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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-methylnicotinamide 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 pyrophosphorylase (29) offers a potential "short circuit" of the cycle between nicotinamide and NAD. However, the
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.

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.

EXPERIMENTAL

Materials

**Labeled Compounds Used**—14C-Labeled nicotinic acid and nicotinamide and aniline-14C were purchased from New England Nuclear. Quinolinic acid-2,3,7,8-14C was synthesized (10) by the condensing glycerol and uniformly labeled aniline 14C. N-methylnicotinic acid-8-14C and N-methyl nicotinamide-8-14C were synthesized as follows. About 10 μmoles of nicotinic acid-
7-\textsuperscript{14}C or nicotinamide-7-\textsuperscript{14}C solution were brought to dryness in a 5-ml round bottom flask. To the residue, 0.5 ml of methylethidium was added and the mixture was refluxed under an air condenser for 6 hours in an oil bath at about 50\degree C. 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-\textsuperscript{14}C and nicotinic acid mononucleotide-7-\textsuperscript{14}C, were prepared from incubation of nicotinic acid-7-\textsuperscript{14}C with extracts of Bacillus subtilis (30) and isolated on a Dowex 1 formate column (26). NAD-7-\textsuperscript{14}C was prepared by incubation of nicotinic acid-7-\textsuperscript{14}C with extracts of Saccharomyces fragilis (27), and was isolated according to the method of Chaykin (31).

Methods

\textit{Incorporation in Vivo of \textsuperscript{14}C-Labeled Precursors into Ricinine—}

Castor seeds of the Cimarron variety were planted about 4 inch beneath the surface of moist sterilized sand in enameled pans and kept at 30\degree C. 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 \textsuperscript{14}C-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 \textmu l 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.

\textit{Formation in Vivo of Nicotinic Acid, N-Methyl nicotinamide, and NAD from Nicotinamide-7-\textsuperscript{14}C—}

In a typical experiment, two castor seedlings (5 days after germination in dark at 30\degree C) were placed in separate test tubes, each containing 0.1 ml of nicotinamide-7-\textsuperscript{14}C, 0.1 PM, 2.22 cpm X 10\textsuperscript{-6}; and 1 ml of the homogenate to a total volume of 2 ml. The mixture was incubated at 30\degree C for 12 hours. An addition of 200 \textmu g 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 cochromatographed with authentic compounds in five solvent systems: 85% isopropyl alcohol, 60% n-propyl alcohol, n-butyl alcohol-water-acetic acid (4: 2: 1), n-butyl alcohol saturated with 3% NH\textsubscript{4}OH, and acetone-H\textsubscript{2}O (7:3). Radioactive peaks on the chromatograms were detected with a Nuclear-Chicago 4\pi 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-\textsuperscript{14}C 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 \textmu m was measured using a spectrophotometer with ammonium bicarbonate (32).

\textit{Conversion in Vivo of Nicotinic Acid-7-\textsuperscript{14}C to NAD and N-Methyl nicotinic Acid—}

Castor seedlings were grown under semisterile conditions. Ten grams of cotyledons obtained from 0-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; MgCl\textsubscript{2}, 10.0 \mu M; EDTA, 8 \mu M; ATP, 20.0 \mu M; L-methionine, 50.0 \mu M; S-adenosyl-L-methionine, 0.1 mM; nicotinic acid-7-\textsuperscript{14}C, 0.1 \mu M, 2.22 cpm X 10\textsuperscript{-4}; and 1 ml of the homogenate to a total volume of 2 ml. The mixture was incubated at 30\degree C for 12 hours. An addition of 200 \textmu g 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 cochromatographed with authentic compounds in five solvent systems: 85% isopropyl alcohol, 60% n-propyl alcohol, n-butyl alcohol-H\textsubscript{2}O-acetic acid (4:2:1), n-butyl alcohol saturated with 15% NH\textsubscript{4}OH 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 \textmu m). Radioactive peaks were located with a Nuclear-Chicago 4\pi Actigraph II paper strip counter. NAD-\textsuperscript{14}C eluted from the paper chromatogram was further identified by conversion into NADH-\textsuperscript{14}C 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-7-\textsuperscript{14}C 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.

\textit{Cleavage in Vivo of NAD-7-\textsuperscript{14}C—}

To each of two uniform 1-week-old castor seedlings, 10 \mu l (specific activity, 13.5 mC per mmole, 6.55 mC per \mu l) of NAD-7-\textsuperscript{14}C were introduced by means of stem absorption. One plant was harvested 1 hour later and the other was harvested 4 hours later. The stem absorption. One plant was harvested 1 hour later and the other was harvested 4 hours later.
Incorporation of quinolinic acid-2,3,7,8-$^{14}$C, nicotinic acid-7-$^{14}$C, nicotinamide-7-$^{14}$C, nicotinic acid mononucleotide-7-$^{14}$C, nicotinic acid adenine dinucleotide-7-$^{14}$C, NAD-7-$^{14}$C, $N'$-methylnicotinamide-8-$^{14}$C, and $N'$-methylnicotinic acid-8-$^{14}$C into ricinine by intact Ricinus communis L. plants.

<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-$^{14}$C</td>
<td>0.28</td>
<td>1,660.0</td>
<td>1.35</td>
</tr>
<tr>
<td>Nicotinic acid-7-$^{14}$C</td>
<td>10.00</td>
<td>92.4</td>
<td>3.91</td>
</tr>
<tr>
<td>Nicotinamide-7-$^{14}$C</td>
<td>11.00</td>
<td>57.7</td>
<td>3.31</td>
</tr>
<tr>
<td>Nicotinic acid mononucleotide-7-$^{14}$C</td>
<td>12.40</td>
<td>19.5</td>
<td>11.85</td>
</tr>
<tr>
<td>Nicotinic acid adenine dinucleotide-7-$^{14}$C</td>
<td>12.40</td>
<td>43.6</td>
<td>3.30</td>
</tr>
<tr>
<td>N'-methylnicotinamide-7-$^{14}$C</td>
<td>13.50</td>
<td>29.7</td>
<td>1.57</td>
</tr>
<tr>
<td>N'-methylnicotinamide-8-$^{14}$C</td>
<td>10.00</td>
<td>61.2</td>
<td>1.89</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.

RESULTS

The results of 96-hour ricinine biosynthesis studies from plants administered nicotinic acid mononucleotide-7-$^{14}$C, nicotinic acid adenine dinucleotide-7-$^{14}$C, NAD-7-$^{14}$C, quinolinic acid-2,3,7,8-$^{14}$C, nicotinamide-7-$^{14}$C, N-methyl nicotinic acid-8-$^{14}$C, and $N'$-methylnicotinamide-8-$^{14}$C are presented in Table I. The amounts of precursors administered were nearly equal except for quinolinic acid-2,3,7,8-$^{14}$C. Considerably more of this compound was administered since its specific activity was so low. 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 are included. $N'$-methylnicotinic acid and $N'$-methylnicotinamide were less efficient precursors of ricinine than were the compounds in the pyridine nucleotide cycle shown in Fig. 1. It is proposed, therefore, that $N'$-methylnicotinic acid and $N'$-methylnicotinamide be placed outside the cycle. $N'$-methylnicotinic acid is known to occur in corn and its occurrence in castor plants is reported herein (Table III). $N'$-methylnicotinic acid can be converted to nicotinic acid, nicotinamide, and NAD (30). $N'$-methylnicotinamide 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 nicotinamide 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 (900 μmolmes 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 (1650 μmolmes 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, e.g., deaminase, transmethy|
nicotinic acid a widely occurring alkaloid in higher plants (37), or it can be stored as niacinogen (38). Results of precursor competition studies between quinolinic acid and nicotinic acid showed that the percentage incorporation of quinolinic acid-2,3,7,8-\(^{14}\)C into ricinine was not markedly affected when diluted 10-μmoles of quinolinic acid-2,3,7,8-\(^{14}\)C (specific activity, 50.7 μC per mmole) with and without 36 μmoles of nicotinic acid were administered to castor plants, and the percentage incorporation

Fig. 2. Time course study showing the relative efficiencies of precursors of ricinine. Experiments were conducted under the conditions described in the text. A, the dosage of each precursor is shown in Column 3 of Table I; B, the dosage of each precursor was 600 μmoles per plant.

1 Experiments were performed under the same conditions used for those in Table I. In the labeled quinolinic acid experiment, 4
Table II
Formation in vivo of nicotinic acid, N-methyl nicotinamide, and NAD from nicotinamide-7-14C in castor plant in 16 hours

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Total radio-activity administered</th>
<th>Total radio-activity recovered</th>
<th>Product</th>
<th>Distribution of radio-activitya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotinamide-7-14C</td>
<td>1 µCi</td>
<td>16.0 µCi</td>
<td>Nicotinic acid</td>
<td>48.8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.4 µCi</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>
</tbody>
</table>

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

Table III
Conversion in vitro of nicotinic acid-7-14C into NAD and N-methyl nicotinic acid

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 cpm X 10⁴; and 1 ml of the homogenate to a total volume of 2 ml. The mixture was incubated at 30° for 12 hours.

<table>
<thead>
<tr>
<th>Components of reaction mixture</th>
<th>Radioactive components recovered</th>
<th>cpm x 10⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nicotinic acid</td>
<td>NAD</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>1906</td>
<td>147</td>
</tr>
</tbody>
</table>

Table IV
Clearance in vivo of NAD-7-14C by young castor plants

<table>
<thead>
<tr>
<th>Duration of experiment</th>
<th>Total radio-activity administered</th>
<th>Distribution of radioactivity</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Product³</td>
<td>Radioactivity recovered</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 hr</td>
<td></td>
</tr>
<tr>
<td></td>
<td>micC</td>
<td>65.5 µCi</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ricinie</td>
<td>0.74 µCi</td>
<td>1.1%</td>
</tr>
<tr>
<td></td>
<td>Nicotinamide</td>
<td>32.5 µCi</td>
<td>49.6%</td>
</tr>
<tr>
<td></td>
<td>N'-methyl nicotinamide</td>
<td>0 µCi</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Unchanged NAD and others</td>
<td>49.3%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96 hr</td>
<td>65.5 µCi</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ricinie</td>
<td>13.9 µCi</td>
<td>21.2%</td>
</tr>
<tr>
<td></td>
<td>Nicotinamide</td>
<td>0 µCi</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>N'-methyl nicotinamide</td>
<td>20.3 µCi</td>
<td>44.7%</td>
</tr>
<tr>
<td></td>
<td>Unchanged NAD and others</td>
<td>34.1%</td>
<td></td>
</tr>
</tbody>
</table>

³ Products were first recovered from DEAE cellulose column chromatography and further identified by paper chromatography in the solvent systems described in the text.

b Calculated by difference.

c The quinolinic acid decarboxylase and nicotinic acid mononucleotide pyrophosphorylase reaction was estimated. The quinolinic acid decarboxylase is about 20 times more active than nicotinic acid mononucleotide pyrophosphorylase in 1-week old castor cotyledons. The cotyledons also have nicotinic acid mononucleotidase activity which catalyzes the formation of free nicotinic acid directly from nicotinic acid mononucleotide and without prior conversion of the latter to NAD.

Table II shows the conversion in vivo of nicotinamide-7-14C to nicotinic acid (48.8%) and N-methyl nicotinamide (37.6%) in 16 hours. Only about 0.5% was converted to NAD, which was presumably formed via nicotinic acid. Most (i.e. 90%) of the remainder of the radioactivity (13%) was found in ricinie. The conversion of nicotinamide to nicotinic acid is catalyzed by a potent deamidase which is found in plants (38, 39) as well as in other biological systems (25, 27, 40). Nicotinic acid mononucleotide and nicotinic acid adenine dimononucleotide are presumably intermediates in the formation of NAD from nicotinic acid instead of from nicotinamide (41, 42), and both are efficient precursors of ricinie (Table IV).

The conversion in vitro of nicotinic acid-7-14C to NAD, N-methyl nicotinic acid, and several unidentified compounds in the presence of a methyl donor is shown in Table III. NAD formation from nicotinic acid was significantly dependent on ATP, whereas in the presence of adenosylmethionine the formation of N-methylnicotinic acid was not. In the absence of EDTA, the formation of NAD was doubled, an indication of the requirement of metal ions in the reaction. The conversion in vivo of nicotinic acid to pyridine nucleotides has also been described by Ghosh, Sarkar, and Guha (38) in rice seedlings.

In Table IV, the clearance in vivo of NAD to nicotinamide in a short term experiment (1 hour) and the subsequent formation of N-methyl nicotinamide in a long term experiment (96 hours) is shown. This experiment clearly indicates that NAD is rapidly cleaved to yield nicotinamide, which is subsequently methylated. These results also indicate that ricinie is formed earlier than N-methyl nicotinamide (1.1% in 1 hour and 21% after 96 hours, compared with none in 1 hour and 45% in 96 hours, respectively). The widespread occurrence of enzymes that cleave NAD has been shown in biological systems (26, 39, 40).
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 racicnine could therefore be functioning in a cyclic manner (Fig. 1). In this study, the following cyclic conversions were shown:

\[
\begin{align*}
\text{In vivo: nicotinamide} & \rightarrow \text{nicotinic acid} \quad (1) \\
\text{In vitro and in vivo: nicotinic acid} & \rightarrow \text{NAD} \quad (2) \\
\text{In vivo: NAD} & \rightarrow \text{nicotinamide} \quad (3)
\end{align*}
\]

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 14C-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-8-14C 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 been implicated in the biosynthesis of ricicine by *Ricinus communis* L. The pathway for the biosynthesis of ricicine is still not completely understood. Although it appears that quinolinic acid and the compounds in the pyridine nucleotide cycle are nearly equivalent as precursors of ricinine, the detailed steps in the conversion of the particular pyridine compound to ricinine remain to be elucidated.

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