NICOTINAMIDE AND ACETONE

I. SYNTHESIS AND PROPERTIES

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In 1940 Najjar and Wood (1) detected the presence of an unknown substance appearing in human urine after the ingestion of nicotinic acid and nicotinamide. This material was isolated from urine by Huff and Perlzweig (2, 3) and was shown to be the ion N1-methylpyridinium-3-carboxylic acid amide, designated as N1-methylnicotinamide (I in Fig. 1).

In an investigation of the chemical properties of this substance it was observed that the quaternary ion form (I) is relatively unreactive, but that the α-carbinol (II) produced by the action of alkali on the quaternary ion is a very reactive substance. It was found to condense with a variety of alcohols, aldehydes, and ketones to yield highly fluorescent derivatives. Those formed with the alcohols are readily decomposed on acidification and are presumably carbinol ethers (III) of the type described by Decker and Kaufmann (4), as formed by the α-carbinols of the N-methylpyridines and N-methylquinolines with alcohols. However, the condensation products of N1-methylnicotinamide with certain aldehydes and ketones are quite stable and can be isolated in crystalline form.

There are presented here the synthesis, properties, and the possible structure of a compound obtained when the α-carbinol of N1-methylnicotinamide is condensed with acetone. This reaction is the basis of new fluorometric methods for the quantitative estimation of N1-methylnicotinamide in urine (6) and of the pyridine nucleotides in the blood cells (7).

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1 Huff, J. W., unpublished data.

2 Najjar, White, and Scott (5) observed independently that F2 (the fluorescent derivative produced from N1-methylnicotinamide by treatment with alkali and butanol) reacts with acetone in alkaline solution to produce a yellow compound which shows a yellow-green fluorescence on extraction into butanol.
EXPERIMENTAL

Synthesis and Isolation—N1-Methylnicotinamide chloride, when treated in aqueous acetone solution at room temperature with KOH followed by boiling with HCl, is converted to a yellow highly fluorescent compound which was isolated from the reaction mixture as its hydrochloride.

To 2.5 gm. of N1-methylnicotinamide chloride, dissolved in 130 ml. of a mixture containing equal volumes of water and acetone (redistilled over KMnO4), were added 8 ml. of 7 N KOH with stirring, and the mixture was allowed to stand for 12 hours at room temperature. The solution became slightly warm and turned a deep orange color upon the addition of the alkali. After 12 hours, 15 ml. of concentrated HCl were added; the flask was covered with a glass funnel and heated in a boiling water bath for 20 minutes. The solution was then evaporated almost to dryness in vacuo. The residue was transferred to a small beaker, just covered with absolute ethanol, and warmed at about 50° for 1 hour with occasional stirring. Absolute alcohol was added to replace that lost during the warming and the mixture kept at —20° for 24 hours. The semisolid mass was drained on a small Büchner funnel, and after several washings with cold (—10°) absolute ethyl alcohol there remained on the filter about 6.1 gm. of light yellow crystalline material consisting of KCl and the condensation product.

![Diagram of probable pathways of condensation reaction of N1-methylnicotinamide with acetone.](http://www.jbc.org/)

Fig. 1. Probable pathways of the condensation reaction of N1-methylnicotinamide with acetone.
amber-colored mother liquor yielded on evaporation a dark fluorescent oil which could not be crystallized. The condensation product was separated from the salt by converting it to the free base and extracting into absolute alcohol in the following manner: To the yellow residue there were added about 15 ml. of water and the solution was titrated potentiometrically to pH 11 with 12 N KOH solution. The alkaline solution was evaporated to near dryness under vacuum and dried in a desiccator. To the dried orange-red residue there were added about 40 ml. of absolute ethanol; the mixture was allowed to stand for about 1 hour at 30° with occasional stirring and was then held at -20° for 24 hours, and the KCl was removed by filtration. The filtrate was brought to a volume of about 100 ml. with absolute ethanol and concentrated HCl added to about pH 3 (indicator paper). The acid solution was heated to boiling and 100 mg. of Darco charcoal were added. After stirring for a few minutes, the hot mixture was filtered. The light yellow filtrate was concentrated to 75 ml. and placed in the refrigerator (5°) for 48 hours. After filtering and washing with cold alcohol there were obtained 980 mg. of crystalline material.

Properties—The substance crystallizes from alcohol as its hydrochloride in long yellow prisms. The melting point is 304–305° with decomposition with a copper block previously heated to 295°. The hydrochloride is very soluble in water, dilute alcohol, and boiling alcohol, sparingly soluble in cold alcohol, and insoluble in ether, chloroform, benzene, and acetone. In an acidic aqueous or alcoholic solution it exhibits in ultraviolet light an intense blue fluorescence which is reversibly converted to a green fluorescence in alkaline reaction. The variation of the fluorescence intensity of this substance with pH is shown in Fig. 2.

There are also included in Fig. 2 the pH-fluorescence curves for the mother liquor remaining after the isolation of the crystalline compound described here, and the curve for the fluorescence derived from 0.9 γ of N1-methylnicotinamide when treated with acetone and alkali in the analytical method (6) for the quantitative determination of this substance. The fluorescence of the crystalline product is stable between pH 1.8 and 5.5 and drops off sharply on the alkaline side. A similar curve is found on the acid side for the fluorescence obtained in the analytical method, but in this case the intensity increases in alkali. It is thus apparent that the crystalline product, as isolated here, does not represent the only compound being measured in the analytical method. The pH-fluorescence curve for the mother liquor indicates the presence of a compound, showing a rise in fluorescence on the alkaline side.

Further evidence that two compounds are present is shown by the fact that a quantitative fluorometric measurement on the reaction mixture, obtained by treating 2.0 gm. of N1-methylnicotinamide with acetone and
alkali, indicates a 100 per cent yield of the fluorescent derivative. However, only 1 gm. of the crystalline product could be isolated, a 30 per cent yield. The remainder of the fluorescence could be accounted for in the mother liquor. These observations lead to the interference that at least two derivatives are formed in the condensation reactions. The possibility of an oxidation-reduction equilibrium is discussed below, in relation to the possible configuration of the products.

An elementary analysis of the crystalline condensation products for C, H, N, and Cl indicates an empirical formula of $C_{10}H_{10}N_2O\cdot HCl$ corresponding to a molecular weight of 210.6. A molecular weight determination by the freezing point lowering of camphor was 206 and 218. An aqueous solution of the compound is neutral. A potentiometric titration (Fig. 3) confirms the molecular weight of 211. The titration indicated the presence of a rather strong basic group with $pK = 9.2$. The substance forms insoluble derivatives with the base-precipitating agent, picric acid, AuCl₃, and HgCl₂. The substance is unchanged on boiling for 1 hour in 2 N HCl. Boiling for 1 hour with 2 N KOH resulted in a 10 per cent loss of the substance, as indicated by fluorometric measurements. Reduction with Na₂S₂O₄, or with Zn-HCl, converts the compound to a non-fluorescent
derivative. The original compound is obtained quantitatively upon the addition of \( \text{H}_2\text{O}_2 \) to the reduced form. The substance does not give a positive reaction with \( \text{FeCl}_3 \) solution, indicating the absence of a free phenolic OH group. An aqueous solution takes up bromine with the formation of a yellow, water-insoluble bromide derivative. The material did not give a positive test with CNBr and aniline, indicating that the N of the pyridine ring remained in the substituted state.

The ultraviolet absorption curve of the condensation product dissolved in water shows absorption maxima at 2530 to 2540 Å and at 3480 to 3520 Å, with extinction coefficients for 1 per cent solutions corresponding to \( \varepsilon = 292 \) and 722, respectively.\(^3\)

The probable configuration of the condensation product on the basis of the available data is believed to be 1,7-dimethyl-5-oxo-(1,5-dihydro-1,6-naphthpyridine) hydrochloride, compound VI in Fig. 1. This substance has an empirical formula of \( \text{C}_{10}\text{H}_{10}\text{N}_2\text{O} \cdot \text{HCl} \) and a molecular weight of 210.6, which is in good agreement with the following elementary analysis.

Calculated. \( \text{C} 57.02, \text{H} 5.25, \text{N} 13.29, \text{Cl} 16.83 \)

Found.\(^4\) \( \text{C} 56.82, \text{H} 5.38, \text{N} 13.00, \text{Cl} 16.96 \)

\( \text{C} 56.60, \text{H} 5.31, \text{N} 13.18, \text{Cl} 16.75 \)

\( ^3 \) These measurements were made on the Beckman quartz spectrophotometer. The author is obliged for them to Dr. W. J. Dann.

\( ^4 \) The elementary analyses reported in this paper were carried out in the Laboratory of Microchemistry, Dr. Carl Tiedcke, New York.
The stability of the compound on heating with acid indicates that the linkage of the carbon in the α position of the pyridine ring with the acetone is a \(-\text{C}–\text{C}–\) linkage (compound IV) rather than a labile ether linkage (III), as produced by the α-carbinol (II) with alcohol. Since the compound is not altered by heating with acid or alkali, the \(-\text{NH}_2\) of the labile amide group has been either eliminated or stabilized in the course of the condensation reaction. That it has not been eliminated is shown by the presence of 2 equivalents of nitrogen in the compound; that it is not present as a free \(-\text{NH}_2\) group is shown by the failure to obtain a colored derivative upon diazotization and coupling with \(\beta\)-naphthol. The conclusion is therefore drawn that the \(-\text{NH}_2\) of the amide group is stabilized by cyclization with the carbonyl group of the acetone (compounds IV and V).

The difference between the proposed structure (VI) and the structure (V) which would necessarily follow from cyclization of compound IV is that the former calls for 11 H atoms, while the latter calls for 13. It is believed that 2 hydrogen atoms have been lost between the carbons in the 8 and 9 positions as a result of a state of oxidation-reduction equilibrium between the isolated crystalline product and the oily substance remaining in the mother liquor. This supposition is supported by the ready and reversible reducibility of the crystalline compound with Zn-HCl and with hydrogensulfite.

The above is all the available evidence that can be presented for the probable structure of the compound. It is not entirely conclusive, and the problem still awaits final solution.

**SUMMARY**

The synthesis and the properties of a highly fluorescent derivative of \(\text{N}^1\)-methylnicotinamide with acetone are described. It is tentatively proposed on the basis of the available evidence that the configuration of the product is \(1,7\text{-dimethyl-5-oxo-(1,5-dihydro-1,6-naphthyridine)}\) hydrochloride.

**BIBLIOGRAPHY**

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