ACTION OF SOME SUBSTITUTED ANTHRANILIC ACIDS ON
ESCHERICHIA COLI

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Rydon (1) has found that 4- and 5-methylanthranilic acids inhibit the
growth of Eberthella typhi and that this inhibition is reversed by anthranilic
acid, indole, and tryptophan. He concluded that the two methyl comp-
ounds interfere with the synthesis of tryptophan at the stage of anthranilic
acid.

Interruption in the conversion of anthranilic acid to indole in Escherichia
coli, caused by chloromycetin, aureomycin, and Terramycin, was recently
observed by Bergmann and Sicher (2) and Bergmann et al. (3). The same
effect is shown by 5-fluorotryptophan (4). It appeared, therefore, inter-
esting to study the response of E. coli to some substituted anthranilic
acids.

Since the fluoro compounds o-, m-,1 p-fluorophenylalanine1 (5) and 3-fluo-
rotyrosine (6, 7) were found to exert antimetabolite activity, 4- and 5-fluo-
roanthranilic acids in addition to 5-methylantranilic acid were chosen as
possible antimetabolites.

The present study shows that these anthranilic acid analogues do indeed
inhibit the biosynthesis of anthranilic acid in E. coli, and that the inhibition
can be overcome not only by the similarly built metabolites, but also by a
number of amino acids, purines, and vitamins.

Methods and Materials

Culture and Media—E. coli (ATCC 9637) was carried as a slant culture
on minimal medium as described by Davis and Mingioli (8), supplemented
with 0.5 per cent of casein enzymatic hydrolysate (Nutritional Biochemicals
Corporation). For the preparation of inocula, a small amount of surface
growth from an 8 hour culture was suspended in sterile 0.9 per cent sodium
chloride solution to a turbidity reading of 60 on the Klett-Summerson
photoelectric colorimeter (red filter No. 64) and was then further diluted
1:33 in saline. Each experimental culture of 2.5 ml. was inoculated with
1 drop of this suspension. The basal medium used for the various assays
was that of Davis and Mingioli (8).

1 Unpublished experiments.
Tested materials were added in desired concentration to 1.25 ml. of double strength medium in 18 × 150 mm. Pyrex culture tubes. The media were then completed with distilled water, to a final volume of 2.5 ml., capped, autoclaved at 15 pounds pressure for 15 minutes, cooled, and inoculated. After incubation at 37° for 17 hours, turbidity readings were made with the Klett-Summerson colorimeter, red filter No. 61. All experiments were carried out in duplicate.

The amounts of each compound needed to reduce growth to 50 per cent and to cause complete inhibition were determined from dose-response curves. The reversal of inhibition, by various metabolites, was tested at 50 per cent, and complete inhibition and the antibacterial indices (micrograms per ml. of inhibitor versus micrograms per ml. of metabolite) for these levels were obtained from the corresponding growth curves.

Preparation of 5-Fluoro-2-aminobenzoic Acid (5-Fluoroanthranilic Acid)—2-Nitro-5-aminotoluene was prepared according to Wibaut (9), and 2-nitro-5-fluorotoluene by the general method of Schiemann and Bolstad (10). The compound has been obtained by a different route by Schiemann (11).

To a mixture of 45 gm. of 2-nitro-5-aminotoluene and 130 ml. of concentrated hydrochloric acid, a solution of 21 gm. of sodium nitrite in 65 ml. of water was added at 5°. After filtration, 78 ml. of 40 per cent fluoroboric acid solution were added, giving a dark yellow precipitate, which was filtered after 30 minutes, washed with fluoroboric acid, alcohol, and ether, and dried. Yield, 55 gm.

The diazonium salt was decomposed at 90–100° in portions of 2.5 gm.; the tarry residue, subjected to steam distillation, gave a yellow oil which distilled at 50–60° at 11 mm.; m.p. 28°. Yield, 17.6 gm. (from 55 gm. of the diazonium salt).

C₇H₆O₂NF. Calculated, C 54.2, H 3.9; found, C 54.4, H 4.0

2-Nitro-5-fluorobenzoic Acid—A mixture of 17.6 gm. of 2-nitro-5-fluorotoluene, 44.7 gm. of potassium permanganate, and 500 ml. of water was heated on the water bath with stirring until the color disappeared (2.5 hours). The mass was then steam-distilled in order to remove unchanged starting material (4.2 gm.) and filtered, and the filtrate was concentrated and acidified with 11 ml. of concentrated hydrochloric acid. The crude precipitate (6 gm.) was recrystallized from slightly acidic water. White crystals, m.p. 132–133°. Yield, 1.5 gm.

C₇H₆O₂NF. Calculated, C 45.4, H 2.2, N 7.8; found, C 45.3, H 2.3, N 8.0

5-Fluoro-2-anthranilic Acid—To 1.32 gm. of nitro acid, suspended in 30 ml. of alcohol, was added 0.2 gm. of palladium on charcoal (5 per cent). This mixture absorbed the theoretical quantity of hydrogen during 90
minutes. Filtration and evaporation yielded 750 mg. of a light brown residue, m.p. 180°.

C₇H₆O₇NF. Calculated, C 54.2, H 4.0, N 9.1; found, C 54.0, H 4.2, N 9.2

Preparation of 4-Fluoro-2-aminobenzoic Acid (4-Fluoroanthranilic Acid)—
2-Nitro-4-aminotoluene was prepared according to Noelting and Collin (12).

\[
\text{Fe}^{3+} + \text{NH}_2 \text{CH}_2 \text{NH}_2 + \text{H}_2 \text{O} \rightarrow \text{Fe}^{2+} + \text{NH}_2 \text{CH} = \text{CN} + \text{H}_2 \text{O}
\]

The crude material was recrystallized from 50 per cent alcohol. Yield, 62 per cent instead of 39 per cent.

2-Nitro-4-fluorotoluene (13).—To a mixture of 15.2 gm. of 2-nitro-4-aminotoluene and 30 ml. of concentrated hydrochloric acid, was added a solution of 7.5 gm. of sodium nitrite in 20 ml. of water at 5°. Addition of 23 ml. of 40 per cent borofluoric acid to the filtered solution gave a light pink precipitate which was filtered after 30 minutes, washed with borofluoric acid, alcohol, and ether, and dried. Yield, 17.5 gm.
The diazonium salt (17.5 gm.) was decomposed in portions of 2.5 gm. at 125°. The residue was steam-distilled and the distillate extracted with ether. Evaporation of the solvent gave 4.5 gm. of the desired product in the form of a heavy oil, b.p. 51–52° (0.7 mm.).

C₇H₄NO₄F. Calculated, C 54.2, H 3.9; found, C 54.7, H 4.1

6-Nitro-4-fluorobenzoic Acid—A mixture of 3.5 gm. of 6-nitro-4-fluoro-

**Table I**

**Reversal of 5-Methylantraniolic Acid Inhibition of E. coli by Amino Acids, Vitamins, and Purines**

Data given for the quantities of metabolites in micrograms per ml., required to restore 50 and 100 per cent inhibition to full growth. Incubation for 17 hours at 37°.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Initial inhibition</th>
<th>Type of antagonism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 per cent (5 gm per ml. inhibitor)</td>
<td>100 per cent (20 gm per ml. inhibitor)</td>
</tr>
<tr>
<td>L-Aspartic acid</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>2000 (maximum 78%*)</td>
<td>2000 (maximum 38%*)</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>0.004–0.4 (maximum 62%*)</td>
<td>0.4</td>
</tr>
<tr>
<td>Folic acid</td>
<td>40</td>
<td>40 (maximum 80%*)</td>
</tr>
<tr>
<td>Thiamine</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Adenine</td>
<td>0.4†</td>
<td>0.4</td>
</tr>
<tr>
<td>Guanine</td>
<td>4†</td>
<td>4</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>0.4†</td>
<td>0.4</td>
</tr>
</tbody>
</table>

* Maximal reversal of inhibition, as percentage of full growth.
† No reversal of inhibition.
‡ Initial inhibition 65 per cent (6 gm per ml. of 5-methylantraniolic acid).

toluene, 8.9 gm. of potassium permanganate, and 80 ml. of water was heated with stirring on a water bath until the color disappeared (4 hours). The mass was then steam-distilled, to free it from unchanged starting material (0.9 gm.), and filtered, and the filtrate was concentrated to a small volume. Acidification with concentrated hydrochloric acid gave a dark yellow precipitate which melted at 141.5° and, after recrystallization from water, at 145°. Yield, 0.8 gm.

C₇H₄O₄NF. Calculated, C 45.4, H 2.2, N 7.6; found, C 46.1, H 2.3, N 7.7

4-Fluoroanthranilic Acid—To 6-nitro-4-fluorobenzoic acid (0.65 gm.) sus-
pended in 30 ml. of alcohol was added 0.5 gm. of palladium charcoal (5 per cent). This mixture absorbed 250 ml. of hydrogen during 45 minutes. Filtration and evaporation gave 425 mg. of a light brown material, m.p. 171°. After recrystallization from water, the acid melted at 178°.

C₇H₆O₂NF. Calculated, C 54.2, H 4.0, N 9.1; found, C 54.1, H 4.1, N 9.0

**Results**

*Effect of 5-Methylanthranilic Acid—E. coli* is inhibited 50 per cent and completely by 5 and 20 γ per ml. of 5-methylanthranilic acid, respectively. As shown in Figs. 1 and 2, the inhibition is reversed by anthranilic acid
and a number of amino acids. The effect of other amino acids, purines, and vitamins is listed in Table I.

**Effect of 5- and 4-Fluoroanthranilic Acids** The inhibition caused by these compounds was greater than that caused by 5-methylanthranilic acid. For the 5- and the 4-fluoro compounds, 0.06 and 1.6 \( \gamma \) per ml. caused 50 per cent inhibition, while 0.4 and 4.0 \( \gamma \) per ml. yielded complete inhibition. The inhibition could be alleviated by the same metabolites, with the excep-

**Table II**

*Reversal of 5-Fluoroanthranilic Acid Inhibition of E. coli by Amino Acids and Vitamins*

Data given for the quantities of metabolites in micrograms per ml., required to restore 50 and 100 per cent inhibition to full growth. Incubation for 17 hours at 37°.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Initial inhibition</th>
<th>Type of antagonism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 per cent (0.06 ( \gamma ) per ml. inhibitor)</td>
<td>100 per cent (0.4 ( \gamma ) per ml. inhibitor)</td>
</tr>
<tr>
<td>DL-Phenylalanine</td>
<td>2</td>
<td>2000*</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>200</td>
<td>400*</td>
</tr>
<tr>
<td>L-Proline</td>
<td>4</td>
<td>200 (maximum 45%†)</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>4</td>
<td>4-2000 (maximum 76%†)</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>4-400 (maximum 65%†)</td>
<td>400*</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>0.004-0.04 (maximum 78%†)</td>
<td>0.4*</td>
</tr>
<tr>
<td>Folic acid</td>
<td>0.4</td>
<td>40 (maximum 15%†)</td>
</tr>
<tr>
<td>Thiamine</td>
<td>0.004</td>
<td>0.04</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>0.4-400 (maximum 85%†)</td>
<td>4-400 (maximum 50%†)</td>
</tr>
</tbody>
</table>

* No reversal of inhibition.
† Maximal reversal of inhibition, as percentage of full growth.

* The antibacterial indices determined for 50 and 100 per cent inhibition were as follows: anthranilic acid (500, 500), indole (125, 70), L-tryptophan (41, 50), DL-phenylalanine (2.5, 10), L-tyrosine (2.5, 5), 2-ketoglutaric acid (1.25, 1), L-glutamic acid (0.25, 0.1), L-proline (0.02, 0.1), L-hydroxyproline (0.25, 0.5), and DL-methionine (25, 0.5).

3 The antibacterial indices determined for 50 and 100 per cent inhibition were as follows for 5-fluoroanthranilic acid: anthranilic acid (3, 2), indole (1.5, 2), L-tryptophan (0.6, 1), L-tyrosine (0.075, 0.1), 2-ketoglutaric acid (0.03, 0.1), L-aspartic acid (0.008, 0.01), L-glutamic acid (0.03, 0.02).
in Table III. It was further found that, whereas nicotinic acid failed to reverse the inhibition caused by the 5-methylantranilic acid, it was par-

**TABLE III**
Reversal of 4-Fluoroantranilic Acid Inhibition of E. coli by Various Amino Acids and Vitamins

Data given for the quantities of metabolites in micrograms per ml., required to restore 50 and 100 per cent inhibition to full growth. Incubation for 17 hours at 37°.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Initial inhibition</th>
<th>Type of antagonism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 per cent (1.6 γ per ml. inhibitor)</td>
<td>100 per cent (4 γ per ml. inhibitor)</td>
</tr>
<tr>
<td>Anthranilic acid</td>
<td>0.04</td>
<td>0.12</td>
</tr>
<tr>
<td>Indole</td>
<td>0.008</td>
<td>0.04</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>0.004</td>
<td>0.04</td>
</tr>
<tr>
<td>DL-Phenylalanine</td>
<td>2000 (maximum 70%*)</td>
<td>2000†</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>2-Ketoglu taric acid</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>L-Aspartic acid</td>
<td>0.12</td>
<td>0.4</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>0.04</td>
<td>0.4</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>50</td>
<td>400 (maximum 90%*)</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>4-2000 (maximum 70%*)</td>
<td>2000†</td>
</tr>
<tr>
<td>L-Proline</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>L-Hydroxyproline</td>
<td>200</td>
<td>400 (maximum 85%*)</td>
</tr>
<tr>
<td>DL-Threonine</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.4</td>
<td>1.2</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>1.2-400 (maximum 80%*)</td>
<td>400†</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>0.02-0.4 (maximum 90%*)</td>
<td>0.4†</td>
</tr>
<tr>
<td>Folic acid</td>
<td>0.4</td>
<td>0.4-40 (maximum 70%*)</td>
</tr>
<tr>
<td>Thiamine</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>4</td>
<td>1.2-400 (maximum 40%*)</td>
</tr>
</tbody>
</table>

* Maximal reversal of inhibition, as percentage of full growth.
† No reversal of inhibition.

Partially effective with both 5- and 4-fluoroantranilic acids. Phenylalanine, while reversing a low level of inhibition by 5-fluoroantranilic acid, was inactive for the 4-fluoro compound. The inhibition caused by any of the
three inhibitors could not be reversed by any of the following compounds: L-arginine, DL-alanine, glutathione, glycine, L-leucine, L-lysine, DL-ornithine, DL-serine, DL-valine (quantities up to 2000 γ per ml.); 3-hydroxyanthranilic acid (80 γ per ml.); p-aminobenzoic acid (1 γ per ml.), biotin (1 γ per ml.), riboflavin (40 γ per ml.), calcium pantothenate (40 γ per ml.), pyridoxine (very slightly effective (4 γ per ml.) in the case of 5 methyl and 4-fluoroanthranilic acids); cytidine, thymine, and uracil (400 γ per ml.).

DISCUSSION

In accordance with the results obtained by Rydon (1) for E. typhi and 5-methylanthranilic acid, the experiments with E. coli have shown that the three substituted anthranilic acids, 5-methylanthranilic acid and 5- and 4-fluoroanthranilic acids, are antagonists of the parent substance, anthranilic acid. The magnitude of the inhibition was greater in the case of the fluoro compounds. In addition to anthranilic acid, indole, tryptophan, and tyrosine restored growth. In the case of 4-fluoroanthranilic acid, tryptophan was the most effective metabolite. Phenylalanine was effective only with 5-methylanthranilic acid. These results accord with the fact that the anthranilic acid → indole synthesis opens the route to the biosynthesis of tryptophan (cf. Rydon (1)), and, on the whole, with the exception of the limited effectiveness of phenylalanine, indicate the possible relationship existing among the various aromatic amino acids (5).

The fact that a number of other metabolites, which are not structurally related to anthranilic acid, are capable of relieving the inhibition caused by the substituted anthranilic acids indicates that their attack is not confined to one single point in the normal metabolism of the bacterial cell, or shows, at least, that the primary attack is followed by a number of secondary disturbances. It is not likely that the fluoro compounds owe their effect to a breakdown to fluoroacetic acid, which then causes phenomena similar to those observed in higher animals; it has been shown4 that fluoroacetic acid is a relatively inefficient inhibi tor of E. coli. It is more likely that the observations represent another example of the fact that "even with the presumably specific competitive inhibition we have examples where more than one enzyme is interfered with by a single metabolite analogue" (16).

For this reason, only a few of the metabolite-antimetabolite relationships which have been observed will be discussed.

It has been suggested (2, 3) that methionine may supply the carbon required for the completion of the indole ring from anthranilic acid. The reversal, by methionine, of the inhibition caused by the substituted anthranilic acids may, therefore, be related to this function of methionine.

4 Personal communication from Dr. J. Mager.
The effectiveness of cysteine can then be ascribed to its interrelationship to methionine, which has been established for *Neurospora crassa* (17), *E. coli* (18), and *Bacillus subtilis* (19). The same may apply to threonine, which is interrelated to methionine through the common precursor, homoserine (17, 20). Since glutamic acid was effective in alleviating the inhibition of the substituted anthranilic acids, the effectiveness of proline could be ascribed to the interrelationship existing between glutamic acid and the latter (21); that of histidine could be correlated to a similar relationship between glutamic acid and histidine (22).

Lastly, it is noteworthy that *p*-aminobenzoic acid does not counteract the inhibition caused by the substituted anthranilic acids (as found previously for the methyl compound by Rydon (1)), while folic acid does.

**SUMMARY**

1. The inhibition caused in *Escherichia coli* by 5-methyl-, 4-fluoro-, and 5-fluoroanthranilic acids was studied. The substances were found to interfere with the biosynthesis of anthranilic acid; the fluoro compounds were the more potent inhibitors.

2. In addition to anthranilic acid, the inhibition could be reversed by indole, tryptophan, tyrosine, phenylalanine, and also by a number of other amino acids, vitamins, and purines. Phenylalanine and purines were effective only in the case of 5-methylanthranilic acid.

3. The reversal of inhibition by the various compounds is discussed in view of the known interrelations among some of the compounds.

4. The synthesis of 4- and 5-fluoroanthranilic acids is reported.

**BIBLIOGRAPHY**

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