THE CONVERSION OF DEUTERO-N\textsuperscript{15}-TRYPTOPHAN TO QUINOLINIC ACID BY THE RAT*

BY RICHARD W. SCHAYER AND L. M. HENDERSON

WITH THE TECHNICAL ASSISTANCE OF ROSA L. SMILEY

(From the Rheumatic Fever Research Institute, Northwestern University Medical School, Chicago, and the Division of Biochemistry, Noyes Laboratory of Chemistry, University of Illinois, Urbana, Illinois)

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Quinolinic acid (pyridine-\(\alpha,\beta\)-dicarboxylic acid) has been isolated from rat urine after administration of tryptophan to rats (1). It has been suggested that quinolinic acid is formed via 3-hydroxyanthranilic acid by oxidative cleavage of the benzene ring in the 3-4 position, followed by closure incorporating the amino nitrogen in a pyridine ring (1).

In this paper are reported the results of studies of the formation of quinolinic acid from tryptophan labeled in the indole ring with N\textsuperscript{15} and in the benzene ring with deuterium.

EXPERIMENTAL

Preparation of Labeled Tryptophan—The synthesis of DL-tryptophan labeled with N\textsuperscript{15} in the indole ring has been described (2).

Synthesis of DL-Tryptophan Labeled in Benzene Ring with Deuterium—To 125 gm. of cold fuming sulfuric acid (65 per cent excess sulfur trioxide) were carefully added 40 gm. of deuterium oxide. The resulting deuterosulfuric acid had a strength of 52 mole per cent, approximately that recommended by Ingold et al. (3) for introducing deuterium into benzene. This deuterosulfuric acid was shaken with 41 gm. of benzene for 5 days, and the deuteronitrobenzene separated and nitrated by adding it in small portions over a period of 30 minutes to a shaken mixture of 58 ml. of concentrated nitric acid and 58 ml. of fuming nitric acid (sp. gr. 1.50) maintained between 40-50°. Use of sulfuric acid during the nitration was avoided to prevent exchange of hydrogen for deuterium. The 47 gm. of deuteronitrobenzene obtained were reduced to 32.4 gm. of deuteroaniline (4), which was converted to 25.9 gm. of deuterophenylhydrazine (5). The deuterophenylhydrazine was allowed to react with the condensation product of acrolein and acetamidomalonic ester (6) and the resulting phenylhydrazone cyclized by the method of Warner and Moe (7). The ethyl \(\alpha\)-acetamido-\(\alpha\)-carbo-

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ethoxy-$\beta$-(3-indole)-propionate thus obtained was converted to tryptophan, as described by Howe et al. (8).

After two recrystallizations 3.4 gm. of DL-tryptophan, containing 27.1 atom per cent excess deuterium in the benzene ring, were obtained, a 3.2 per cent yield from benzene.

Calculated, C 64.7, H 5.93, N 13.72; found, C 64.5; H 6.25, N 13.86

Administration of Tryptophan—Some preliminary experiments were conducted to determine the conditions for maximum conversion of a test dose of tryptophan to quinolinic acid. Since there had been reports that the protein content of the diet was a factor (9, 10), two levels of casein, 9 and 25 per cent, were tested. Various amounts of DL- and L-tryptophan were administered by injection or stomach tube or were mixed in the diet. The results of these experiments are summarized in Table I. The mean values for three or four animals are expressed as the percentage of the tryptophan which was excreted as quinolinic acid during the 24 hour period following the beginning of tryptophan administration. The “microbiologically available” nicotinic acid values ranged from 0.09 mg. per day for the animals.

1 We are indebted to Dr. H. S. Anker of the University of Chicago for the isotope analyses.

2 Analysis by the Micro-Tech Laboratories.

### Table I

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Casein content of diet</th>
<th>Sex</th>
<th>Amount and form of tryptophan given</th>
<th>Mode of administration</th>
<th>Per cent of test dose of tryptophan excreted as quinolinic acid in 24 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>Mixed</td>
<td>179 (DL)</td>
<td>2.04% of diet</td>
<td>6.4</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>Male</td>
<td>151 &quot;</td>
<td>2.04% &quot; &quot;</td>
<td>12.5</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>Female</td>
<td>141 &quot;</td>
<td>2.04% &quot; &quot;</td>
<td>9.3</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>Mixed</td>
<td>204 &quot;</td>
<td>Stomach tube, 3 doses</td>
<td>7.0</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>&quot;</td>
<td>204 &quot;</td>
<td>Intraperitoneal, 1 dose</td>
<td>14.1</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>&quot;</td>
<td>204 &quot;</td>
<td>&quot;</td>
<td>5 doses</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>&quot;</td>
<td>204 &quot;</td>
<td>&quot;</td>
<td>1 dose</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>&quot;</td>
<td>510 &quot;</td>
<td>&quot;</td>
<td>5 doses</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>&quot;</td>
<td>204 &quot;</td>
<td>&quot;</td>
<td>1 dose</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>&quot;</td>
<td>51 &quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>11</td>
<td>9</td>
<td>&quot;</td>
<td>204 (L)</td>
<td>&quot;</td>
<td>15.1</td>
</tr>
<tr>
<td>12</td>
<td>9</td>
<td>&quot;</td>
<td>51 &quot;</td>
<td>&quot;</td>
<td>16.4</td>
</tr>
</tbody>
</table>
receiving 0.1 mM of tryptophan to 0.53 mg. for those receiving 2.5 mM of dl-tryptophan (Group 8, Table I), intermediate dosages yielding approximately 0.2 per cent of the tryptophan as "free" nicotinic acid.

The results of these experiments indicated that nearly all of the quinolinic acid was excreted during the first 24 hours following tryptophan administration, the values during the 2nd day ranging from 2 to 10 per cent of those the 1st day. The sex did not appear to influence the conversion. The results suggested that a larger percentage of administered tryptophan was recovered as quinolinic acid at low dosage levels. Many more such experiments would be required to establish with certainty the effect of sex, dosage, and mode and frequency of administration because of the rather large variation in excretion of individual animals in the same group. However, the results showed that 0.25 mM (51 mg.) of tryptophan per day was a satisfactory amount for good yields of urinary quinolinic acid.

Six male rats approximately 2 months of age (187 to 227 gm.), which had been kept on a stock ration since weaning, were placed in individual metabolism cages and fed a purified diet containing 9 per cent casein plus 0.2 per cent L-cystine (11) for 1 week prior to the experiment. Each rat then received by intraperitoneal injection 51 mg. of doubly labeled dl-tryptophan per day on 2 successive days. The injections were made at 10 a.m. and 3 p.m. each day, in four equal doses each dissolved in 2 ml. of isotonic saline. The tryptophan was prepared by recrystallizing a mixture of the sample containing N16 and the one containing deuterium. The administered tryptophan contained 13.3 atom per cent excess deuterium in the benzene ring and 1.89 atom per cent excess N15 in the indole nucleus. Urine was collected under toluene throughout a 48 hour period following the first injection of tryptophan. The pooled sample was filtered, diluted to 500 ml., and analyzed for nicotinic acid and for quinolinic acid following decarboxylation by autoclaving with acetic acid (12) by use of Lactobacillus arabinosus (13). The urine was found to contain 1.5 mg. of free nicotinic acid or equivalent of other compounds active for the test organism and 93.0 mg. of quinolinic acid. This represented an 18.7 per cent yield from the administered tryptophan. Quinolinic acid (675 mg.) was dissolved in an aliquot of the urine calculated to contain 75 mg., to give a 1:10 dilution of the excreted quinolinic acid. This compound was then isolated as described previously (1), except that the ion exchange step was omitted and 50 per cent ethanol was used as a solvent in crystallizing the product eluted from the alumina column. After two recrystallizations from 50 per cent ethanol, 140 mg. of pure quinolinic acid, m.p. 187–188°, were obtained.

Calculated, C 50.28, H 3.02, N 8.39; found, C 50.54, H 3.26, N 8.39
A portion of the isolated quinolinic acid was diluted with an equal weight of carrier quinolinic acid for deuterium analyses and another portion was used directly for N\textsuperscript{15} determination.\textsuperscript{1}

**Results**

Deuterium analysis of a quinolinic acid sample diluted 20:1 = 0.250 atom per cent excess = 5.00 atom per cent excess corrected for carrier.

N\textsuperscript{15} analysis of a quinolinic acid sample diluted 10:1 = 0.169 per cent excess = 1.69 per cent excess corrected for carrier. Found, D:N\textsuperscript{15} = 5.00/1.69 = 2.96.

The tryptophan contained 13.3 atom per cent excess D in the benzene ring. Assuming that 2 of the original 4 deuterium atoms are retained, quinolinic acid, which has a total of 5 hydrogen atoms, would contain 13.3 X 2/5 = 5.32 atom per cent D.

The N\textsuperscript{15} of quinolinic acid would be expected to be formed entirely from the N\textsuperscript{15} of the indole ring of tryptophan which contained 1.89 per cent excess N\textsuperscript{15}. Calculated, D:N\textsuperscript{15} = 5.32/1.89 = 2.81.

Based on the N\textsuperscript{15} determinations, the isolated quinolinic acid was formed from the isotopic tryptophan to the extent of 1.69/1.89 = 89 per cent.

Based on the deuterium determinations, the isolated quinolinic acid was formed from the isotopic tryptophan to the extent of 5.00/5.32 = 94 per cent.

In another experiment, qualitatively similar results were obtained, but, owing to an unexplained failure of duplicate N\textsuperscript{15} samples to agree closely, the data do not warrant quantitative treatment. Insufficient sample was available for repeat determinations.

**DISCUSSION**

That the indole ring N of tryptophan is the major source of the nitrogen attached to the ring in kynurenine and of the ring nitrogen in kynurenic acid and xanthurenic acid has been demonstrated (2). The appearance of N\textsuperscript{15} from ring-labeled tryptophan in quinolinic acid with only slight dilution is in agreement with the view that the latter compound arises from tryptophan directly through kynurenine and 3-hydroxyanthranilate. The dilution of deuterium in the course of the reactions was such as to point to an exchange of hydrogen for 2 of the 4 deuterium atoms in tryptophan. Assuming such an exchange, the recovery of 94 per cent for deuterium was very close to the 89 per cent found for N\textsuperscript{15}. This slight discrepancy might reflect failure to get complete exchange of hydrogen for deuterium on carbon atom 6 of quinolinic acid.

The results presented provide strong evidence that tryptophan is converted to quinolinic acid, by the rat, by reactions involving entry of the
indole nitrogen of tryptophan into the broken benzene ring of tryptophan to form a doubly labeled pyridine derivative. The findings on the ratio of deuterium to N15 are consistent with the postulated (1) opening of the benzenoid nucleus of 3-hydroxyanthranilic acid in the 3-4 position, followed by closure of carbon atom 4 with the nitrogen to form quinolinic acid.

SUMMARY

Tryptophan labeled in the benzene ring with deuterium has been synthesized.

Tryptophan doubly labeled with deuterium and N15 was administered to rats by intraperitoneal injection, and quinolinic acid was isolated from the urine with the aid of carrier.

The isotope concentrations in the isolated quinolinic acid indicate that (a) tryptophan is probably the only source of quinolinic acid, (b) nitrogen from the indole ring of tryptophan is incorporated into the disrupted benzene ring of tryptophan to form a pyridine derivative, quinolinic acid, and (c) 2 of the 4 deuterium atoms of the original tryptophan benzene ring are lost in the conversion to quinolinic acid. These findings are consistent with mechanisms previously postulated for the conversion of tryptophan to nicotinic acid.

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