Dipicolinic Acid Synthesis in Penicillium citreo-viride

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Received for publication 7 September 1965

ABSTRACT

HODSON, PHILLIP H. (University of Texas, Austin), AND J. W. FOSTER. Dipicolinic acid synthesis in Penicillium citreo-viride. J. Bacteriol. 91:562-569. 1966.—Dipicolinic acid (DPA) accumulation in culture filtrates of the mold Penicillium citreo-viride was studied in surface and submerged cultures. Good DPA yields were obtained in suspensions of washed, submerged mycelium in the presence of a carbon and a nitrogen source but in the absence of other minerals essential for growth. Fumaric acid was the only other acid formed in significant amounts. Glucose and glycerol were superior to various salts of organic acids as carbon sources, and certain amino acids were excellent nitrogen sources. L-Leucine, L-norvaline, L-tyrosine, and L-histidine were superior to urea, NH₄Cl, or NaNO₃ as nitrogen precursors for DPA production. D-Norvaline was useless for DPA production. Glycerol-2-C¹⁴ and -L-C¹⁴, C¹⁴O₂, and L-leucine-C¹⁴, L-tyrosine-C¹⁴, and L-histidine-C¹⁴ were tested as precursors in conjunction with suitable carbon and nitrogen sources. The DPA was decarboxylated chemically, and the distribution of C¹⁴ was determined in the pyridine-C and in the carboxyl-C. The data are consistent with Martin and Foster's suggestion for bacteria that the DPA molecule is formed by a condensation of C₄ plus C₄ precursors, the resulting 2-keto, 6-aminopimelic acid derivative undergoing ring closure to form a heterocyclic precursor of DPA. The C¹⁴O₂ experiments indicate that oxaloacetate is formed by β-carboxylation of pyruvate, this in turn probably becoming aspartic acid β-semialdehide, the C₈ compound which condenses with a second pyruvate. The enhancement of DPA formation by L-norvaline, L-leucine, and L-histidine is not ascribable to their functioning either as a source of nitrogen or carbon. L-Tyrosine, in a glycerol medium, contributed nearly 40% of the DPA carbon. The mechanism of biosynthesis of C₇ straight-chain and cyclic compounds is discussed.

Dipicolinic acid (pyridine-2,6-dicarboxylic acid, DPA) evidently occurs in all bacterial spores, where it is believed to be involved in the resistance properties characteristic of those specialized cells (21). Thought before 1960 to be unique to bacterial spores, DPA was discovered by Ooyama to be a mold fermentation product accumulating in substantial amounts in culture filtrates of certain penicillia (23-26). The mechanism of DPA biosynthesis in bacteria had been studied prior to this (17, 28, 30), and the corresponding study in a mold by Tanenbaum and Kaneko (35) has, in general, given results similar to the findings obtained with bacteria. Many aspects, nevertheless, remain to be elucidated. The following work was undertaken as a prelude to biochemical mechanism studies and was completed before the appearance of Tanenbaum and Kaneko's report. At least two routes exist for biogenesis of the pyridine ring—the tryptophan pathway and the condensation of C₄ and C₃ precursors (19).

MATERIALS AND METHODS

Microbiological. Penicillium citreo-viride 4174 was kindly furnished by J. Ooyama of the Fermentation Research Institute, Inage, Chiba City, Japan. The stocks were carried on Czapek's agar. The basal medium for production of DPA consisted of: glucose, 100 g; KH₂PO₄, 5 g; MgSO₄·7H₂O, 1 g; corn steep liquor solids, 0.5 g; urea, 1 g; and distilled water, 1 liter. The urea solution was filter-sterilized before
addition to the autoclaved medium. Surface and submerged growth cultures were inoculated with a light suspension of conidiospores washed from the surface of a Czapek's agar slant. Submerged growth was obtained on a reciprocating shaking machine operating at ninety-two 3.5-inch (8.9-cm) strokes per min.

Most often, experiments were done with washed, preformed submerged mycelium under conditions in which minerals essential for growth were omitted. The mycelial suspensions for these were obtained from 3-day-old shaken cultures harvested from 200 ml of growth medium in 2-liter flasks. The hyphal mass was filtered on paper in a Buchner funnel and was washed on the funnel with 200 ml of sterile, distilled water. Further details are given in the tables. Contamination was rarely a problem. DPA analyses were made on culture filtrates pooled with mycelial hot-water extracts and washings. DPA concentrations are based on the initial volume of culture medium; in the longer experiments, evaporation losses were restored by periodic additions of sterile, distilled water.

Chemical. DPA was assayed colorimetrically (13), and carbohydrate was determined by the anthrone method (20). Detection of acidic spots on paper chromatograms was achieved by spraying with 0.05% aqueous bromocresol green. Ether-soluble organic acids were separated on a silicic acid partition column (37).

Decarboxylation of DPA. The method was adapted from Martin and Foster (17), with the use of the solvent phenanthrene omitted. A 10- to 12-mg amount of dry DPA and 10 mg of copper chromite were mixed in a micro porcelain boat, which was then inserted in a horizontal Pyrex tube, with a thermometer along the inside. The tube temperature was brought to 290 C with an electrical heating element wound around an asbestos tape tube-wrapping. The pyridine and CO2 resulting from the decarboxylation were removed from the reaction area by a slow, continuing stream of CO2 and oxygen-free N2. The pyridine was trapped by passing the gas stream through an attached vessel containing 2.5 ml of an ice-cold, saturated, aqueous solution of picric acid. Crystalline pyridine picrate was recovered by filtration. The reaction CO2 was absorbed by passing the gas serially through three vessels containing 1 N NaOH. The absorbed CO2 was converted to BaCO3 in the usual manner and was recovered by filtration. Usually, 10 , used per labeled precursor. Radioisotope measurements were made with a thin-window Geiger-Müller tube and a Tracerlab "64" scaler and were corrected for self-absorption when necessary. Carrier DPA was added for the decarboxylation reaction and the specific radioactivity data were corrected accordingly.

RESULTS

DPA production in growth media. To determine DPA production in surface cultures, several replicate cultures were incubated in parallel, and a different one was analyzed daily. The mycelium was boiled to extract crystals of DPA which often accumulated in older mycelial masses. It was apparent that DPA formation paralleled growth, that both were almost proportional to glucose consumption, and that both ceased upon exhaustion of the glucose (Fig. 1). Maximal weight conversion yield of glucose to DPA was 12.4% (equivalent to 13.8% molar yield) at which stage the pH of the culture was 2.5.

An experiment similar to that described above, but carried out on a shaker, revealed that DPA was produced in submerged culture but that both the rates of glucose consumption and DPA formation were lower than in the surface culture (Fig. 2). Even so, the conversion efficiency was approximately the same. A paper chromatogram of the culture filtrate revealed appreciable quantities of

FIG. 1. Changes in surface growth cultures of a DPA-producing Penicillium citreo-viride. Twelve replicate 250-ml Erlenmeyer flasks, each containing 30 ml of medium, were inoculated and incubated at 30 C. Each day a different culture was analyzed.

FIG. 2. Changes in submerged growth cultures of a DPA-producing Penicillium citreo-viride. Eleven replicate 250-ml Erlenmeyer flasks, each containing 30 ml of medium, were inoculated and incubated at 30 C on a continuous shaker. Each day a different culture was analyzed.
a second acid. This acid was ether-soluble and reduced aqueous KMnO₄ in the cold. It was isolated in pure, crystalline form by partition column chromatography and was identified as fumaric acid by RF values in three solvent systems, by melting points, and by infrared absorption spectra. Fumaric acid was a common product along with DPA in P. citreo-viride cultures, and was the only other acid encountered in appreciable amounts (Fig. 3).

**DPA production by washed, submerged mycelium.** Based on the precursor studies of Martin and Foster (17), various organic acids and glucose were tested for conversion to DPA under conditions where growth was minimized by the absence of nutrients other than a source of nitrogen and phosphate buffer (0.2 m, pH 6 to 8). Appreciable DPA was formed under these conditions, and this technique was adjudged useful for mechanism studies. Glucose was decidedly superior to the organic acids with respect to accumulation of DPA. However, the consumption of the acids was not measured, and it is possible that slowly consumed organic acids (due to permeability?) might actually be efficient precursors of DPA.

Numerous nitrogenous substances were tested for efficacy as nitrogen sources for DPA production. Data dealing with the best of these are presented in Table 1. Several features of this experiment suggest that certain amino acids enhance DPA production by means other than simply furnishing nitrogen. Thus, all of the amino acids were deaminated, with the ammonia content of the filtrates ranging from 0.1 to 1.0 mg/ml; yet, a few of the amino acids were better than the others for DPA production. Likewise, some good nitrogen donors, e.g., glutamic acid, aspartic acid, and urea, were inferior for DPA production. Direct evidence for a carbon precursor role for certain amino acids is presented later. Histidine, phenylalanine, tyrosine, tryptophan, leucine, and norvaline were unexpectedly useful for DPA production. The L isomers of leucine and phenylalanine were much better than the D forms, but there was little difference between the efficacy of DL- and L-tyrosine. The efficacy of DL-norvaline was unexpected; this compound does not occur naturally. Its utility for DPA formation was contingent upon a concomitant carbon source, e.g., glucose.

Two of the best nitrogenous precursors, namely, DL-norvaline and L-leucine, were used in experiments designed to compare various carbon sources for DPA production. Glycerol was the best of those tested (Table 2), but interesting specificities were revealed. Whereas the neutral carbon sources as a class were superior to the organic acids, the nitrogen source appeared to have a determinative role in certain cases. The outstanding example of this is acetone; it was worthless in conjunction with DL-norvaline but excellent with L-leucine. Some of the organic acids were much better DPA precursors in the presence of L-leucine than of DL-norvaline.

The L enantiomorph of norvaline proved to be
Table 2. DPA production from various carbon sources with DL-norvaline and L-leucine as nitrogen sources

| Carbon source† | DPA in culture filtrate
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DL-Norvaline† mg/ml</td>
</tr>
<tr>
<td>None</td>
<td>0.3</td>
</tr>
<tr>
<td>K citrate</td>
<td>2.2</td>
</tr>
<tr>
<td>K α-ketoglutarate</td>
<td>1.2</td>
</tr>
<tr>
<td>K succinate</td>
<td>1.1</td>
</tr>
<tr>
<td>K fumarate</td>
<td>1.7</td>
</tr>
<tr>
<td>K malate</td>
<td>0.7</td>
</tr>
<tr>
<td>K pyruvate</td>
<td>0.4</td>
</tr>
<tr>
<td>K lactate</td>
<td>0.4</td>
</tr>
<tr>
<td>Glucose</td>
<td>6.0</td>
</tr>
<tr>
<td>Fructose</td>
<td>5.7</td>
</tr>
<tr>
<td>Glycerol</td>
<td>7.7</td>
</tr>
<tr>
<td>Acetone</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* Determined on 50-ml cultures in 0.2 M phosphate buffer (pH 7.0) in 250-ml Erlenmeyer flasks; 2 g of submerged mycelium (wet weight); incubation, 9 days on shaker.
† Amounts of 139.0 mmoles. The acetone was filter-sterilized and added to the autoclaved medium.
‡ An amount of 5.6 mmoles.

far superior to the D form (Table 3); the effectiveness of the racemic mixture was evidently attributable to the L component. The exceptional concentration of 14 mg of DPA per ml was reached in this experiment. On the basis of glycerol supplied, the weight yield of DPA was 28%. Paper chromatograms on the 16th day showed that all of the L-norvaline had been utilized, but not the D or the DL forms. In the latter two filtrates only, there were detected appreciable quantities of an unidentified acid. It had an \( R_f \) value of 0.80 in \( n \)-butanol-acetic acid-water (4:1:1, \( v/v \)), and did not react with ninhydrin, 2,4-dinitrophenyl-hydrazine, or aqueous K\( \text{MnO}_4 \) in the cold.

Various analogues of norvaline were furnished as ammonium salts in 5% glycerol and were compared with norvaline on an equivalent basis. Ammonium \( \alpha \)-hydroxy-n-valerate and ammonium \( \alpha \)-keto-n-valerate were no better than urea as nitrogen sources. These results imply that norvaline enhancement of DPA formation does not depend on a deamination and utilization of the corresponding keto or hydroxy acid.

Radioactive tracer experiments. With the exception of norvaline, which was unavailable as a \( ^{14} \)C-labeled compound, the best precursors studied in the foregoing experiments were used in labeled form in tracer experiments. The cultures were incubated individually in 10-liter desiccators, in which the atmospheres were enriched with 5% \( \text{CO}_2 (v/v) \) to reduce by dilution the reassimilation of radioactivity in the form of respiratory \( \text{CO}_2 \). The radioactivity of the atmospheric \( \text{CO}_2 \) was measured after collection as Ba\( \text{CO}_3 \) at the end of the experiment.

Besides DPA, the only significant radioactive product found in these cultures was fumaric acid (Fig. 3). The radiopurity of the DPA isolated from the partition column is indicated by the parallelism between titration and counts of the various eluate fractions and by radioautography of paper chromatograms. The purified DPA was recrystallized from water, dried in vacuo over P\( _2 \)O\( _5 \), and used in the decarboxylation procedure described in Materials and Methods.

The experiments with glycerol-\( ^{14} \)C and unlabeled norvaline as cosubstrates show that the DPA-C was derived practically entirely from the glycerol-C (Table 4), since the specific radioactivity of the DPA was nearly the same as that of the substrate glycerol. Some of the glycerol was respired, with more of the 1-C than the 2-C being lost as \( \text{CO}_2 \). Norvaline's contribution to the DPA-C was, therefore, negligible, a conclusion foreshadowed by the previous knowledge that the amino acid itself does not support DPA synthesis and that its deaminated analogues are ineffective DPA precursors.

The specific radioactivity data indicate likewise that leucine and histidine, despite their enhancement of DPA formation, contributed carbon to that synthesis only to a minor degree. That more of the uniformly labeled than the specifically labeled amino acids should be incorporated in DPA is not surprising. The carbonyl-C would be readily lost early, and extensive carbon chain metabolism undoubtedly mixes short-chain inter-
mediates from the breakdown of the amino acids with the same intermediates from glycerol that are used in the biosynthesis of DPA. In any event, formation of only 11.2% of the DPA from uniformly labeled leucine falls short of the magnitude of the DPA-promoting effect of this amino acid. If only the L isomer of leucine-2-C\textsuperscript{14} were metabolized, the incorporation value becomes 28.4%, i.e., the equivalent of two of the seven carbon atoms of DPA.

Uniformly labeled tyrosine, on the other hand, contributed nearly 40% of the DPA-C, even in the presence of as favorable a carbon source as glycerol. The phenyl group obviously is extensively degraded to biosynthetic precursors of DPA, which contain the 2-C, in addition to the amino group's contribution of nitrogen. Fumaric and acetooacetic acids, known as oxidation products of tyrosine metabolized via the homogentic acid pathway (19), would be readily converted to known precursors of DPA. The occurrence of fumaric acid as the only significant coproduct of DPA in cultures of \textit{P. citreo-viride} is likewise consistent with its being involved in DPA metabolism. Tricarboxylic acid cycle intermediates are excellent progenitors of DPA (17, 35), and the tricarboxylic acid cycle has been demonstrated in a number of filamentous fungi (9, 16, 22).

Further implicating the tricarboxylic acid cycle, CO\textsubscript{2} fixation provided a significant portion of the mold DPA-C (Table 4). The highest value (17%) represents one-sixth of the DPA-C and corresponds closely to the findings in \textit{Bacillus megaterium} (17). Tanenbaum and Kaneko's (35) data, although showing CO\textsubscript{2}-C incorporation in mold DPA, do not reveal the extent to which CO\textsubscript{2}-C contributed to the total.

In agreement with previous reports for the bacterial and the mold systems, all of the labeled precursors tested in this work contributed to both the ring- and the carboxyl-C of DPA (Table 5). This is the result expected for a total synthesis from short carbon chains. A combination of any two chains formed via a respiratory cycle would redistribute the labeling so that both pyridine- and carboxyl-C would be labeled. The fairly equal distribution between ring- and carboxyl-C of DPA derived from the two specifically labeled glycerols, and from the uniformly labeled leucine, tyrosine, and histidine, points strongly to the formation of short-chain degradation products, intracellular randomization of label via respiratory cycling, and synthesis of DPA from the pool of small precursors. Formation of appreciable amounts of labeled fumaric acid from uniformly labeled leucine (Fig. 3) bears that out.

A marked disproportion between label found in the pyridine and the carboxyl groups was, on the other hand, obtained when the amino acids were specifically labeled, and also when C\textsuperscript{14}O\textsubscript{2} was used. Here, the immediate precursors of DPA must have been asymmetrically labeled predomi-

<table>
<thead>
<tr>
<th>Compound</th>
<th>Precursor added</th>
<th>Dipicolinic acid</th>
<th>Carbon dioxide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amt (mmoles)</td>
<td>Count/ min X 10^4</td>
<td>Count per min per atom of C</td>
</tr>
<tr>
<td>Glycerol-2-C\textsuperscript{14}†</td>
<td>27.14</td>
<td>47.4</td>
<td>5.6</td>
</tr>
<tr>
<td>Glycerol-1-C\textsuperscript{14}†</td>
<td>27.14</td>
<td>30.5</td>
<td>3.6</td>
</tr>
<tr>
<td>L-Leucine-\textit{U}-C\textsuperscript{14}</td>
<td>5.72</td>
<td>94.8</td>
<td>27.6</td>
</tr>
<tr>
<td>L-Leucine-\textit{L}-C\textsuperscript{14}</td>
<td>5.72</td>
<td>156.5</td>
<td>45.5</td>
</tr>
<tr>
<td>L-Tyrosine-\textit{U}-C\textsuperscript{14}</td>
<td>5.86</td>
<td>74.9</td>
<td>14.5</td>
</tr>
<tr>
<td>L-Tyrosine-\textit{L}-C\textsuperscript{14}</td>
<td>2.81</td>
<td>23.9</td>
<td>9.4</td>
</tr>
<tr>
<td>DL-Tyrosine-\textsuperscript{2}-C\textsuperscript{14}</td>
<td>2.81</td>
<td>30.4</td>
<td>12.0</td>
</tr>
<tr>
<td>L-Histidine-\textit{U}-C\textsuperscript{14}</td>
<td>1.85</td>
<td>43.9</td>
<td>39.0</td>
</tr>
<tr>
<td>L-Histidine-2(ring)-\textsuperscript{2}</td>
<td>1.87</td>
<td>32.1</td>
<td>28.6</td>
</tr>
</tbody>
</table>

* Determined on 50-ml cultures in 0.2 m phosphate buffer (pH 7.0) in 250-ml Erlenmeyer flasks; glycerol, 27.2 mmoles, as carbon source; 2 g of submerged mycelium (wet weight); incubation, 7.5 days on shaker in separate 10-liter descicators. Except in the two which had C\textsuperscript{14}O\textsubscript{2} as a precursor, the air in each descicator contained 5% CO\textsubscript{2} (v/v).
† DL-Norvaline, 5.6 mmoles, as N source.
‡ Urea, 2.85 mmoles, as N source; glucose, 13.9 mmoles, as carbon source.

Table 4. Conversion of C\textsuperscript{14}-labeled precursors by \textit{Penicillium citreo-viride} 4174*
nantly in a terminal carbon destined to become the DPA carboxyl-C. The data make it clear that some recycling and partial randomization of label took place but not enough to obscure the radioactive asymmetry of the precursor(s).

**DISCUSSION**

The effect of tyrosine appears to have a different basis from that of the other DPA-promoting amino acids. The latter do not contribute materially to the carbon of DPA, whereas the former does. Instances are legion in which amino acids influence biochemical pathways, e.g., through competitive inhibition (3, 11, 12, 32, 33), non-competitive inhibition (31), feedback control (1, 36, 41), and nutritional functions (2, 34).

The carbon-14 data indicate that the precursors of DPA are "C₃" compounds and CO₂, at least when glycerol or glucose is substrate. Pyridine ring biosynthesis de novo from short, aliphatic carbon chains is well established (10, 27, 38). Martin and Foster (17) proposed that the biosynthesis of the DPA skeleton in *Bacillus megaterium* occurs by a condensation of an aspartyl compound and pyruvic acid, yielding a pimelate compound which then cyclizes to form a pyridine dicarboxylic acid. Aspartic semialdehyde and pyruvic acid were recently reported to serve for the net synthesis of DPA in cell-free bacterial extracts (Bach and Gilvarg, Federation Proc. 23:313, 1964). Evidence for ring closure of a pimelate compound in DPA biosynthesis was advanced by Perry and Foster (28). Similar experiments by Finlayson and Simpson (4) were less clear cut, but those of Tanenbaum and Kaneko (35) were decisive.

The identity and the formation route of the pimelate precursors are the main problems in DPA biosynthesis. Regarding the identity, Tanenbaum and Kaneko (35) proposed that *P. citreoviride* uses the spontaneous diketopimelic acid-ammonia cyclization described by Powell and Strange (30). They isolated 2,6-diketopimelic acid (DKP) from the mold, and ascribed a precursor function to it. On the other hand, 2-keto, 6-aminopimelic acid (KAP) has been considered to be the key intermediate (5, 17), and none of the available bacterial and mold data are inconsistent with that idea. The precursor origins of the carbon chains of KAP and DPA are strikingly similar (8, 17, 35).

KAP may well represent the focal biogenetic origin of the various pimelic acid compounds in cells. It is convertible to 2,6-diaminopimelic acid (7, 8, 15, 29), and probably to DPA and to DKP. The DKP found in *P. citreoviride* may originate from intermediate metabolic KAP by the action of the mold's same strong amino acid oxidase that removes both amino groups from added 2,6-diaminopimelic acid (35). When intermediate KAP is N-acetylated, the open chain essential for 2,6-diaminopimelic acid (and, presumably, DKP) formation is conserved (8). DPA formation, on the other hand, would require a deacetylated KAP for ring closure to occur. Indirect evidence for KAP as an intermediate in DPA formation was obtained in the oxidative deamination of 2,6-diaminopimelic acid in extracts of *Neurospora*

### Table 5. Distribution of radioactivity between pyridine-carbons and carboxyl-carbons*

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Precursor specific radioactivity (count per min per μatom of C)</th>
<th>Specific radioactivity of Dipicolinic acid (count per min per μatom of C)</th>
<th>Specific radioactivity, pyridine-C/carboxyl-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol-2-C¹⁴†</td>
<td>5.6</td>
<td>7.7</td>
<td>5.1</td>
</tr>
<tr>
<td>Glycerol-1-C¹⁴†</td>
<td>3.6</td>
<td>2.9</td>
<td>3.4</td>
</tr>
<tr>
<td>L-Leucine-U-C¹⁴</td>
<td>27.6</td>
<td>3.5</td>
<td>3.0</td>
</tr>
<tr>
<td>L-Leucine-1-C¹⁴</td>
<td>45.5</td>
<td>4.4</td>
<td>2.4</td>
</tr>
<tr>
<td>L-Tyrosine-U-C¹⁴</td>
<td>14.5</td>
<td>0.5</td>
<td>1.6</td>
</tr>
<tr>
<td>L-Tyrosine-1-C¹⁴</td>
<td>9.4</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>DL-Tyrosine-2-C¹⁴</td>
<td>12.0</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>L-Histidine-U-C¹⁴</td>
<td>39.0</td>
<td>4.1</td>
<td>3.9</td>
</tr>
<tr>
<td>L-Histidine-2-(ring)-C¹⁴</td>
<td>28.6</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>C¹⁴O₂†</td>
<td>11.3 X 10³</td>
<td>15.2</td>
<td>39.6</td>
</tr>
<tr>
<td>C¹⁴O₂‡</td>
<td>14.5 X 10³</td>
<td>7.4</td>
<td>29.6</td>
</tr>
</tbody>
</table>

* Conditions were the same as in Table 4.
† DL-Orvaline, 5.6 mmoles, as N source.
‡ Urea, 2.85 mmoles, as N source; glucose, 13.9 mmoles, as carbon source.
(39, 40). The O₂, ammonia, and 2,6-diaminopimelic acid stoichiometry indicated that only one amino group had been attacked. Keto and amino groups could not be detected. The expected KAP could not be detected, presumably because of rapid ring closure to the putative dihydriodipicolinic acid (6, 18). The above pathways are depicted in Schema 1, modified from Foster (5), and Martin and Foster (17).

With regard to the synthesis of the pimelic acid carbon chains, viewpoints are necessarily conjectural in the absence of direct proof. The evidence from bacterial and mold systems is consistent with the C₃ + C₄ mechanism (14, 17). However, a novel pathway for this biosynthesis has also been suggested (35), which does not take into account the above-described substantial body of information developed by Gilvarg.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grant AI-03564 from the National Institute of Allergy and Infectious Diseases, by grant No. 375(12) from the Office of Naval Research, and by grant 14568 from the National Science Foundation.

LITERATURE CITED


