SYNTHESIS OF 6-SUCCINOAMINOPURINE*

BY CHARLES E. CARTER

(From the Department of Pharmacology, Yale University, New Haven, Connecticut)

(Received for publication, April 5, 1956)

The structure proposed for adenylosuccinic acid (6-succinoaminopurine-9-riboyl-5'-phosphate), the product of the enzymatic reaction of adenosine-5'-phosphate with fumaric acid (1, 2), is supported by a synthesis of the aglycone (III) from 6-chloropurine (II) and aspartic acid (I) reported in this paper. This reaction, which appears applicable to the condensation of several amino acids with 6-chloropurine, is conducted in aqueous solution at pH 9.5. The compounds formed exhibit an ultraviolet absorption maximum at 270 to 275 μ in acid and those bearing two carboxyl substituents (derived from aspartic acid or glutamic acid) or a sulfonic and a carboxyl group (cysteic acid) are characterized by lability of the purine ring to acid hydrolysis with resultant formation of a diazotizable amine. The amine exhibits an ultraviolet absorption maximum at 267 μ in acid, and is chromatographically identical with 4-amino-5-imidazolecarboxamide (V) and different from 4-amino-5-imidazolecarboxylic acid and 4-aminoimidazole, according to the chromatographic and spectral data for these compounds reported by Rabinowitz (3). In the case of the acid hydrolysis of 6-succinoaminopurine, which has been most extensively studied, aspartic acid is formed in addition to the diazotizable amine. It is suggested that the acid hydrolysis of 6-succinoaminopurine involves the formation of an

* Supported by grants from the United States Public Health Service and the Atomic Energy Commission.
intermediate anhydride (IV) by the internal condensation of a substituent carboxyl with the nitrogen of the imidazole ring corresponding to N-7 of the purine. The breakdown of such an intermediate would account for the acid lability of 6-succinoaminopurine. However, chromatographic or spectrophotometric evidence has not been obtained in support of the existence of such an intermediate.

The identity of the natural aglycone, produced by mild acid hydrolysis of adenylosuccinic acid and 6-succinoaminopurine of synthetic origin, is based upon corresponding chromatographic behavior of the compounds in several solvent systems and with the anion exchange resin, on similar rates of degradation in acid with resultant formation of the same diazotizable amine, and on identical characteristics of spectrophotometric titration.

EXPERIMENTAL

Synthesis of 6-Succinoaminopurine—Preliminary investigation of the reaction of 6-chloropurine and aspartic acid in aqueous solution indicated that conditions were optimal at pH 9.5, and that both yield and ease of isolation of product were superior to those employing non-aqueous solvents with basic catalysis.

2 gm. of 6-chloropurine (Dougherty Chemicals) and 1.2 gm. of L-aspartic acid were added to 35 ml. of water, and the mixture was brought to pH 9.5 with concentrated KOH. The solution was refluxed for 3 hours, cooled, and added to 1 liter of absolute ethanol. After standing overnight at 4°, the precipitate was separated by filtration and washed with 100 ml. of cold absolute ethanol. After the precipitate was dried in vacuo, 2.15 gm. of crude product were obtained; this exhibited an absorption maximum at 276 mμ in acid. From a predicted extinction coefficient at this wavelength for 6-succinoaminopurine of 17.2 × 10^4, based on a quantitative conversion of the parent nucleotide to the aglycone by acid hydrolysis (1)
and the presence of aspartic acid as determined by the ninhydrin reaction, the crude product was estimated to contain about 60 per cent 6-succino-
aminopurine. Neither unchanged 6-chloropurine nor hypoxanthine could be detected in the crude product by paper chromatography.

Purification of 6-succinoaminopurine was achieved by dissolving 400 mg. of the crude product in 20 ml. of water (pH 8.0) and allowing this solution to percolate through a column 12 X 200 mm. composed of 2 parts charcoal and 1 part Celite. The effluent contained no ultraviolet-absorbing material, and over 90 per cent of the aspartic acid of the crude product was recovered in this fraction. The column was then washed with water and successive 10 ml. fractions of effluent were collected. The first two fractions, which contained 20 mg. of 6-succinoaminopurine and a small amount of aspartic acid, were discarded. The subsequent 100 ml. of effluent contained no amino acid and all of the remaining ultraviolet-absorbing material. This fraction was concentrated to 4 ml. and added to 300 ml. of absolute ethanol. After standing overnight at 4°, the crystalline precipitate was removed by filtration, washed with absolute ethanol, and dried in vacuo. Purified 6-succinoaminopurine was obtained in a yield of 175 mg. as the dipotassium·2H₂O salt with an extinction coefficient of 17.6 X 10³ in acid at 276 mμ (Table I). Analysis¹ of the compound dried in vacuo at 25° indicates 2 molecules of water of crystallization. Calculated for C₉H₇O₅N₆K₂·2H₂O, C 29.8, H 3.6, O 26.6, N 19.4; found, C 28.5, H 3.07, O 26.7, N 18.8. The compound progressively darkens above 255° and decomposes with evolution of gas at 298°.

The reaction of 6-chloropurine with amino acids has been applied to glutamic acid, cysteic acid, glycine, lysine, and histidine. Conditions similar to the reaction described for the synthesis of 6-succinoaminopurine were employed with 200 mg. of 6-chloropurine and a relative excess of amino acid of 0.5 M dissolved in 3.5 ml. of water at pH 9.5. After refluxing for 3 hours, the mixture was poured into excess absolute ethanol and the precipitate collected by filtration. For the amino acids listed above, the ethanol precipitate contained crude substituted aminopurine, estimated by spectro-
photometry to be equivalent to about 25 to 35 per cent of the theoretical yield. The principal contaminant was in each case the unchanged amino acid. Paper chromatography in several solvent systems showed a single ultraviolet-absorbing component (Table I). Because of the small amounts of these compounds and their high solubility in aqueous and non-aqueous solvents, further purification could not be achieved by crystallization, and the data presented for these compounds are intended to demonstrate the potential scope of the reaction rather than the preparation of definitive compounds. Each of the compounds shows an absorption maximum in the

¹ Huffman Microanalytical Laboratories.
SYNTHESIS OF 6-SUCCINOAMINOPURINE

275 m\(\mu\) region, and, by comparison with the natural and synthetic aglycone, is assumed to have an extinction coefficient at the maximum of approximately 16 to 17 \(\times 10^3\). On this basis, the crude products for which data are presented in Table I are approximately 50 to 60 per cent pure.

Comparison of 6-Succinoaminopurine with Aglycone Derived from Adenylosuccinyl-

### Table I

**Paper Chromatography**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Absorbance (\times 10^3) in 0.1 N HCl</th>
<th>(R_F) paper chromatography</th>
<th>(R_F) amine product of acid degradation</th>
<th>Ultraviolet absorption, m(\mu)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\mu_l, 276)</td>
<td>Solvent 1</td>
<td>Solvent 2</td>
<td>Solvent 1</td>
</tr>
<tr>
<td>6-Succinoaminopurine.............</td>
<td>17.6</td>
<td>0.26</td>
<td>0.53</td>
<td>0.37</td>
</tr>
<tr>
<td>Aglycone from adenylosuccinate...</td>
<td>17.2</td>
<td>0.26</td>
<td>0.53</td>
<td>0.37</td>
</tr>
<tr>
<td>6-Glutamyl purine.................</td>
<td>10.9</td>
<td>0.27</td>
<td>0.67</td>
<td>0.46</td>
</tr>
<tr>
<td>6-Glycyl purine..................</td>
<td>12.5</td>
<td>0.40</td>
<td>0.61</td>
<td>0.46</td>
</tr>
<tr>
<td>6-Cysteic acid purine............</td>
<td>10.9</td>
<td>0.26</td>
<td>0.33</td>
<td>0.31</td>
</tr>
<tr>
<td>6-Histidyl purine.................</td>
<td>8.9</td>
<td>0.42</td>
<td>0.57</td>
<td>0.46</td>
</tr>
<tr>
<td>6-Lysyl purine...................</td>
<td>9.5</td>
<td>0.45</td>
<td>0.56</td>
<td>0.53</td>
</tr>
<tr>
<td>6-Chloropurine...................</td>
<td>8.4</td>
<td>0.69</td>
<td>0.76</td>
<td>0.85</td>
</tr>
<tr>
<td>Hypoxanthine.....................</td>
<td>0.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Amino-5-imidazolecarboxamide...</td>
<td>0.54</td>
<td>0.38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Solvent 1. Concentration, NH\(_4\)OH, 10; H\(_2\)O, 20; isopropyl alcohol, 70. Double zoning in this solvent may be encountered, depending on the amount of inorganic cation present. Solvent 2, butanol, 50; H\(_2\)O, 25; acetic acid, 25. The 4-amino-5-imidazolecarboxamide standard was put on the paper in 1 N HCl solution. Solvent 3, 1 m NH\(_4\)Ac, 3.5; ethanol, 7.0.

osuccinic Acid by Acid Hydrolysis—The aglycone of adenylosuccinic acid has not been recovered as a solid, following acid hydrolysis of the parent nucleotide (1, 2), because of the limited amounts of the nucleotide available and the high solubility of the aglycone and its salts in a variety of solvents. The identification of the aglycone with 6-succinoaminopurine is based on chromatographic and spectrophotometric criteria and the characteristic lability of the compound in acid to yield a diazotizable amine. Table I
summarizes the ultraviolet absorption characteristics and the chromatographic behavior of the natural and synthetic aglycone and the diazotizable amine derived by acid hydrolysis.

These compounds may also be isolated and determined by anion exchange chromatography. On a Dowex 1, 2 per cent cross-linked, 200 to 400 mesh resin column in the acetate form, 10 cm. long and with a 1 cm. diameter, the products of a 10 hour hydrolysis of adenylosuccinic acid (10 μmoles) and an equivalent amount of the synthetic aglycone in 0.5 N HCl at 100° were compared. Under these conditions some of the aglycone and the derived diazotizable amine remained at the end of the hydrolysis period. In both cases the diazotizable amine with an ultraviolet absorption maximum at 267 mμ was eluted from the column with 0.05 M NH4Ac adjusted to pH 4.0. Treatment of the column with 0.25 M NH4Ac, pH 4.0, removed 6-succinoaminopurine and the aglycone derived from adenylosuccinic acid in a single symmetrical peak between 60 and 120 ml. of effluent.

Spectrophotometric characteristics dependent upon the dissociating groups of the natural and synthetic aglycone of adenylosuccinic acid are shown in Fig. 1. The data demonstrate the correspondence of the natural and synthetic aglycone and suggest the presence of two groups with pK values of 4.0 and 5.3. These dissociations are assigned to the substituted amino and the secondary carboxyl group, though not necessarily respectively. In contrast to the nucleotide, in which a dissociation at pH 2.1 attributed to a carboxyl group clearly influences spectrophotometric ratios (2), no corresponding spectrophotometric evidence for this dissociation is seen in the data for the aglycone presented in Fig. 2. However, electrometric titration with HCl of the purified synthetic aglycone, 6-succinoaminopurine, resulted in the consumption of 2.92 equivalents of acid per mole of 6-succinoaminopurine between pH 8.0 and 2.0 and indicates the presence of two free carboxyl groups and the substituted amino group.

The lability of the purine ring of 6-succinoaminopurine and the corresponding glutamic acid and cysteic acid derivatives to acid hydrolysis characterizes this group of compounds and affords another basis of correspondence between the natural and synthetic aglycones. In Fig. 2 the production of diazotizable amine by acid hydrolysis from a series of related compounds is shown. In the case of the aglycone of adenylosuccinic acid, synthetic 6-succinoaminopurine, and the analogous glutamic acid and cysteic acid derivatives, acid hydrolysis is associated with a decreasing absorption at 265 and 275 mμ, a decrease in the ratio of absorption of 265 mμ to 275 mμ, and the appearance and destruction of diazotizable amine. These data for 6-succinoaminopurine are recorded in Table II. Paper and ion exchange chromatography of hydrolysis mixtures of these compounds show the progressive disappearance of the substituted purine with the
production of a common ultraviolet-absorbing component which exhibits a maximum at 265 μM in acid and reacts with the Bratton-Marshall reagent. The diazotizable amine accounts for about 40 per cent of the original purine, and is chromatographically identical with 4-amino-5-imidazolecarboxamide (Table I). The amine product of acid degradation of the substituted purines has not been further characterized, but from the foregoing evidence and the spectral data for 4-amino-5-imidazolecarboxylic acid and 4-aminoimidazole, as well as the extreme lability of these compounds reported by Rabinowitz (3), the amine is believed to be 4-amino-5-imidazolecarboxamide.

While amino acid-substituted purines such as kinetin (6-furfurylamino-purine) (4) and 6-methylaminopurine (5) are resistant in the purine ring...
to mild acid degradation, Fig. 2 shows that the glycine-substituted purine is to some extent degraded in 1 N acid to diazotizable amine. In the case of 6-chloropurine, small amounts of diazotizable amine are formed, but

**Table II**

*Acid Hydrolysis of 6-Succinoaminopurine*

10 μmoles of the purified dipotassium salt of 6-succinoaminopurine were dissolved in 2 ml. of 1 N HCl and heated in a stoppered tube in a boiling water bath. Samples of 0.05 ml. were withdrawn at intervals for spectrophotometry, after dilution to 4 ml. with H₂O, and for diazotizable amine determination by the Bratton-Marshall method.

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Ratio of absorbance</th>
<th>Diazotizable amine (μmoles)</th>
<th>Time (min.)</th>
<th>Ratio of absorbance</th>
<th>Diazotizable amine (μmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.25</td>
<td>0</td>
<td>180</td>
<td>1.09</td>
<td>4.40</td>
</tr>
<tr>
<td>30</td>
<td>1.24</td>
<td>0.75</td>
<td>210</td>
<td>1.00</td>
<td>3.6</td>
</tr>
<tr>
<td>60</td>
<td>1.75</td>
<td>2.30</td>
<td>240</td>
<td>0.95</td>
<td>3.5</td>
</tr>
<tr>
<td>90</td>
<td>1.17</td>
<td>3.20</td>
<td>270</td>
<td>0.94</td>
<td>3.2</td>
</tr>
<tr>
<td>120</td>
<td>1.13</td>
<td>3.60</td>
<td>330</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table III**

*Chromatography of Acid Degradation Products of Carboxyl-Labeled Adenylosuccinic Acid*

Carboxyl-labeled adenylosuccinic acid was added to the unlabeled compound to give a final concentration of 14 mg. of adenylosuccinic acid in 4 ml. of 1.0 N HCl containing a total radioactivity of 264,000 c.p.m. as determined for an infinitely thin layer in a windowless counter. The solution was refluxed for 5 hours, diluted to 100 ml., and adjusted to pH 3.0. This solution was then percolated through a column of 1 cm. diameter and 10 cm. long of Dowex 50-H⁺, 200 to 400 mesh.

<table>
<thead>
<tr>
<th>Eluting agent</th>
<th>Compound</th>
<th>Radioactivity</th>
<th>Ninhydrin test</th>
<th>Atyramine test</th>
<th>Ultraviolet maximum, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 N HCl</td>
<td>Aspartic acid</td>
<td>74</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.0 &quot; &quot; &quot;</td>
<td>Succinoaminopurine</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>276</td>
</tr>
<tr>
<td>2.0 &quot; &quot; &quot;</td>
<td>4-Amino-5-imidazolecarboxamide</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>267</td>
</tr>
</tbody>
</table>

Lability is to a great extent masked by the rapid transformation of this compound in acid solution to hypoxanthine, which is resistant to acid degradation.

The products of acid hydrolysis were further characterized by employing a sample of adenylosuccinic acid labeled with C¹⁴ in the two carboxyl groups
SYNTHESIS OF 6-SUCCINOAMINOPURINE

(1, 2). The hydrolysate was resolved on a cation exchange resin in the H+ form. The 0.5 N HCl eluate, which was shown by paper chromatography to contain only aspartic acid (Table III), accounted for most of the original radioactivity, and this was identified with aspartic acid. A small amount of activity was associated with the unhydrolyzed 6-succinoaminopurine, but the only other ultraviolet-absorbing component, the aminoimidazole product of hydrolysis, was devoid of radioactivity. In acid hydrolysates of 6-succinoaminopurine, which result from prolonged heating in 3 N HCl, small amounts of β-alanine arising from decarboxylation of aspartic acid, and glycine arising from degradation of the imidazole nucleus, have been detected by paper chromatography.

Alkaline hydrolysis destroys 6-succinoaminopurine without formation of diazotizable amine, but the products of this reaction have not been identified.

SUMMARY

A compound chromatographically and spectrophotometrically identified as the aglycone of adenylosuccinic acid was synthesized from 6-chloropurine and aspartic acid and assigned the structure, 6 succinoaminopurine. The reaction appears applicable to several amino acids.

Synthetic 6-succinoaminopurine and the natural compound derived from adenylosuccinic acid exhibited lability of the purine ring to 1 N acid hydrolysis at 100° and yielded 4-amino-5-imidazolecarboxamide and aspartic acid under these conditions.

BIBLIOGRAPHY

SYNTHESIS OF
6-SUCCINOAMINOPURINE
Charles E. Carter

J. Biol. Chem. 1956, 223:139-146.

Access the most updated version of this article at http://www.jbc.org/content/223/1/139.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/223/1/139.citation.full.html#ref-list-1