The Pyridine Nucleotide Cycle and Its Role in the Biosynthesis of Ricinine by Ricinus communis L.*

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SUMMARY

The pyridine moieties of the pyridine nucleotides, nicotinic acid mononucleotide, nicotinic acid adenine dinucleotide, and nicotinamide adenine dinucleotide, were shown to be incorporated into ricinine by Ricinus communis L. with an efficiency comparable to that of quinolinic acid, nicotinic acid, and nicotinamide, which had been established previously as being efficient precursors of this alkaloid. All of these pyridine compounds except quinolinic acid were postulated to be closely related in a cyclic form, and they could function as a source of metabolic intermediates for the biosynthesis of ricinine. It was shown that nicotinamide was readily converted to nicotinic acid and N-methylnicotinamide in vivo; nicotinic acid was converted to nicotinamide adenine dinucleotide and N-methylnicotinic acid (trigonelline) in vitro; nicotinamide adenine dinucleotide was readily converted to nicotinamide and N-methylnicotinamide in vivo.

The biosynthesis of pyridine compounds in higher plants has been studied extensively by determining labeling patterns which characterize the incorporation of various isotopically labeled precursors into the pyridine alkaloids, ricinine (1-7), nicotine (8-11), and anabasine (12, 13). The present evidence indicates that the pyridine ring of these alkaloids and of nicotinic acid (14) produced by microorganisms is formed from aspartic acid and a glycerol derivative. Recent experiments on the biosynthesis in vitro of quinolinic acid in a system prepared from Escherichia coli (15) also support the theory of the formation of the pyridine ring from glycerol and aspartate. However, the reports that aspartate-2-14C gives rise to nicotinic acid-7-14C in Serratia marcescens (16) and that formate-14C and acetate-14C but not glycerol-14C are incorporated into the ring carbons of nicotinic acid in a partially purified enzyme system from Clostridium butyricum (17) suggest that alternate pathways for pyridine ring biosynthesis may be operating in some organisms. Pertinent review articles by Ramstad and Agurell (18) and by Leete (19) have appeared recently.

In previous studies from this laboratory (7, 20), it was shown that quinolinic acid was an efficient precursor of ricinine in intact Ricinus communis L. plants. The evidence indicated that quinolinic acid may serve as a key intermediate in the formation of pyridine compounds in higher plants. The conversion of quinolinic acid into nicotinic acid mononucleotide was also shown in vitro in plants (20), microorganisms (21, 22), and animals (23, 24).

It was suggested by Joshi and Handler (25) that a portion of the nicotinamide produced in vivo by the action of nicotinamide adenine dinucleotide glycohydrolase may be reutilized by deamidation of nicotinamide and conversion to NAD via the Priess-Handler pathway (26). Cyclic schemes for the degradation and resynthesis of NAD have been proposed by Joshi and Handler (25) and Sarma, Rajalakshmi, and Sarma (27). These schemes were postulated when the available evidence indicated that the nicotinyl moiety enters the pathway of NAD biosynthesis de novo at the level of free nicotinic acid. The recent discovery that the nicotinyl moiety enters the pathway de novo as nicotinic acid mononucleotide which is formed from quinolinic acid and 5-phosphoribosylpyrophosphate (20-24) provides the basis for the formulation of a pyridine nucleotide cycle as shown in Fig. 1. Gholson (28) has recently summarized the evidence for the formation and degradation of the pyridine nucleotides in biological systems and has suggested that the cycle exists in plants, animals, and microorganisms.

The recent discovery of hepatic nicotinamide mononucleotide phosphorylase (29) offers a potential "short circuit" of the cycle between nicotinamide and NAD. However, the

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**Experimental**

**Materials**

*Labeled Compounds Used*—$^{14}$C-Labeled nicotinic acid and nicotinamide and aniline-$^{14}$C were purchased from New England Nuclear. Quinolinic acid-$2,3,7,8$-$^{14}$C was synthesized (10) by the condensing glycerol and uniformly labeled aniline-$^{14}$C. N-methylnicotinic acid-$8$-$^{14}$C and N-methylnicotinamide-$8$-$^{14}$C were synthesized as follows. About 10 μmoles of nicotinic acid-

The objective of this investigation was to determine whether the pyridine ring moiety of the pyridine nucleotides was incorporated into ricinine and to measure the efficiency of these compounds as alkaloid precursors. This study was also designed to investigate the occurrence of the pyridine nucleotide cycle in the castor plant and its relationship to the biosynthesis of ricinine.

Fig. 1. The pyridine nucleotide cycle and proposed biosynthetic pathway for ricinine.

quantitative contribution of this reaction to NAD biosynthesis has yet to be evaluated.

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7-14C or nicotinamide-7-14C solution were brought to dryness in a 5-ml round bottom flask. To the residue, 0.5 ml of methyliodide was added and the mixture was refluxed under an air condenser for 6 hours in an oil bath at about 50°. The product was taken to dryness and the residue was dissolved in a minimum amount of water. The labeled N-methylnicotinic acid or N-methylnicotinamide was further purified by paper chromatography with Whatman No. 3MM filter paper. The radiochemical yield of each compound was 80%.

The pyridine nucleotides, nicotinic acid adenine dinucleotide-7-14C and nicotinic acid mononucleotide-7-14C, were prepared from incubation of nicotinic acid-7-14C with extracts of Bacillus subtilis (30) and isolated on a Dowex 1 formate column (26). NAD-7-14C was prepared by incubation of nicotinic acid-7-14C with extracts of Saccharomyces fragilis Cen, and was isolated according to the method of Chaykin (31).

Methods

Incorporation in Vivo of 14C-Labeled Precursors into Ricinine—Castor seeds of the Cimarron variety were planted about 1 inch beneath the surface of moist sterilized sand in enamel pans and kept at 30°. The sand was kept moist by spraying daily with distilled water. Four to five days later, the seeds began to germinate and the stem elongated to 15 to 20 cm 2 days after germination. The plants were placed under Gro-Lux lamps (or in a plant growth chamber) for another week. A solution of the 14C-labeled precursor was introduced into a single plant by means of absorption through the stem. An opening was made with a hypodermic needle in the upper part of the stem and the desired amount of solution was applied. Uptake of 20 to 40 μl of solution was usually complete in 3 to 5 min. Plants were harvested at appropriate intervals. Ricinine was isolated and purified according to the published procedures (3, 7), except that prior to extraction with chloroform the plants were cut into 1-cm pieces and ground to a fine powder with the aid of liquid nitrogen and a mortar and pestle.

Formation in Vivo of Nicotinic Acid, N-Methyl nicotinamide, and NAD from Nicotinamide-7-14C—In a typical experiment, two castor seedlings (5 days after germination in dark at 30°) were placed in separate test tubes, each containing 0.2 ml of nicotinamide-7-14C (specific activity, 5.96 mCi per mmole, 5.0 μCi per ml). The plants were returned to the dark at 30° for 3 to 4 hours and exposed to Gro-Lux lamps for 14 hours. Then 0.5 ml of distilled water was added to each tube and they were returned to darkness at 30°. After 16½ hours, the plants were rinsed thoroughly with distilled water and weighed (1.53 g). The washings and unabsorbed nicotinamide solution were combined for counting. The plants were ground to a fine powder in a mortar after freezing with liquid nitrogen and were treated with 15 ml of 0.6% ethanol, 0.8 mg of NAD, and 0.4 mg of NADP (carrier). The mixture was boiled for 15 min. The supernatant solution was recovered by centrifugation. Most of the ethanol was removed on a steam bath under a stream of nitrogen and the remainder was removed in a desiccator over P2O5 under vacuum. Dried residues obtained from plant extracts were extracted with 7 ml, 1.5 ml, 1.0 ml, 1.0 ml volumes of water; after each treatment the supernatant fraction was recovered by centrifugation. All supernatant fractions were combined and an aliquot was used for counting the total radioactivity recovered (Packard model 314A Tri-Carb liquid scintillation spectrometer). Samples of the plant extracts (0.1 ml) were spotted on 1-inch strips of Whatman No. 1 paper and chromatographed in four different solvent systems: 55% isopropyl alcohol, n-butyl alcohol-H2O-acetic acid (4:2:1), n-butyl alcohol saturated with 3% NH4OH, and acetone-H2O (7:3). Radioactive peaks on the chromatograms were detected with a Nuclear-Chicago 4π Actigraph II paper strip counter and were identified by comparing them to standard chromatograms of authentic compounds obtained under the same conditions.

The NAD spot was eluted and further identified by conversion to NADH with alcohol dehydrogenase as follows. To 0.35 ml of NAD-14C eluent were added 2.7 ml of Tris buffer (pH 10.0, 1.0 mM), 0.1 ml of alcohol dehydrogenase (3.0 mg per ml, Sigma), and 0.1 ml of ethanol (95%) to start the reaction in a 1-cm spectrophotometric cell. The absorption at 340 μM rose significantly with a small concomitant fall at 260 μM. The reaction was terminated by exposure of the contents of the cell to boiling water for 1 min. NADH and unchanged NAD were separated on a DEAE-cellulose column (0.7 X 12 cm) by batchwise elution with ammonium bicarbonate (32).

Conversion in Vitro of Nicotinic Acid-7-14C to NAD and N-Methylnicotinamide—Castor seedlings were grown under semi sterile conditions. Ten grams of cotyledons obtained from 7-day old seedlings were ground in a mortar with sand and 20 ml of 0.01 M potassium phosphate buffer, pH 7.0. The homogenate was filtered through four thicknesses of cheesecloth and the filtrate was adjusted to pH 7.4 with 1 N NaOH.

The standard reaction mixture consisted of phosphate buffer (pH 7.0), 0.67 mM; MgCl2, 10.0 μM; EDTA, 8 μM; ATP, 20.0 μM; L-methionine, 5.0 μM; S-adenosyl-L-methionine, 0.1 μM; nicotinic acid-7-14C, 0.1 μM, 2.22 κm X 10-4; and 1 ml of the homogenate to a total volume of 2 ml. The mixture was incubated at 30° for 12 hours. An addition of 200 μg of streptomycin and penicillin was made in some experiments to eliminate the possibility of microbial action during the long incubation period. The antibiotics did not affect the results. The reaction was terminated with 30% perchloric acid and centrifuged. The supernatant solution was neutralized with 1 N KOH. The clear supernatant solution was cocrchromatographed with authentic compounds in five solvent systems: 55% isopropyl alcohol, 60% n-propyl alcohol, n-butyl alcohol-H2O-acetic acid (4:2:1), n-butyl alcohol saturated with 15% NH4OH and 95% ethanol-1 M ammonium acetate (7:3), pH 5.0. The products were detected on the basis of fluorescence in ultraviolet light (254 μM). Radioactive peaks were located with a Nuclear-Chicago 4π Actigraph II paper strip counter. NAD-14C eluted from the paper chromatogram was further identified by conversion into NADH-14C with alcohol dehydrogenase as described previously. The product was placed on a DEAE-cellulose column in the presence of carrier NADH. The NADH recovered from the column was found to contain all the radioactivity. N-Methyl nicotinic acid-14C was diluted with 50.0 mg of carrier N-Methyl nicotinic acid and purified by recrystallization to constant specific activity. Other radioactive peaks found on the paper chromatograms were not identified.

Chlorination in Vivo of NAD-7-14C—To each of two uniform 1-weekold castor seedlings, 10 μl (specific activity, 13.5 nM per nanomole, 6.55 μCi per μl) of NAD-7-14C were introduced by means of stem absorption. One plant was harvested 1 hour later and...
TABLE I

Biosynthesis of Ricinine

<table>
<thead>
<tr>
<th>Compound</th>
<th>Precursor</th>
<th>Ricinine</th>
<th>Incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific activity</td>
<td>Amount administered</td>
<td>Specific activity</td>
</tr>
<tr>
<td>Quinolinic acid-2,3,7,8-14C</td>
<td>0.26</td>
<td>1,660.0</td>
<td>1.35</td>
</tr>
<tr>
<td>Nicotinic acid-7-14C</td>
<td>10.00</td>
<td>92.4</td>
<td>3.91</td>
</tr>
<tr>
<td>Nicotinamide-7-14C</td>
<td>11.00</td>
<td>57.7</td>
<td>3.31</td>
</tr>
<tr>
<td>Nicotinic acid mononucleotide-7-14C</td>
<td>12.40</td>
<td>19.5</td>
<td>11.85</td>
</tr>
<tr>
<td>Nicotinic acid adenine dinucleotide-7-14C</td>
<td>12.40</td>
<td>43.6</td>
<td>3.30</td>
</tr>
<tr>
<td>NAD-7-14C</td>
<td>13.50</td>
<td>29.7</td>
<td>1.57</td>
</tr>
<tr>
<td>N methyl nicotinolnic acid-8-14C</td>
<td>10.00</td>
<td>61.2</td>
<td>1.89</td>
</tr>
<tr>
<td>N methyl nicotininamide-8-14C</td>
<td>7.50</td>
<td>79.4</td>
<td>0.79</td>
</tr>
</tbody>
</table>

* Ten-day-old plants were used. The duration of the experiment was 96 hours. Percentage incorporation was calculated by dividing the total radioactivity recovered by the total radioactivity administered.

b This figure has been corrected for the loss of activity which originally resided on the carboxyl group attached to carbon 2 of the pyridine ring.

RESULTS

The results of the 96-hour ricinine biosynthesis studies from plants administered nicotinic acid mononucleotide-7-14C, nicotinic acid adenine dinucleotide-7-14C, NAD-7-14C, quinolinic acid-2,3,7,8-14C, nicotinamide-7-14C, N-methyl nicotinic acid-8-14C, and N-methyl nicotinamide-8-14C into ricinine by intact Ricinus communis L. plants are presented in Table I. The amounts of precursors administered were nearly equal except for quinolinic acid-2,3,7,8-14C. Considerably more of this compound was administered since its specific activity was less. In Fig. 2A, data are shown comparing the efficiencies of certain of the same precursors listed in Table I, but in the former instances information from experiments conducted for shorter times is included. N-methyl nicotinamide and N-methyl nicotinamide were less efficient precursors of ricinine than were the compounds in the pyridine nucleotide cycle shown in Fig. 1. It is proposed, therefore, that N-methyl nicotinamide and N-methyl nicotinamide be placed outside the cycle. N-methyl nicotinic acid is known to occur in corn and its occurrence in castor plants is reported herein (Table III). N-methyl nicotinic acid can be converted to nicotinic acid, nicotinamide, and NAD (30). N-methyl nicotinamide has not been previously reported in plants, and its occurrence in castor plants is shown in Table II. The roles of nicotinic acid mononucleotide and nicotinic acid adenine dinucleotide in the biosynthesis of NAD in animal systems (26, 34, 35) and bacteria (36) have been well established, and it is likely that the same pathway occurs in higher plants.

In an attempt to understand better the role of NAD, nicotinic acid, nicotinamide, and quinolinic acid in the biosynthesis of ricinine, an experiment was performed in which the same dosage of each precursor was used, and the results from this experiment are presented in Fig. 2B. The percentage incorporation of NAD, nicotinic acid, and nicotinamide at the same dosage level (1000 mmoles per plant) was nearly the same. Quinolinic acid was the most efficient precursor of ricinine; however, previous studies have shown that the efficiency of quinolinic acid and nicotinic acid varies inversely with the amount administered (7). Thus, the significance of the higher efficiency of quinolinic acid shown in Fig. 2B is difficult to assess at this time. The high concentration of quinolinic acid (1050 mmoles per plant) administered to castor plants used in the experiment reported in Fig. 2A is the reason for the slightly lower incorporation as compared with NAD and nicotinamide in that series of experiments. The relative percentage incorporation of these precursors may depend upon the level and activity of the enzymes involved in their metabolism, i.e. deaminase, transmethylase, phosphatase, and NAD glycohydrolase; however, all precursors from the cycle in Fig. 1 and quinolinic acid are incorporated rapidly into ricinine as shown in Fig. 2A. It has been proposed that quinolinic acid is one of the first pyridine compounds formed in the biosynthesis of pyridine compounds in plants (7, 10, 20) and microorganisms (21). From experiments in vitro with castor plant homogenates, it was found that quinolinic acid was converted to nicotinic acid mononucleotide and subsequently to nicotinic acid (30). One possible explanation for the higher efficiency of quinolinic acid as a precursor can be that free nicotinic acid is not in the direct route of ricinine biosynthesis and that it is utilized only by competition with quinolinic acid for the formation of nicotinic acid mononucleotide (Fig. 1). Also, free nicotinic acid can be utilized for the production of N-methyl-
nicotinic acid a widely occurring alkaloid in higher plants (37), or it can be stored as niacinogen (38). Results of precursor competition experiments between quinolinic acid and nicotinic acid showed that the percentage incorporation of quinolinic acid-2,3,7,8\textsuperscript{14}C into ricinine was not markedly affected when diluted 10-μmoles of quinolinic acid-2,3,7,8\textsuperscript{14}C (specific activity, 50.7 μCi per mmole) with and without 36 μmoles of nicotinic acid were administered to castor plants, and the percentage incorporation
Duration of experiment

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Total radioactivity administered</th>
<th>Total radioactivity recovered</th>
<th>Product</th>
<th>Distribution of radioactivity (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotinamide-7-(^{14})C</td>
<td>(1\mu C)</td>
<td>(10.4\mu C)</td>
<td>Nicotinic acid</td>
<td>48.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N-methyl nicotinamide</td>
<td>37.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NAD</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ricinine and others</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(1\) Percentages of distribution of radioactivity reported here are averaged values taken from paper chromatograms of each solvent system described under "Experimental."

Table II

Formation in vivo of nicotinic acid, N-methyl nicotinamide, and NAD from nicotinamide-7-\(^{14}\)C in castor plant in 16 hours

<table>
<thead>
<tr>
<th>Components of reaction mixture</th>
<th>Radioactive components recovered</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotinic acid</td>
<td>NAD</td>
<td>N-methyl nicotinamide</td>
</tr>
<tr>
<td>Complete</td>
<td>1871</td>
<td>62</td>
</tr>
<tr>
<td>Minus ATP</td>
<td>1803</td>
<td>26</td>
</tr>
<tr>
<td>Minus EDTA</td>
<td>1996</td>
<td>147</td>
</tr>
</tbody>
</table>

Table III

Conversion in vitro of nicotinic acid-7-\(^{14}\)C into NAD and N-methyl nicotinic acid

The standard reaction mixture consisted of phosphate buffer (pH 7.0), 0.67 mM; MgC\(_2\), 10.0 mM; EDTA, 8 \(\mu\)M; ATP, 20.0 \(\mu\)M; L-methionine, 5.0 \(\mu\)M; S-adenosyl-L-methionine, 0.1 \(\mu\)M; nicotinic acid-7-\(^{14}\)C, 0.1 \(\mu\)M, 2.22 cpm \(\times 10^{-4}\); and 1 ml of the homogenate to a total volume of 2 ml. The mixture was incubated at 30\(^\circ\) for 12 hours.

Table IV

Clearance in vivo of NAD-7-\(^{14}\)C by young castor plants

<table>
<thead>
<tr>
<th>Duration of experiment</th>
<th>Total radioactivity administered</th>
<th>Distribution of radioactivity</th>
<th>Radioactivity recovered</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>hr</td>
<td>(\mu)C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>65.5</td>
<td>Ricinine</td>
<td>(\mu)C</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nicotinamide</td>
<td></td>
<td>32.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N'-methyl nicotinamide</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unchanged NAD and others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>65.5</td>
<td>Ricinine</td>
<td></td>
<td>13.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nicotinamide</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N'-methyl nicotinamide</td>
<td></td>
<td>20.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unchanged NAD and others</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(2\) Castor cotyledons were homogenized in a Potter homogenizer with 4 volumes of 0.1 \(\times\) potassium phosphate buffer, pH 7.0. After centrifugation at 10,000 \(\times\) \(g\) for 20 \(\text{min}\) for supernatant, fractions were assayed for protein, quinolinic acid dehydrogenase (21), and nicotinic acid mononucleotide pyrophosphorylase (35). Nicotinic acid mononucleotide was determined by the formation of nicotinic acid-7-\(^{14}\)C from nicotinic acid mononucleotide-7-\(^{14}\)C as detected by paper chromatography. The values obtained in units of millimicromoles of nicotinic acid mononucleotide per hour per mg of protein were: quinolinic acid dehydrogenase, 2.6 to 6.8; nicotinic acid mononucleotide pyrophosphorylase, 0.15 to 0.24; and nicotinic acid mononucleotidase, 2.6.

of \(\mu\)C was 4.3 and 5.4, respectively. In the labeled nicotinic acid experiment, 60.6 millimoles of nicotinic acid 7-\(^{14}\)C (specific activity, 12.4 \(\mu\)C per millimole) with and without 720 millimoles of quinolinic acid were administered to castor plants, and the percentage incorporation of \(\mu\)C was 11.9 and 20.7, respectively.

\(3\) Castor cotyledons were homogenized in a Potter homogenizer with 4 volumes of 0.1 \(\times\) potassium phosphate buffer, pH 7.0. After centrifugation at 10,000 \(\times\) \(g\) for 20 \(\text{min}\) for supernatant, fractions were assayed for protein, quinolinic acid dehydrogenase (21), and nicotinic acid mononucleotide pyrophosphorylase (35). Nicotinic acid mononucleotide was determined by the formation of nicotinic acid-7-\(^{14}\)C from nicotinic acid mononucleotide-7-\(^{14}\)C as detected by paper chromatography. The values obtained in units of millimicromoles of nicotinic acid mononucleotide per hour per mg of protein were: quinolinic acid dehydrogenase, 2.6 to 6.8; nicotinic acid mononucleotide pyrophosphorylase, 0.15 to 0.24; and nicotinic acid mononucleotidase, 2.6.
43–45). In rabbit tissue, NAD is cleaved into fragments by two distinct pathways, i.e. to nicotinamide by NAD nucleotidase and to nicotinamide mononucleotide by NAD pyrophosphatase (43). Nicotinamide mononucleotide was not isolated in the experiment in Table IV. It would be included under “others” in that table.

**DISCUSSION**

In the present studies, it was shown that the pyridine ring moieties of nicotinic acid mononucleotide and NAD can be readily incorporated into ricinine with efficiencies of the same order of magnitude as those of nicotinic acid and nicotinamide which have been previously reported (46). These efficient precursors of ricinine could therefore be functioning in a cyclic manner (Fig. 1). In this study, the following cyclic conversions were shown:

In vivo: nicotinamide $\rightarrow$ nicotinic acid (1)

In vivo and in vitro: nicotinic acid $\rightarrow$ NAD (2)

In vivo: NAD $\rightarrow$ nicotinamide (3)

Reactions 2 and 3 may have involved the mononucleotides, nicotinic acid mononucleotide and nicotinamide mononucleotide, as intermediates which remained undetected by the methods used.

Leete and Leitz (2) first suggested that the pyridine nucleotides might be involved in the biosynthesis of ricinine; however, the experimental proof was not attempted until recently when the $^{14}$C-labeled compounds became available. They suggested a biogenetic scheme which employed nucleophilic attack by hydroxide ion at carbon atoms 2 and 4 of NAD. Recently, Robinson (47) reported the occurrence of an enzyme in young castor seedlings that catalyzed the oxidation of 1-methylnicotinonitrile to the corresponding 4- and 6-pyridones. It was proposed that since this enzyme is not highly specific for the 1-alkyl group it might be active in the formation of nicotinonitrile nucleotide analogue which may be a key intermediate in the biosynthesis of ricinine. The nicotinonitrile nucleotide analogue is not available; however, preliminary experiments with N-methylnicotinonitrile-$\text{H}^{14}$C as a precursor of ricinine indicated that it was about 10% as effective as N-methylnicotinamide. Work is in progress to determine the effectiveness of several pyridine nitriles as precursors of ricinine.

The metabolic relationships of quinolinic acid, nicotinic acid, N-methyl nicotinic acid, nicotinamide, N-methyl nicotinamide, nicotinic acid mononucleotide, nicotinic acid adenine dinucleotide, and NAD have been examined and these compounds have readily incorporated into ricinine with efficiencies of the same order of magnitude as those of nicotinic acid and nicotinamide which have been previously reported (46). These efficient precursors of ricinine could therefore be functioning in a cyclic manner (Fig. 1). In this study, the following cyclic conversions were shown:

**REFERENCES**


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