STUDIES OF PYRIDOXINE DISPLACEMENT

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Relatively little work has been done with pyridoxine displacers. Ott (1) found 2,4-dimethyl-3-hydroxy-5-hydroxymethylpyridoxine (desoxypyridoxine) to be a very potent inhibitor of pyridoxine in the metabolism of the chick. 2 molecules of inhibitor were sufficient to offset the vitamin activity of 1 molecule of pyridoxine. In the course of an investigation of antimalarials, McCasland et al. (2) synthesized 2-methyl-4-hydroxy-6-hydroxymethylpyrimidine and a number of closely related compounds which are analogues of pyridoxine. No statement was made of their activity. The second paper by this group (3), reports the synthesis of 2,6-di-(hydroxymethyl)-4-hydroxy-5-methylpyrimidine hydrochloride which is the pyrimidine analogue of pyridoxine. Tests for antipyridoxine activity of this compound showed it to be inactive. Ott (1) found 2-methyl-3-hydroxy-4-methoxymethyl-5-hydroxymethylpyridine (methoxypyridoxine) to be nearly as effective a pyridoxine displacer in the chick as was desoxypyridoxine. Mushett et al. (4) have reported on the pathologic effects produced by these two analogues of pyridoxine. Atrophy of the lymphoid tissues seemed to characterize the histopathological picture. The effectiveness of desoxypyridoxine in the rat has been reported (5). The Merck group (6) has studied the effect of pyridoxine-displacing agents on the metabolism of tryptophan. From this study, it was concluded that desoxypyridoxine interfered with some phase of tryptophan metabolism. Recently, Beiler and Martin (7) found desoxypyridoxine to be ineffective as an inhibitor of the action of tyrosine decarboxylase. Phosphorylated desoxypyridoxine, on the other hand, displaces pyridoxal phosphate in the tyrosine decarboxylase system.

EXPERIMENTAL

Several analogues of pyridoxine were prepared and tested with desoxypyridoxine as the standard. These chemicals included 2-acetoxyl-3,5-diacetoxymethyltoluene, 2-methyl-3-hydroxy-4-dimethylaminomethylpyridine, 2-methyl-3-hydroxy-4-hydroxymethylpyridine, and 2-ethyl-3-amino-4-ethoxymethyl-5-aminomethylpyridine. The compounds were prepared in accordance with the following procedures.

1 Ott, W. H., personal communication; cited in (4).
2-Acetoxy-3,5-diacetoxymethyltoluene was prepared according to the method of Bruson and MacMullen (8). 110 gm. of 40 per cent formaldehyde were added with stirring to a mixture of 54 gm. of o-cresol and 150 gm. of 33 per cent dimethylamine over a period of 1 hour. The temperature was kept at 25-30° during the addition and then raised to 90-95°. After 1.5 hours the product was separated, dried, and distilled under reduced pressure. The yield of the pure product, b.p. 115-117° at 0.3 mm. pressure, was 31 gm. of 2-hydroxy-3,5-bis(dimethylaminomethyl)-toluene.

A mixture of 30 gm. of 2-hydroxy-3,5-bis(dimethylaminomethyl)toluene and 50 gm. of acetic anhydride was heated at 90-95° for 2 hours and then refluxed for 1.5 hours. Distillation yielded 30 gm. of product boiling at 155-160° at 0.3 mm. This was extracted with 1 N hydrochloric acid and redistilled to give 27 gm. of 2-acetoxy-3,5-diacetoxymethyltoluene boiling at 158-159° at 0.3 mm.; nD 1.4997.

Analysis—C₁₅H₁₈O₅. Calculated, CH₂CO— 43.8; found, CH₂CO— 43.0

2-Methyl-3-hydroxy-4-hydroxymethylpyridine Hydrochloride—A solution of 23 gm. of 4-diethylaminomethyl-3-hydroxy-2-methylpyridine (9) in 31 gm. of acetic anhydride was heated at 90-95° for 3 hours and then refluxed for 1 hour. The resulting mixture was dissolved in water, neutralized, and extracted with ether. Distillation gave 23 gm. of product boiling at 135-136° at 0.3 mm. The hydrochloride melted at 161-162°.

5 gm. of 4-acetoxymethyl-3-acetoxy-2-methylpyridine were dissolved in 200 cc. of 2 N hydrochloric acid and the solution refluxed for 12 hours. The solvent was evaporated under reduced pressure and the residue recrystallized from ethyl alcohol; m.p. 165-166°.

Analysis—C₁₀H₁₀O₂N-HCl. Calculated, N 7.98; found, N 8.16

2-Methyl-3-hydroxy-4-dimethylaminomethylpyridine—A mixture of 16.4 gm. of 2-methyl-3-hydroxypyridine (10) and 18 gm. of 33 per cent dimethylamine was dissolved in 40 cc. of water and 13.5 gm. of 40 per cent formaldehyde were added over a period of 1 hour. The mixture was allowed to stand overnight; the clear solution was heated to 90-95°, saturated with sodium chloride, and the oily layer separated. Distillation gave 11 gm. of product boiling at 75-76° at 0.5 mm. The hydrochloride melted at 223-224°.

Analysis—C₁₀H₁₀N₂. Calculated, N 16.86; found, N 16.93

Preparation of 2-ethyl-3-amino-4-ethoxymethyl-5-aminomethylpyridine dihydrochloride occurred by a series of reactions analogous to those used by Harris and Folkers (11) in the preparation of pyridoxine.
To the stirred suspension of sodium amide was added in 5 minutes a solution of 0.3 mole of ethyl methyl ketone in 100 cc. of ether. After 5 minutes 0.4 mole of ethyl ethoxyacetate in 100 cc. of ether was added and the stirring was continued for 2 hours at reflux temperature. The mixture was poured into 300 cc. of water, neutralized with dilute hydrochloric acid, and extracted with ether. The solvent was distilled from the ether solution, the residue added to a hot filtered solution of 40 gm. of copper acetate in 350 cc. of water, and the mixture allowed to stand for 4 hours. The copper salt of the diketone was filtered, washed with ligroin, and recrystallized from methyl alcohol. The yield of pure product, m.p. 137-138°, was 20 gm. or 33 per cent.

The copper salt was stirred with 300 cc. of 10 per cent sulfuric acid and 200 cc. of ether. The acid layer was again extracted with ether and the combined ether solution dried over sodium sulfate. The solvent was distilled and the residue fractionated in vacuo through a 30 cm. Vigier column, b.p. 93-95° at 13 mm. The product was 1-ethoxy-2,4-hexadione.

To 63 gm. of cyanoacetamide, dissolved in 450 cc. of hot ethyl alcohol, 102 gm. of 1-ethoxy-2,4-hexadione and 10 cc. of piperidine were added with shaking. The mixture was allowed to stand overnight, cooled, and filtered. The yield of 2-ethyl-4-ethoxymethyl-5-cyano-6-pyridone, after filtration and crystallization from alcohol, was 115 gm., m.p. 174-175°.

To 10 gm. of 2-ethyl-4-ethoxymethyl-5-cyano-6-pyridone in 30 cc. of acetic anhydride containing a little urea were added, with cooling and stirring, 4.5 cc. of fuming nitric acid. The reaction temperature was kept between 5-10° during the addition. The reaction mixture was kept at this temperature for 10 minutes and then at 25-30° for half an hour. The mixture was poured into ice and the crystalline product filtered after 3 hours. The yield of 2-ethyl-3-nitro-4-ethoxymethyl-5-cyano-6-pyridone, m.p. 125-127°, was 4 gm. Recrystallization raised the melting point to 127-128°.

A mixture of 30 gm. of 2-ethyl-3-nitro-4-ethoxymethyl-5-cyano-6-pyridone, 35 gm. of phosphorus pentachloride, and 250 cc. of chlorobenzene was heated at reflux temperature for half an hour and then at such a rate as to distil 150 cc. of the chlorobenzene in 3 to 4 hours. The remaining solvent was removed at 10 to 15 mm. The viscous residue was heated with 80 cc. of 10 per cent ethyl alcohol; the resulting mixture was extracted with ether and dried over sodium sulfate. The ether was distilled and the residue extracted twice with 250 cc. of boiling petroleum ether. This extract was concentrated on a steam bath and then cooled slowly to 10°. The precipitate 2-ethyl-3-nitro-4-ethoxymethyl-5-cyano-6-chloropyridine, after filtration and recrystallization, melted at 52-53°. The yield was 9.5 gm.
A solution of 27 gm. of 2-ethyl-3-nitro-4-ethoxymethyl-5-cyano-6-chloropyridine (m.p. 50–52°) in 175 cc. of alcohol was shaken in the presence of 0.5 gm. of Adams' catalyst with hydrogen at a pressure of 50 pounds. The hydrogenation was stopped after 3 moles of hydrogen had been absorbed and the mixture was allowed to cool. The alcohol was decanted and the product extracted with hot alcohol. Thus 14 gm. of pure 2-ethyl-3-amino-4-ethoxymethyl-5-cyano-6-chloropyridine, m.p. 121–122°, was obtained.

A solution of 3.5 gm. of 2-ethyl-3-amino-4-ethoxymethyl-5-cyano-6-chloropyridine in 140 cc. of glacial acetic acid with 1.2 gm. of sodium acetate, 0.3 gm. of Adams' catalyst, and 5 gm. of 10 per cent palladium charcoal catalyst was shaken with hydrogen at a pressure of 50 pounds until 3 moles had been absorbed (5 hours). After filtering off the catalyst the solvent was evaporated under reduced pressure, and the residue extracted with alcohol and separated from sodium chloride. The alcoholic solution was saturated with dry hydrogen chloride and the dihydrochloride precipitated by addition of acetone. The yield of the pure dihydrochloride of 2-ethyl-3-amino-4-ethoxymethyl-5-aminomethylpyridine, m.p. 214–215°, was 1.2 gm.

Analysis—C_{17}H_{19}ON_{2}·(2HCl). Calculated, N 14.89; found, N 14.93

The structure of 2-ethyl-3-amino-4-ethoxymethyl-5-aminomethylpyridine was established through the following reactions.

\[
\text{CH}_2\text{CH}_2\text{Br} \quad \text{CH}_2\text{Br}
\]

A solution of 1 gm. of the dihydrochloride of 2-ethyl-3-amino-4-ethoxymethyl-5-aminomethylpyridine in 20 cc. of water was added simultaneously with a solution of 2 gm. of sodium nitrite to 40 cc. of hot 2.5 N hydrochloric acid. The solution was concentrated to dryness under reduced pressure and the residue washed with acetone. The dihydroxy hydrochloride was extracted from sodium chloride with absolute alcohol and the extract evaporated to dryness.

The crude residue was refluxed with 25 cc. of 48 per cent hydrobromic acid for half an hour, cooled in ice water, and filtered. Two crystallizations from ethyl alcohol gave the pure compound with the same melting point as that given by Harris (12) for 2-ethyl-3-hydroxy-4,5-dibromomethylpyridine hydrobromide.

The method used in testing these agents was to employ the medium of
Atkin et al. (13) and the technique of Williams et al. (14). The test micro-organism was *Saccharomyces cerevisiae*, G. M.

2-Acetoxyl-3,5-diacetoxymethyltoluene and 2-methyl-3-hydroxy-4-di-

**Table I**

2-Ethyl-3-amino-4-ethoxymethyl-5-aminomethylpyridine As Pyridoxine Displacing Agent

The results are expressed in readings on the Klett photoelectric colorimeter.

<table>
<thead>
<tr>
<th>Pyridoxine concentration</th>
<th>Concentrations, γ per 10 ml.</th>
<th>0</th>
<th>1</th>
<th>10</th>
<th>100</th>
<th>1000</th>
<th>10,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Ethyl-3-amino-4-ethoxymethyl-5-aminomethylpyridine</td>
<td>0</td>
<td>102</td>
<td>85</td>
<td>70</td>
<td>63</td>
<td>60</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>146</td>
<td>103</td>
<td>90</td>
<td>85</td>
<td>68</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>100.04</td>
<td>151</td>
<td>108</td>
<td>102</td>
<td>89</td>
<td>90</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>1,000.04</td>
<td>155</td>
<td>128</td>
<td>125</td>
<td>108</td>
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<td>95</td>
</tr>
<tr>
<td></td>
<td>10,000.04</td>
<td>158</td>
<td>143</td>
<td>123</td>
<td>130</td>
<td>102</td>
<td></td>
</tr>
</tbody>
</table>

Desoxypyridoxine concentrations

| | | 0 | 123 | 110 | 80 | 66 | 43 |
| | | 0.04 | 160 | 123 | 111 | 80 | 74 | 65 |
| | | 100.04 | 180 | 138 | 123 | 118 | 100 | 92 |
| | | 1,000.04 | 185 | 154 | 150 | 148 | 145 | 125 |
| | | 10,000.04 | 195 | 185 | 185 | 180 | 175 | 135 |

**Table II**

Displacement of Pyridoxal by Desoxypyridoxine

Klett photoelectric colorimeter readings.

<table>
<thead>
<tr>
<th>Pyridoxal concentration</th>
<th>Desoxypyridoxine concentrations, γ per 10 ml.</th>
<th>0</th>
<th>1</th>
<th>10</th>
<th>100</th>
<th>1000</th>
<th>10,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ per 10 ml.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>90</td>
<td>120</td>
<td>108</td>
<td>72</td>
<td>53</td>
<td>40</td>
</tr>
<tr>
<td>0.0005</td>
<td></td>
<td>162</td>
<td>140</td>
<td>135</td>
<td>70</td>
<td>58</td>
<td>43</td>
</tr>
<tr>
<td>100.0005</td>
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<td>200</td>
<td>160</td>
<td>132</td>
<td>80</td>
<td>72</td>
</tr>
<tr>
<td>1000.0005</td>
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<td>257</td>
<td>246</td>
<td>245</td>
<td>225</td>
<td>120</td>
<td>101</td>
</tr>
</tbody>
</table>

The ratio is approximately 1:1 in higher concentrations of metabolite and displacer.

methylaminomethylpyridine were inactive. 2-Methyl-3-hydroxy-4-hydroxymethylpyridine was active with an inhibitor-metabolite ratio of 250. 2-Ethyl-3-amino-4-ethoxymethyl-5-aminomethylpyridine was found to be
a more powerful inhibitor of pyridoxine than was desoxypyridoxine. Table I presents an experiment showing comparative results.

The effectiveness of desoxypyridoxine was tested against pyridoxal in order to check the possibility that displacers for one form of vitamin B₆ might not be displacers of the other forms. The results presented in Table II clearly show the effectiveness of desoxypyridoxine in displacing pyridoxal.

To date, only seven compounds have been tested as pyridoxine-displacing agents. It is therefore impossible to generalize concerning the structure of active agents.

SUMMARY

Four compounds structurally related to pyridoxine were synthesized and tested as possible displacing agents. Of these, 2-methyl-3-hydroxy-4-hydroxymethylpyridine and 2-ethyl-3-amino-4-ethoxymethyl-5-amino-methylpyridine were found active.

BIBLIOGRAPHY

10. Wulff, O., U. S. patents 1,880,645 and 1,880,646 (1932).
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