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Synthesis of 1,4-dihydro-1-methyl-4-oxonicotinonitrile-8-^{14}C * +

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SUMMARY

The possibility that 1,4-dihydro-1-methyl-4-oxonicotinonitrile (V) is an intermediate in the biosynthesis of the alkaloid ricinine from nicotinic or quinolinic acid in Ricinus communis *L. led to our interest in preparing the carbon-14 labeled compound. The 4-oxonicotinonitrile (V) and the corresponding 6-oxonicotinonitrile were shown to be oxidation products of nicotinonitrile methiodide by a crude enzyme preparation from castor seedlings (1, 2). The 6-oxonicotinonitrile was shown to be identical to the alkaloid nudiflorine isolated from* Trewia nudiflora *(3).*

EXPERIMENTAL.

The synthesis of 1,4-dihydro-*1*-methyl-4-oxonicotinonitrile-8-^{14}C (V) was accomplished in a manner similar to published procedures (2, 4, 5) with a few exceptions. Changes in reaction conditions were necessary because of the micro scale of the final step in the reaction sequence where the carbon-14 label was introduced. Changes in the purification steps were necessary because of the inability to reproduce the results of earlier authors.

The flow diagram describing the steps used in the synthesis of V is shown in Figure 1. Three grams (31.6 mmoles of 4-pyridinol (I) was added to a mixture of fuming nitric acid and fuming sulfuric acid containing 20 % sulfur trioxide. This mixture was heated gently until turbulence ceased and then boiled for one hour. The cooled mixture was poured into cold water with stirring and adjusted to pH 4-5 with ammonium hydroxide. The resulting

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+ Taken from the M. S. Thesis of H. E. Johnson, Oklahoma State University, May 1968 (work completed November 1967).

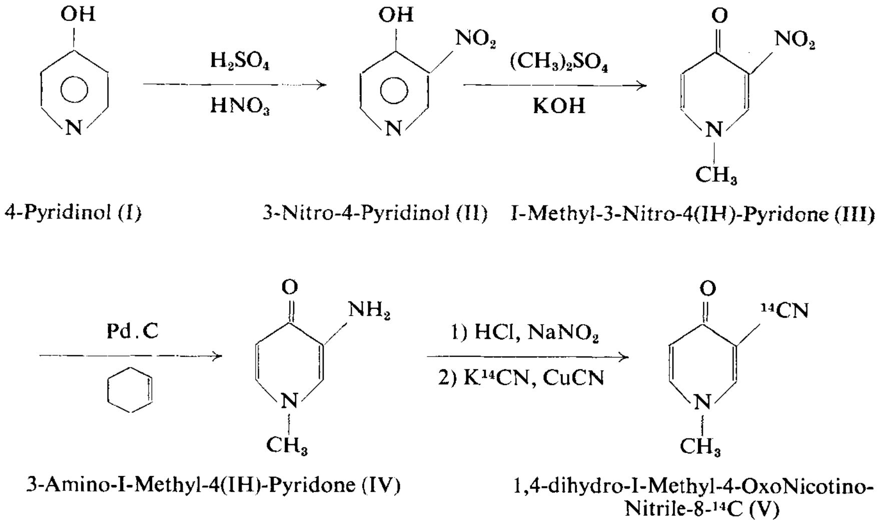

FIG. 1. Synthesis of 1,4-dihydro-1-methyl-4-oxonicotinonitrile-8-^{14}C

precipitate of 3-nitro-4-pyridinol (II) was collected by centrifugation, washed and recrystallized from water.

3-Nitro-4-pyridinol (II), 1.85 grams (13.2 mmoles), was added to a mixture of 5 ml of dimethyl sulfate in 20 ml of water. The mixture was heated, treated with solid potassium hydroxide until solution was complete, and allowed to stand overnight at room temperature. The yellow crystalline product of 1-methyl-3-nitro-4(1H)-pyridone (III) was collected, washed and recrystallized from water.

III was reduced to the corresponding amine by a hydrogentransfer method. A 10 % palladium-on-charcoal catalyst (0.5 gram) and 2.2 ml of cyclohexene were added to a solution of 0.7 gram (4.5 mmoles) of III in methanol. After refluxing for 24 hours the catalyst was removed by filtration, and the filtrate was evaporated. The residue was dissolved in acetone, and the resulting solution was decolorized by passing it over activated charcoal. Crystallization of 3-amino-1-methyl-4(1H)-pyridone (IV) from this solution was induced by addition of diethyl ether.

V was synthesized from IV by a Sandmeyer reaction (2). Two drops of 20 % sodium nitrite solution was added to a solution of 25 mg (200 μmoles) of freshly recrystallized IV in 0.3 ml of 2 *N* hydrochloric acid. The formation of the diazonium salt was considered to be complete when a positive test for excess nitrous acid was obtained using starch-iodide paper. The reaction mixture was kept in a salt-ice bath during the addition of sodium nitrite and was allowed to stand at 0° for one hour after the addition of sodium nitrite. This solution was neutralized with potassium carbonate and added to a solution of 15 mg (230 μmoles) of carbon-14 labeled potassium cyanide, specific activity 17.5 mμc/μmoles, purchased from New England Nuclear Corp., and 0.5 mg (6 μmoles) of cuprous cyanide. This mixture was heated gently until the evolution of nitrogen gas was complete. The solution was then extracted twice with 5 ml portions of diethyl ether, and the aqueous solution was evaporated to dryness. The residue which contained crude carbon-14 labeled V was extracted several times with hot acetone, and the acetone solution was passed through activated charcoal and evaporated to dryness. Further purification of the 1,4-dihydro-1-methyl-4-oxonicotinonitrile-8-^{14}C (V) was effected by extraction with small volumes of chloroform followed by preparative paper chromatography using Whatman No. 1 paper and *N*-butanol saturated with 3 % ammonia as the solvent. The purified V had a specific activity of 12.7 mμc/μmole.

The chloroform extract containing V had two impurities, one detectable on paper chromatograms by its absorption at 254 mμ and another by its radioactive label. The radioactive impurity contained about 5 % of the total activity in the extract.

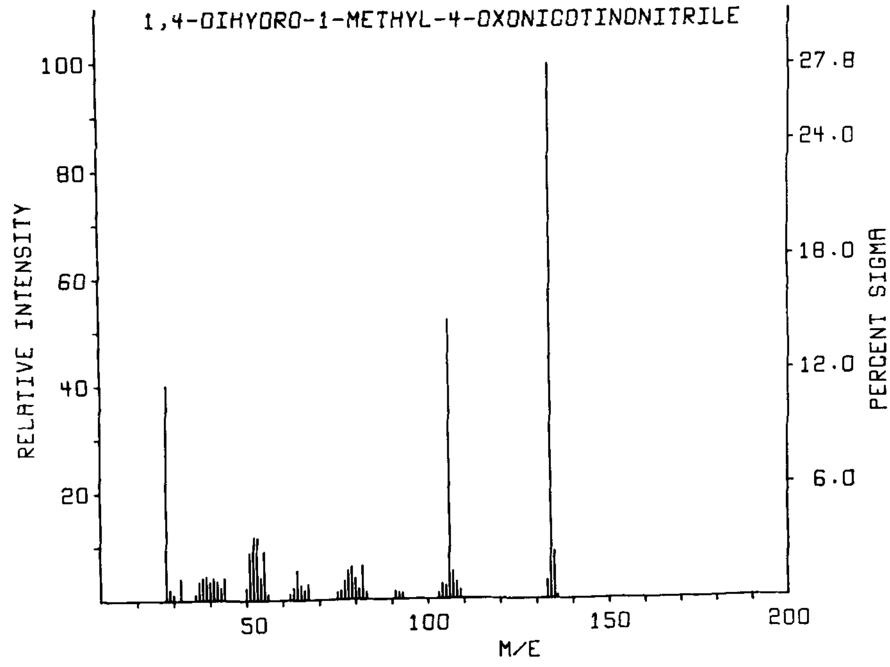

FIG. 2. Mass spectrum of 1,4-dihydro-1-methyl-4-oxonicotinonitrile

This spectrum was computer plotted from tabular intensity data. A CalComp 565 plotter driven by an IBM 1620 computer required about 3 min. of ploting time and 6 min. of computer time (for a Fortran II-D program).

After purification by paper chromatography V was tested for radiochemical purity with the aid of a Nuclear-Chicago Actigraph III paper strip counter on chromatograms developed in the following solvent systems : 85 % 2-propanol, *N*-butanol-water-acetic acid (4 : 2 : 1), *N*-butanol saturated with 3 % ammonia, and acetone-water (7 : 3). In each case only one radioactive spot was observed. Ultraviolet spectral data obtained on V with a Beckman DB spectrophotometer agreed well with published data (2). The absorption peak at 256 mμ has a log ε of 4.15 and the shoulder at 282 mμ a log ε of 3.70. The mass spectrum (Fig. 2) of V obtained with a prototype of the LKB Model 9000A mass spectrometer (6.7) using the direct probe gave the expected mol. wt. of 134. The molecular ion peak was the most intense peak of the 70 ev spectrum.

REFERENCES

1. Robinson, T. — *Phytochemistry*, **4** : 67 (1965).
2. Robinson, T. and Cepurneek, C. — *Phytochemistry*, **4** : 75 (1965).
3. Mukherjee, R. and Chatterjee, A. — *Chem. and Ind.* (*London*), **35** : 1524 (1964).
4. Ahmad, Y. and Hey, D. H. — *J. Chem. Soc.* : 4516 (1954).
5. Kruger, S. and Mann, F. G. — *J. Chem. Soc.* : 2755 (1955).
6. Waller, G. R. — *Proc. Okla. Acad. Sci.*, **47** : in press (1967).
7. Ryhage, R. — *Arkiv Kemi*, **26** : 305 (1967).