Studies on the Metabolism of Kynurenic Acid

I. THE FORMATION OF L-GLUTAMIC ACID, D- AND L-ALANINE, AND ACETIC ACID FROM KYNURENIC ACID BY PSEUDOMONAS EXTRACTS*

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Kynurenic acid was first isolated from the urine of a dog by Lübig in 1853 (1) and was later shown to be a metabolite of tryptophan by Ellinger (2). However, its further metabolism has not been investigated until recently. The formation of quinaldic acid from kynurenic acid in mammals has been reported by Brown and Price (3). Its extensive degradation by a pseudomonad has been described (4), but the exact metabolic sequence has not been elucidated.

In preliminary reports from our laboratory, we have described briefly an enzyme system in extracts of tryptophan-adapted cells of Pseudomonas that catalyzed the formation of L-glutamic acid from kynurenic acid (5, 6). A similar preliminary observation was independently reported by Behrman and Tanaka (7, 8).

The purpose of this paper is to describe in detail the preparation and properties of a kynurenic acid-degrading crude extract from cells of Pseudomonas fluorescens, an organism which uses the so-called "quinoline pathway" of tryptophan degradation (9). L-Glutamic acid, D- and L-alanine, and acetic acid have been isolated as products of the enzymatic degradation. The succeeding report will deal with the results of isotopic experiments that are pertinent to the mechanism of enzymatic formation of these products.

EXPERIMENTAL PROCEDURE

Chemicals—The DL-tryptophan used as a major carbon and nitrogen source for the cultivation of Pseudomonas was a gift from the Sigma Chemical Company and the Takeda Chemical Industries, Ltd. Kynurenic acid was chemically synthesized from aniline and ethylacetacetate (10, 11). Kynurenic acid purchased from the Nutritional Biochemicals Corporation was also used. Quinaldic acid was obtained from the Eastman Kodak Company. 7-Hydroxykynurenic acid, 8-hydroxykynurenic acid, and 7,8-dihydroxykynurenic acid were synthesized by Senoh, Tokuyama, and Sakan.1 DL-Tryptophan-3'-C14 was obtained from the Nuclear-Chicago Corporation.2 L-Glutamic acid, DL- and L-alanine, and γ-aminobutyric acid were obtained from the Nutritional Biochemicals Corporation. DL-γ- and β-Hydroxyglutamic acids were kindly furnished by Dr. T. Kaneko of Osaka University.

Biological Materials—Pseudomonas fluorescens (ATCC 11299B) was used as an enzyme source. Stock cultures were maintained on slants prepared from 3% nutrient agar media. Inoculation was carried out by transferring the cells from a slant to 1.5 liters of the growth medium, which contained 0.1% DL-tryptophan, 0.1% Difco yeast extract, 0.02% MgSO4·7H2O, 0.15% K2HPO4, and 0.05% KH2PO4 in a 5-liter Erlenmeyer flask. The cells were grown at 23°C for approximately 14 hours with mechanical shaking. The cells were then harvested with the aid of a Sharples centrifuge and washed once with 0.85% potassium chloride solution. The yield of wet packed cells was about 1.5 g per liter of the medium. All the subsequent manipulations were carried out at 0-4°C unless specified otherwise. Extracts were prepared by suspending 1 part by weight, of the washed cells in 2 parts of 0.02 M potassium phosphate buffer, pH 7.5, and disrupting the bacterial mass in batches of 15 ml for 15 minutes in a Kubota 9-ke sonic oscillator. The resulting material was centrifuged for 10 minutes in an International model PR II centrifuge at 15,000 × g. The supernatant solution thus obtained was referred to as the "crude extract" throughout this paper. The crude extract was further centrifuged in a Spinco model L supercentrifuge at 105,000 × g for 1 hour. The supernatant solution was a clear, straw-colored solution and will be referred to as the "supernatant fraction" hereafter. Cells were also disrupted by grinding with alumina and extracted with 2 parts by weight of 0.02 M potassium phosphate buffer, pH 7.5, as previously described (12). L-Glutamic dehydrogenase was prepared from pig kidney (15), and lactic dehydrogenase was purified from heart muscle (16).

Preparation of C14-labeled Kynurenic Acid—The principle of the enzymatic synthesis of C14-labeled kynurenic acid is based upon the previously reported enzymatic conversion of L-tryptophan to L-kynurenine by Pseudomonas tryptophan oxygenase and formamidase (17), followed by the transformation of the latter compound to kynurenic acid by kynurenine transaminase of Pseudomonas in the presence of α-ketoglutarate (18). The incubation mixture contained in a total volume of 2.0 ml the following components: 5.35 μmoles of DL-tryptophan 1'-C14 (4.67 μc per μmole), 100 μmoles of potassium phosphate buffer,
n-Tryptophan, which did not react in this system, was eluted in the
natant solution was neutralized by the addition of 2 g KOH. The precipitate was washed twice with 1 ml of 6% perchloric acid, and the combined super-
натant solution was neutralized by the addition of 2 N KOH in the cold. After the potassium perchlorate was removed by centrifugation at 0°, the supernatant solution containing approximately 14,550,000 c.p.m. was applied to a Dowex 1-formate column (200 to 400 mesh, 8% cross-links, 1 sq cm x 5 cm). Gradient elution was carried out with 6 N formic acid in a reservoir and 300 ml of water in a mixing flask. Kynurenic acid, which was eluted between 249 and 393 ml, contained 5,430,000 c.p.m., representing approximately 75% of the theoretical yield.

D-Tryptophan, which did not react in this system, was eluted in a single peak between 36 and 78 ml. The fractions containing kynurenic acid were combined and evaporated at 40° under reduced pressure by means of a flash evaporator. The dried material was dissolved in 4 ml of water and neutralized by the addition of 0.1 N KOH. To test the purity of the material and to calculate the specific activity, a 0.1 ml aliquot was removed for spectrophotometric examination. The specific activity was calculated to be 3,100,000 c.p.m. per pmole on the basis of the optical density of kynurenic acid at 333 nm (= 11,100, pH 7.0) (19). Unlabeled kynurenic acid, 16.4 pmoles, was then added to the remaining solution, and crystallization was achieved by the addition of concentrated HCl. The final specific activity of kynurenic acid was 292,000 c.p.m. per pmole. Recrystallization from water yielded about 15 pmoles of kynurenic acid monohydrate, specific activity unchanged. The purity of this material was further examined by means of paper chromatography with three different solvent systems. Rf values of kynurenic acid, as revealed by its characteristic fluorescence and the radioactivity, were: 0.58, butanol-acetic acid-water (4:1:1); 0.53, ethanol-ammonia-water (18:1:1); 0.83, methanol-pyridine-water (80:4:20). No radioactive or fluorescent spots other than kynurenic acid were detectable. A similar method of preparation of kynurenic acid-C14 has recently been described (20).

The yield of kynurenic acid from L-tryptophan based on the radio-
activity ranged between 60 to 80% in several runs.

Determination—Radioactivity was determined with samples of
infinite thinness in a Nuclear-Chicago gas flow counter equipped with a micromil thin window; the radioactive areas on paper chromatograms were recorded by means of an automatic recorder, the Actigraph. Spectrophotometric experiments were carried out with a Beckman model DU spectrophotometer and a Shimazu recording spectrophotometer. The infrared spectra were taken in potassium bromide pellets with a Shimazu spectrophotometer. Manometric assays were done in a conventional Warburg apparatus; CO2 output was determined by the direct method. Melting points were determined with the aid of a Yanagimoto micromelting point apparatus. Optical rotation was deter-
mined with the Rudolph model 400 photoelectric polarimeter. The concentration of cell suspensions was determined with the aid of a Klett-Summerson spectrophotometer with filter No. 66. Amino acids were assayed by the ninhydrin color reaction (21). Protein was determined by a spectrophotometric method (22). Ammonia was determined by the direct nesslerization (23).

FIG. 1. Manometric experiments with resting cell suspensions.

The washed bacterial cells were suspended in 0.1 M potassium phosphate buffer, pH 6.4. The absorbancy of cell suspensions was 1.06 at 600 mµ. The assay system contained in a volume of 2.0 ml, 1 pmole of substrate, and 1.6 ml of a cell suspension. Incubation was carried out at 30°. The rate of the endogenous oxygen uptake was below that with kynurenic acid. O---O, kynurenic acid; □-□, 7-hydroxykynurenic acid; △-△, 8-hydroxykynurenic acid; O---O, 7,8-dihydroxykynurenic acid; ×---×, quinaldic acid.

Chromatographic Procedures—Whatman No. 1 filter paper was used for paper chromatography by the descending technique at room temperature. Whatman No. 31 (extra thick) was also used for preparative purposes. Partition chromatography of acetic acid was conducted by the use of a Celite column according to the method of Peterson and Johnson (21).

RESULTS

Experiments with Intact Cell Suspensions—When cells were grown at the expense of L-tryptophan, the resting cell suspension was found to metabolize kynurenic acid without any lag period as described before by Stanier and Tsuchida (4). Approximately 5.0 moles of oxygen were consumed per mole of kynurenic acid utilized (Fig. 1), and about 4.5 moles of CO2 were released. 7,8-Dihydroxykynurenic acid was also oxidized at about the same rate and without any lag period with the consumption of approximately 4 moles of oxygen per mole of substrate. 7- and 8-hydroxykynurenic acid were both oxidized but the initial velocity was approximately \( \frac{1}{2} \) that of kynurenic acid, respectively. There was a short but definite lag period with the two latter substrates. Quinaldic acid, which has been reported to be a metabolite of kynurenic acid in mammals (3), was metabolized at a rate approximately \( \frac{1}{2} \) that of kynurenic acid.

Experiments with Cell-free Extracts—When the cells were disrupted by means of alumina or by sonic oscillation, the resulting cell-free extracts were usually inactive and incapable of metabolizing kynurenic acid at an appreciable rate as judged by manometric experiments or by the disappearance of the characteristic ultraviolet absorption. The addition of an energy source or various coenzymes, such as DPN, TPN, DPNH, TPNH, etc., was found to be uniformly ineffective. Finally, attempts were made to remove or inhibit cytochrome systems which might be competing with either various oxygenases, hydroxylases, or both, essential for initiating the primary reaction in kynurenic acid degradation. When the crude extract was centrifuged at 105,000 X g for 1 hour, the supernatant solution was found to be quite...
capable of metabolizing kynurenic acid; approximately 2.5 moles of oxygen were consumed with the output of 2.5 moles of CO₂ per mole of substrate (Fig. 2). Ammonia formation was negligible. 7- and 8-Hydroxykynurenic acid were both metabolized without an appreciable lag period, the total oxygen consumption being almost equal to that in the presence of kynurenic acid. When 7,8-dihydroxykynurenic acid was used as a substrate, the initial velocity was much greater, usually more than three times the rate of kynurenic acid oxidation. The total amount of oxygen consumed was about 2 moles per mole of substrate.

Quinaldic acid was not metabolized at all under the same conditions. The extracts, however, must be sufficiently concentrated; a protein concentration of the order of 10 mg per ml seems to be necessary. Upon dilution, marked loss of activity has been consistently observed. When the precipitate obtained by high speed centrifugation was resuspended in a small amount of buffer and added back to the incubation mixture, almost complete inhibition was observed (Fig. 3). However, when the particulate fraction was heated at 100° for 5 minutes, the inhibitory effect was almost abolished, indicating that the inhibition was due to some heat-labile factor, presumably an enzyme, present in the particulate fraction. To try to confirm this supposition, the effect of several inhibitors was investigated. Cyanide and azide (10⁻³ M), which are known to inhibit cytochrome systems, caused 100 to 200% stimulation, especially when the crude extract prepared from fresh cells possessed some ability to oxidize kynurenic acid. However, in confirmation of the above interpretation, the same amount of cyanide or azide did not stimulate the activity of the supernatant fraction obtained after high speed centrifugation. Instead, the addition of these inhibitors to the supernatant caused a marked lag period and inhibited the reaction considerably.

Isolation of Metabolites—Kynurenic acid, 548 μmoles, was added to a 138 ml reaction mixture containing 1.81 μmoles of kynurenic acid-3-C¹⁴ (329,000 c.p.m.) and 120 ml of the supernatant fraction containing 1.61 g of protein and 2.40 mmoles of potassium phosphate buffer, pH 7.5.

Incubation was carried out with mechanical shaking at 34⁻37° for 2.5 hours; a parallel experiment in a Warburg vessel indicated that 2.5 moles of O₂ were consumed per mole of substrate. A small aliquot was then removed for analysis and paper chromatography. All of the kynurenic acid added had disappeared, as judged by the absence of its characteristic absorption spectrum, with the formation of a considerable amount of glutamic acid. H₂SO₄, 8 N, was added to the incubation mixture until the final concentration reached 0.2 N. After the precipitate was removed by centrifugation, the supernatant solution was neutralized with Ba(OH)₂, and the precipitate was again removed by centrifugation. Then the supernatant solution, which contained about 10% of the original, was adjusted to pH 2 by the cautious addition of 8 N H₂SO₄ and was extracted twice with 50 ml of ether each time. The combined ether fractions were shaken vigorously twice with 50 ml of 5% sodium bicarbonate solution. The combined water layers containing approximately 200,000 c.p.m. (37.8% of the original), was then adjusted to pH 2 by the cautious addition of 8 N H₂SO₄ and was lyophylized. The distillate thus obtained contained approximately 1 nmole of acidic material as titrated by 0.1 N NaOH. The neutralized distillate was evaporated in a vacuum to dryness by means of a flash evaporator (Fraction I).

The water layer, after ether extraction, contained 121,000 c.p.m. (22.9%) and was neutralized with Ba(OH)₂. After a brief centrifugation, the supernatant fraction was evaporated in a vacuum at 45° by means of a flash evaporator. The residue was dissolved in about 10 ml of water. The solution, after brief centrifugation in order to remove a small amount of insoluble material, was subjected to ion exchange chromatography on a Dowex 1-formate column (1 sq. cm × 10 cm, 8% cross-linkage, 200 to 400 mesh). After application of the solution, the column was washed with about 50 ml of water. Approximately 83,100 c.p.m. (15.7%) came through the column (Fraction II); this
fraction contained ninhydrin-reacting substances. A gradient elution was then carried out with 0.1 N HCOOH in a reservoir and 400 ml of water in a mixing flask. A major radioactive peak containing about 15,200 c.p.m. (2.87%) was eluted between 40 and 67 ml (Fraction III) and was ninhydrin-positive. Fraction III was put through a Dowex 50-H+ column (1 sq. cm x 5 cm, 2% cross-linkage, 200 to 400 mesh), and elution was carried out with 6 N HCl in a reservoir and 400 ml of water in a mixing flask. The fraction containing most of the radioactivity (12,500 c.p.m., 2.36%) appeared between 77 and 101 ml. These were combined, evaporated in a vacuum by the aid of a flask evaporator at 40°C and dried finally over KOH and P2O5.

Fraction II was adjusted to pH 3.0 by the addition of 1 N HCl and was placed on a Dowex 50-H+ column (10 cm x 1 sq. cm, 200 to 400 mesh, 2% cross-linkage). The column was washed with 50 ml of water, and gradient elution was carried out with 3 N HCl in a reservoir and 400 ml of water in a mixing flask. A major radioactive and ninhydrin-positive peak came out between 21 and 50 ml. These fractions were combined and evaporated to dryness under reduced pressure at 50°C. The dried material was dissolved in about 5 ml of water and was evaporated again by the same procedure in order to remove HCl.

**Identification of L-Glutamic Acid**—The white semicrystalline material that was isolated from Fraction III was recrystallized from hot 6 N HCl. The melting point (corrected) was 202–203°C with decomposition. Authentic glutamic acid hydrochloride melted at 203–204°C with decomposition. The melting point was 202–204°C with decomposition. Optical rotation was [a]D = +31.7° (in 6 N HCl)

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\[ C_6H_9O_3N\cdot HCl \]

Calculated: C 32.71, H 5.49, N 7.63

Found: C 32.91, H 5.39, N 7.67

The compound gave exactly the same Rf values as did an authentic sample of L-glutamic acid with three solvent systems (Table I). When it was treated with a partially purified preparation of L-glutamate decarboxylase, a stoichiometric amount of CO2 was evolved with the formation of a new ninhydrin-reacting substance which, on paper chromatography, was indistinguishable from L-glutamic acid or L-aminobutyric acid. γ- and β-Hydroxyglutamic acid were both clearly distinguishable from the isolated material.

The specific activity of the L-glutamic acid was 140 c.p.m. per pmole on the basis of ninhydrin assay method. This figure represented about 6.8-fold dilution as compared with the original specific activity of kynurenic acid (962 c.p.m. per pmole). A dilution of specific activity in the isolated glutamic acid was observed consistently, although to a varying extent, in several similar experiments as shown in Table II.

**Identification of Acetic Acid**—The compound isolated from Fraction I was purified by partition chromatography on a Celite column. The isolated volatile material showed pK\textsubscript{a} of 4.65 and was identified as acetic acid by the use of acetokinase and by the formation of a p-bromophenacyl ester (23). The latter melted at 84.5–86°C, as did an authentic sample and the mixture of the two. The analysis was:

\[ C_3H_6O_2Br \]

Calculated: C 46.72, H 3.530

Found: C 47.02, H 3.753

**Table I**

**Paper chromatography of isolated glutamic acid and γ-amino butyric acid obtained by decarboxylation reaction**

The assay system for decarboxylation of L-glutamic acid contained in a volume of 2.0 ml, 10 mg of partially purified decarboxylase, 120 μmoles of potassium phosphate (pH 5.8), 15 μg of pyridoxal phosphate, and isolated L-glutamic acid (0.15 μmoles, 8800 c.p.m.). After completion of CO2 evolution, 0.1 ml of 2 N H2SO4 in the side arm was tipped into the main compartment. The amount of CO2 evolved was 144 μl. After neutralization of the acidified reaction mixture with Ba(OH)2, the supernatant solution was separated by centrifugation. Almost all the radioactivity remained in the solution. Aliquots (0.1 ml) of the neutralized solution were subjected to paper chromatography. With both glutamic acid and γ-aminobutyric acid, the ninhydrin-positive spots were coincident with the radioactive spots. There were no ninhydrin-positive or radioactive spots other than L-glutamic acid or γ-aminobutyric acid in each case.

<table>
<thead>
<tr>
<th>Specific activity</th>
<th>Sample</th>
<th>Authentic glutamic acid</th>
<th>Acetic acid</th>
<th>γ-Hydroxy glutamic acid</th>
<th>γ-Aldolic acid</th>
<th>γ-γ-Hydroxy glutamic acid</th>
<th>Sample</th>
<th>Authentic glutamic acid</th>
<th>Acetic acid</th>
<th>γ-Hydroxy glutamic acid</th>
<th>γ-γ-Hydroxy glutamic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butanol-acetic acid-water (1:1:1)</td>
<td>0.28</td>
<td>0.28</td>
<td>0.14</td>
<td>0.17</td>
<td>0.63</td>
<td>0.63</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water-saturated phenol</td>
<td>0.15</td>
<td>0.15</td>
<td>0.07</td>
<td>0.07</td>
<td>0.56</td>
<td>0.56</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol-ammonia-water (18:1:1)</td>
<td>0.35</td>
<td>0.35</td>
<td>0.20</td>
<td>0.27</td>
<td>0.52</td>
<td>0.52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Both DL-γ-hydroxy glutamic acid and DL-γ,γ-hydroxy glutamic acid gave identical Rf values.
† Both DL-threo- and DL-erythro-β-hydroxy glutamic acids gave identical Rf values.
& After treatment with glutamic decarboxylase.

**Table II**

**Specific activity of isolated glutamic acid, alanine, and acetic acid**

The specific activity (c.p.m. per pmole) of glutamic acid and alanine was determined on the basis of ninhydrin assay, and that of acetic acid by titration.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Specific activity</th>
<th>Total counts</th>
<th>Specific activity</th>
<th>Total counts</th>
<th>Specific activity</th>
<th>Total counts</th>
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<tr>
<td>1</td>
<td>2,740</td>
<td>758,000</td>
<td>1,430</td>
<td>101,000</td>
<td>1,300</td>
<td>47,000</td>
<td>13,000</td>
<td>400</td>
<td>211,000</td>
<td></td>
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<tr>
<td>2</td>
<td>1,035</td>
<td>455,000</td>
<td>215</td>
<td>41,200</td>
<td>417</td>
<td>15,900</td>
<td>15,100</td>
<td>200</td>
<td>200,000</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>962</td>
<td>629,000</td>
<td>140</td>
<td>12,500</td>
<td>271</td>
<td>15,100</td>
<td>15,100</td>
<td>200</td>
<td>200,000</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>473</td>
<td>182,000</td>
<td>139</td>
<td>10,000</td>
<td>136</td>
<td>12,900</td>
<td>12,900</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>274</td>
<td>150,000</td>
<td>52</td>
<td>12,100</td>
<td>52</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

The specific activity of acetic acid was also diluted 2- to 4-fold as compared with that of kynurenic acid (Table II). It should be noted, however, that the extent of dilution was always somewhat less than that of glutamic acid in the same experiment.

**Identification of D- and L-Alanine**—The dried material isolated from Fraction II was dissolved in 4 ml of H2O and was subjected to preparative paper chromatography on Whatman No. 31 (extra thick) papers with two successive solvent systems. The Rf values of an authentic sample of L-alanine were: ethanol-...
ammonia-water (18:1:1), 0.30, n-butanol-acetic acid-water (4:1:1), 0.23. The radioactive and ninhydrin-positive bands corresponding to L-alanine were eluted with approximately 5 ml of H2O each time. Finally the solution was condensed over P2O5 and crystallization was achieved by adding 99% ethanol to a small amount of water solution. The yield of white crystalline material was approximately 50 µmoles and contained 15,100 c.p.m. representing 2.9% of the radioactivity of the starting substrate, kynurenic acid.

Values of this material, as determined by the radioactivity and by ninhydrin color, were in essential agreement with those given by an authentic sample of L-α-alanine with six different solvent systems: phenol-water (8:2), 0.53; with isopropanol-water (8:2), 0.27; n-butanol-acetic acid-water (4:1:1), 0.23; ethanol-ammonia-water (18:1:1), 0.31; n-butanol-pyridine-water (1:1:1), 0.28; and with ethanol-diethylamine-water (77:1:22), 0.27. Paper electrophoresis was carried out on Whatman no. 1 paper (13.5 X 45 cm, pyridine-acetic acid-water (1:10:89), pH 3.5, 2000 volts, 30 ma, 40 minutes). Both L-alanine and the sample moved approximately 1.0 cm to the cathode.

Preparation usually contained alanine racemase, the extent of racemization appeared to depend upon the experimental conditions used and the alanine racemase activity.

DISCUSSION

The extensive degradation of kynurenic acid by a strain of *Pseudomonas* was first described in 1949 (4), but attempts to elucidate the intermediary steps were uniformly unsuccessful simply because it was not possible to obtain a cell-free enzyme system active on kynurenic acid. In the present communication, we have described an active enzyme system which was found in a soluble fraction of the crude extract and was inhibited markedly in the presence of a particulate fraction. The nature of this inhibition is not clearly understood at the present time except that the inhibition could be largely removed by heating the particulate fraction at 100° for 5 minutes. However, it seems to be a reasonable conjecture that this inhibitory effect may be largely due to the presence of a cytochrome system in the particulate fraction which is depleting the system of the reduced form of pyridine nucleotide coenzymes and is, therefore, competing with the hydroxylases required to initiate kynurenic acid degradation. This supposition is consistent with our preliminary observation that the primary reaction of kynurenic acid degradation requires DPNH and oxygen (26).

Quinaldic acid, which has been shown to be a metabolite of kynurenic acid in mammals (3), was degraded rather slowly by resting cell suspension of this organism. Although the rate of this reaction was approximately ½ that of kynurenic acid degradation, quinaldic acid was definitely metabolized since, after the termination of the experiment, its characteristic ultraviolet absorption had completely disappeared. However, with the cell-free enzyme preparation, quinaldic acid was not metabolized at all, thus excluding the possibility of its being an intermediate metabolite in the *Pseudomonas* system.

The oxidation of kynurenic acid by cell-free preparations obtained from *Pseudomonas* degrading tryptophan by way of the quinoline pathway was concurrently reported by Behrmann and Tanaka who found that 1 mole of kynurenic acid was oxidized with the consumption of 3 moles of oxygen, yielding 3 moles of CO2 together with 1 mole of L-glutamate. During the course of our investigation we carried out the isolation of L-glutamic acid seven times; the yield of isolated l-glutamic acid ranged from 17 to 50% of the added substrate. One curious aspect of the results, which was observed by both groups of investigators, was the dilution of specific activity of L-glutamate as compared with that of kynurenic acid added. Although considerable amounts of L-glutamate were produced endogenously, this source was far from sufficient to account for the dilution, indicating the possibility that the skeleton of L-glutamate was formed from more than one source during the experiment. Because kynurenic acid labeled in carbon 3 yielded labeled L-glutamic acid ranged from 17 to 50% of the added substrate, one curious aspect of the results, which was observed by both groups of investigators, was the dilution of specific activity of L-glutamate as compared with that of kynurenic acid added. Although considerable amounts of L-glutamate were produced endogenously, this source was far from sufficient to account for the dilution, indicating the possibility that the skeleton of L-glutamate was formed from more than one source during the experiment. Because kynurenic acid labeled in carbon 3 yielded labeled L-glutamic acid, it might be conjectured that the carbon skeleton of the pyridine ring is the source of glutamate, if the assumption is made that the nitrogen atom of L-glutamate is derived from that of kynurenic acid and that the conversion occurs without breaking the carbon-nitrogen bond. It is clear from the results in Table II, however, that the specific activity of glutamate was generally higher than or the same as that of glutamate, indicating the possibility that kynurenic acid was first converted to C-2 or C-3 fragments which could then be resynthesized to C-5 units. The subsequent paper will deal with the fate of the carbon atoms of kynurenic acid as studied with isotopic techniques.
SUMMARY

1. From tryptophan-adapted cells of Pseudomonas, an enzyme system that catalyzes partial degradation of kynurenic acid was prepared and its properties described.

2. Enzymatic synthesis of C\textsuperscript{14} labeled kynurenic acid from C\textsuperscript{14} labeled tryptophan was described.

3. L-Glutamic acid, d- and L-\alpha-alanine, and acetic acid were shown to be among the main radioactive end products of kynurenic acid degradation by this enzyme.

4. The specific activity of glutamic acid was diluted approximately 2- to 7-fold as compared with that of kynurenic acid. The specific activity of alanine and acetate was generally higher than or the same as that of glutamate indicating the possibility that kynurenic acid was first degraded to C-2 or C-3 fragments that could then be resynthesized to C-5 units.

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Studies on the Metabolism of Kynurenic Acid: I. THE FORMATION OF L-GLUTAMIC ACID, d- AND L-ALANINE, AND ACETIC ACID FROM KYNURENIC ACID BY PSEUDOMONAS EXTRACTS
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