DEAZAPTERIDINES AND DEAZAPURINES, INHIBITORY ANALOGUES OF FOLIC ACID AND GUANINE*

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In a search for new inhibitory analogues, several compounds containing a pyridine ring instead of the pyrimidine ring of folic acid and certain precursors of nucleic acids have been synthesized. Two of these compounds, 3-deazafolic acid (I) and 1-deazaguanine (II), have been found in the present investigation to be antagonists of folic acid and guanine, respectively.

\[
\text{OH} \quad \text{COOH}
\]
\[
\text{NH}_2 - \text{CH} - \text{NH} - \text{C}_4 \text{H}_4 - \text{CO} - \text{NH} - \text{CH}
\]
\[
\text{COOH}
\]

The synthesis of 3-deazafolic acid, 6-amino-8-hydroxy-2,3-diphenylpyrido-(2,3)pyrazine (III), and 5-amino-7-hydroxypyrdo(2,3-d)-v-triazole (IV), together with the biological properties of these compounds and 1-deazaguanine, is presented here. The synthesis of 1-deazaguanine has been reported elsewhere (1, 2).

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EXPERIMENTAL

Methods—In determining the microbiological activity of the compounds as growth inhibitors, the assay procedure and medium for the lactic acid bacteria were the same as those previously described (3) with the following exceptions: The tabulated amounts of the vitamins plus 3 mg. of calcium pantothenate were dissolved in 30 ml. of 50 per cent ethanol instead of 100 ml. of water, the purine-pyrimidine supplement was decreased to 0.8 ml. per 100 ml. of basal medium, the total volume of the assay tubes was reduced from 10 to 5 ml., the samples were added with water to make 2.5 ml. instead of 5 ml., and for the Leuconostoc strains the concentration of phosphate was increased 4-fold. For *Leuconostoc dextranicum* the medium was further modified by the addition of 0.1 y of pantethine per 5 ml. per assay tube, and for *Lactobacillus casei* L-glutamine (100 y per assay tube) in a sterile solution was added aseptically to the autoclaved assay. The assay procedure (4) and medium (4, 5) for *Escherichia coli* were the same as those previously described. In all assays, the amount of growth was determined turbidimetrically in terms of galvanometer readings so adjusted in the particular instrument that distilled water reads 0, an opaque object 100.

5-Deazajolic Acid—Aminobenzoyl-L-glutamic acid (80 mg.) (Hoffmann-La Roche and Company, Basel, Switzerland) was added to 10 ml. of a sodium acetate buffer solution (1 gm. of sodium acetate in 100 ml. of water with sufficient glacial acetic acid to adjust the pH to 4.5), and hydrogen gas was bubbled through the solution for several minutes before adding 2,3,6-triamino-4-pyridinol hydrochloride (1) (50 mg.). 2,3-Dibromo-propionaldehyde (64 mg.) in 5 ml. of ethanol was added dropwise to the mixture through which hydrogen was bubbled during the addition and for an additional hour. Air was then bubbled through the solution for 4 hours, and the reaction mixture was diluted with water and placed in a refrigerator overnight. The resulting precipitate was collected by centrifugation and washed twice with water and dried under reduced pressure to yield 42 mg. of brownish purple material which did not melt below 300°. Based on the activity of 3-deazafolic acid subsequently isolated, this crude precipitate contained about 16 per cent of the expected product.

The purification of the deazafolic acid was followed by its ability to inhibit the growth of *L. casei* stimulated by 0.001 y of folic acid per ml. of medium during a growth period of 20 to 24 hours at 37°.

A sample of 440 mg. of such a crude precipitate containing about 12 per cent of the active principle was ground with 10 ml. of 5 per cent aqueous sodium bicarbonate. The resulting suspension was centrifuged, and the insoluble material was again treated with an additional 5 ml. of the sodium bicarbonate solution. The combined soluble fractions were concentrated
under reduced pressure to 5 ml. which were placed on a 12 cm. × 31 mm.
column of 30/60 mesh Florisil (Floridin Company, Warren, Pennsylvania)
prepared in water. The column was developed with water. A reddish
purple band was strongly adsorbed at the top of the column, while a
brownish orange band which moved through the column contained the
folic acid antagonist as determined microbiologically. Continued develop-
ment of the column with water slowly moved the reddish purple band
which was not inhibitory in the microbiological assays.

The fraction containing the brownish orange band was concentrated
under pressure to 2 ml., centrifuged to remove suspended Florisil, and
again placed on a Florisil column in the same manner as described above.
The brownish orange band was freed of all traces of the reddish purple
material in this manner, and the active fraction was again concentrated to
2 ml. and centrifuged to remove inactive material.

The active fraction was placed on a 6 cm. × 31 mm. column of Alphacel
(Nutritional Biochemicals Corporation, Cleveland, Ohio) and developed
with 80 per cent ethanol. A biologically inactive dark brown band came
directly through the column. A light yellow band possessing slight activity
followed, and moving more slowly through the column was an orange
band which contained approximately 60 per cent of the original activity.
A light brownish purple band remained adsorbed on the column.

The ethanol fraction containing the majority of the biologically active
material was centrifuged to remove suspended cellulose and then evaporated
to dryness under reduced pressure. The residue was dissolved in 2 ml.
of distilled water and again centrifuged to remove foreign material. The
solution was finally acidified to pH 3 with 1 N hydrochloric acid to obtain
an orange precipitate. After standing in the ice box overnight, the pre-
cipitate was recovered by centrifugation and washed thrice with distilled
water. The precipitate was dried under reduced pressure to yield 19 mg.
of an orange powder which in bioassay gave half maximal inhibition of
growth at a concentration of 0.14 γ per ml. and which did not melt below
300°; λ_max (in 0.1 N sodium hydroxide) at 346 μ, log ε 4.15; λ_max at 265
μ, log ε 4.45; inflection at 276 μ, log ε 4.42; λ_min at 329 μ, log ε 4.12;
λ_min at 242 μ, log ε 4.23. A sample for analysis was obtained by dissolving
5 mg. of the material in a warm solution of 50 per cent ethanol, cen-
trifuging, evaporating the solvent, and drying the residue under reduced
pressure.

C_{15}H_{20}N_{5}O_{13}.1.5H_2O. Calculated. C 51.4, H 4.96, N 17.98
Found. C 51.1, H 4.50, N 17.5

5-Amino-7-hydroxypyrido(2,3-d)-1,2,4-triazole—To a solution of 137 mg. of
2,3,6-triamino-4-pyridinol hydrochloride in 5 ml. of water held at 3° in an
Ice bath were added 51 mg. of sodium nitrite in 2 ml. of water. The resulting solution was immediately poured into 15 ml. of hot 5 N potassium hydroxide. After boiling for 2 minutes, a light orange-red color developed, and the solution was then cooled and neutralized with 5 N hydrochloric acid. A light tan precipitate formed which was filtered and washed with water. The solid was extracted with hot water, and the solution was decolorized with Darco G-60. After reduction in volume and cooling, very fine clusters of colorless needles separated which were washed and dried at 110°. The product, 11 mg., did not melt below 300°; λ_{max} (in 0.1 N sodium hydroxide) at 283 μ, log ε 3.75.

C_{6}H_{4}N_{6}O. Calculated. C 39.7, H 3.33, N 46.3
Found. " 39.6, " 3.70, " 46.1

The diazotization and cyclization steps of this procedure are attended with several difficulties, owing to the ease of nitrosation of the pyridine ring and the reactivity of the diazonium salt. The rate of addition of sodium nitrite was found to be critical. If addition was too slow, the diazonium salt would form a bluish purple precipitate before the solution could be poured into alkali. Adding the sodium nitrite too fast generated excess nitrous acid, and nitrosation would occur, a turquoise precipitate being formed. As in the case of 1-deazaguanine, the belief that closure had occurred to the 2-amino group to form the triazole, rather than to the 4-hydroxy group to form the diazo oxide, was supported by the observations that the product was colorless and possessed an acidic group.

6-Amino-8-hydroxy-2,3-diphenylpyrido[2,3]-pyrazine—To a solution of 248 mg. of benzil in 100 ml. of glacial acetic acid were added 25 ml. of absolute ethanol containing 2,3,6-triamino-4-pyridinol resulting from the reduction of 200 mg. of 2,6-diamino-3-nitro-4-pyridinol (1). During the addition of the ethanol solution and for several minutes after placing the mixture on a steam bath, a stream of hydrogen was bubbled through the mixture. The solution was then placed on a hot plate, and the volume of the solution was reduced until the boiling temperature rose to 115°. Heated to reflux for 12 hours, the solution was decolorized with Darco G-60, cooled, and poured into an equal volume of cool water. The cream-colored flocculent precipitate which formed was recovered by filtration and washed with water. The cake was extracted with 95 per cent ethanol which dissolved most of the solids. The ethanol solution was then evaporated to dryness on a steam bath, and the residue was extracted with 0.1 N sodium hydroxide. The extract was neutralized with 0.5 N hydrochloric acid to form a precipitate which was filtered, dried, and extracted with ether. The residue from ether extraction was dissolved in 2 ml. of hot 95 per cent ethanol, treated with Darco G-60, and finally with additions
of water until the solution became turbid. After being warmed and allowed to cool slowly, the solution deposited fine granular crystals, which when washed and dried amounted to 7.7 mg. of light orange powder, melting with decomposition at 310–315°; $\lambda_{\text{max}}$ (in 0.1 N sodium hydroxide) at 280 μ, log ε 4.92.

C$_{19}$H$_{14}$N$_{4}$O. Calculated, N 17.8; found, N 17.6

RESULTS AND DISCUSSION

The growth-inhibitory effects of certain of the deaza analogues are indicated in Table I. 1-Deazaguanine is a very effective inhibitor of growth of L. casei and moderately effective against certain other organisms; however, Streptococcus faecalis and E. coli are not inhibited in their growth by the compound even in moderately high concentrations. The 8-aza derivative of 1-deazaguanine, 5-amino-7-hydroxypyrido(2,3-d)-1,3,4-triazole, is ineffective in inhibiting the growth of most of the organisms except L. dextranicum, for which the derivative is about one-fourth as effective as 1-deazaguanine as a growth inhibitor. 3-Deazafolic acid is a very effective growth inhibitor of L. casei and is moderately effective in inhibiting growth of Leuconostoc mesenteroides, but is ineffective in inhibiting growth of S. faecalis. Also, the folinic acid-requiring organism, Leuconostoc citrovorum, is not affected by moderately high concentrations of 3-deaza-folic acid.

The reversal by purines of 1-deazaguanine toxicity for L. casei is indicated in Table II. Although adenine, hypoxanthine, and xanthine each have some effect in reversing the toxicity, guanine reverses the toxicity over a broad range of concentrations, while other purines tend to become less effective at higher concentrations. These results strongly suggest that 1-deazaguanine exerts its primary effect as a guanine antagonist, even though data concerning a competitive relationship show some irregularities. The toxicity of 1-deazaguanine for L. dextranicum is also prevented by guanine.

The fact that guanine is more effective than guanosine, deoxyguanosine, or a mixture of the two nucleosides in reversing 1-deazaguanine toxicity for L. casei suggests that, unless the nucleosides are not utilized by the organism, inhibition of a function of guanine other than nucleic acid synthesis may be involved. Guanosine does not exert a growth-stimulatory effect analogous to that of guanine in the basal medium, but deoxyguanosine does exert such an effect; hence, it is possible that the rate of utilization of guanosine limits its ability to replace guanine in ribonucleic acid synthesis.

Kidder and Dewey (6) noted that the testing of their preparation of 1-deazaguanine (2) was hindered by its limited solubility, which prevented
### Table I

**Growth Inhibitions by 3-Deazafolic Acid, 1-Deazaguanine, and 5-Amino-7-hydroxy pyrido(2,3-d)-v-triazole**

<table>
<thead>
<tr>
<th>Organism*</th>
<th>1-Deazaguanine†</th>
<th>5-Amino-7-hydroxy pyrido(2,3-d)-v-triazole‡</th>
<th>3-Deazafolic acid§</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. faecalis 8043</td>
<td>&gt;500</td>
<td>&gt;300</td>
<td>&gt;100</td>
</tr>
<tr>
<td>L. casei 7469</td>
<td>1</td>
<td>&gt;300</td>
<td>0.3</td>
</tr>
<tr>
<td>&quot; arabinosus 17-5</td>
<td>300</td>
<td>&gt;300</td>
<td></td>
</tr>
<tr>
<td>S. lactis 8039</td>
<td>300</td>
<td>&gt;300</td>
<td></td>
</tr>
<tr>
<td>L. dextranicum 8086</td>
<td>50</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>E. coli, Texas strain</td>
<td>&gt;1000</td>
<td>&gt;300</td>
<td></td>
</tr>
<tr>
<td>L. mesenteroides 8293</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>&quot; citrovorum 8081</td>
<td></td>
<td></td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

* Incubated at 30° for 20 hours, except L. casei and E. coli, which were incubated at 37°.
† Purines omitted from the basal medium.
‡ Added without heat treatment to autoclaved assay tubes.
§ In the presence of 0.003 γ per 5 ml. of folic acid, except for the L. citrovorum medium which contained 0.003 γ per 5 ml. of folinic acid.

### Table II

**Reversal of 1-Deazaguanine Toxicity with Purines**

Test organism, L. casei 7469, incubated for 24 hours at 37°.*

<table>
<thead>
<tr>
<th>1-Deazaguanine, γ per 5 ml.</th>
<th>Guanine, γ per 5 ml.</th>
<th>Adenine</th>
<th>Hypoxanthine</th>
<th>Xanthine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>42</td>
<td>47</td>
<td>70</td>
<td>80</td>
</tr>
<tr>
<td>1</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>22</td>
<td>56</td>
<td>80</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>15</td>
<td>59</td>
<td>70</td>
</tr>
<tr>
<td>30</td>
<td>11</td>
<td>35</td>
<td>40</td>
<td>34</td>
</tr>
<tr>
<td>100</td>
<td>11</td>
<td>25</td>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td>300</td>
<td>8</td>
<td>20</td>
<td>33</td>
<td>1</td>
</tr>
<tr>
<td>1000</td>
<td></td>
<td>9</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

* Purines omitted from the basal medium.
† A measure of culture turbidity; distilled water reads 0, an opaque object 100.

Their obtaining even half maximal inhibition of *Tetrahymena pyriformis* W. The slight inhibition obtained was reversed by guanine.

1-Deazaguanine showed some antitumor activity against mouse mam-
mary carcinoma C3H strain grown in eggs. At a concentration of 0.8 mg. per egg, the survival of the eggs was 98 per cent of the control, tumor size was 68 per cent of the control, and the embryo size was 90 per cent of the control. These data suggest that the inhibitory effect is not specific for the tumor. 3-Deaza folic acid (100 γ per egg) was inactive against the tumor although inhibiting the embryo growth to 80 per cent of the control.

The toxicity of 3-deaza folic acid for _L. casei_ is reversed in a competitive manner by folic acid as indicated in Table III. Also, a similar relationship exists in the case of _L. mesenteroides._

**TABLE III**

_Reversal of 3-Deaza folic Acid Toxicity with Folic Acid_

<table>
<thead>
<tr>
<th>Test organism, <em>L. casei</em> 7469, incubated for 20 hours at 37°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic acid, γ per 5 ml.</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Galvanometer readings*</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>0.1</td>
</tr>
<tr>
<td>0.3</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>30</td>
</tr>
</tbody>
</table>

* See the corresponding footnote in Table II.
† Control without folic acid supplement read 3.

Substitution of a pyridine ring for the pyrimidine ring results in deaza derivatives of guanine and folic acid which are very good metabolite antagonists for some organisms but relatively ineffective for others. Such specificity for particular organisms is not uncommon among specific antagonists of metabolites.

**SUMMARY**

3-Deaza folic acid, synthesized by the condensation of 2,3,6-triamino-4-pyridinol with 2,3-dibromopropionaldehyde and _p_-aminobenzoyl-L-glutamic acid, inhibits in a competitive manner the utilization of folic acid by _Lactobacillus casei_ 7469 and _Leuconostoc mesenteroides_ 8293 but not by _Streptococcus faecalis_ 8043 at the concentrations tested. 6-Amino-

1 The authors are indebted to Dr. Alfred Taylor for the data on the effect of the deaza derivatives on egg-grown tumors.
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8-hydroxy-2, 3-diphenylpyrido(2,3)pyrazine synthesized from the triaminopyridinol and benzil was inactive under similar conditions. Of several organisms studied, the growths of L. casei and Leuconostoc dextranicum 8086 are most effectively inhibited by 1-deazaguanine, and the inhibition is reversed by guanine in a competitive manner. Other purines have some effect on the toxicity. 5-Amino-7-hydroxypyrirdo(2,3-d)-v-triazole synthesized by the action of nitrous acid on triaminopyridinol is somewhat less effective than 1-deazaguanine in inhibiting L. dextranicum, but it is ineffective against several other organisms.

BIBLIOGRAPHY

DEAZAPTERIDINES AND DEAZAPURINES, INHIBITORY ANALOGUES OF FOLIC ACID AND GUANINE
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