DERIVATIVES OF $\alpha,\alpha$-DI(GLYCYLAMINO)PROPIONIC ACID

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$\alpha,\alpha$-Di(glycylamino)propionic acid hydrochloride, \((\text{NH}_2\text{CH}_2\text{CONH})_2\text{C}-(\text{CH}_2)\text{COOH}\cdot\text{HCl}\), is enzymatically hydrolyzed in aqueous extracts of rat kidney to yield, as a maximum, 1 mole of ammonia and 1 mole of pyruvic acid per mole of substrate (1). $\alpha$-(dl-Chloropropionylamino)-$\alpha$-(dl-alanyl-amino)propionic acid and $\alpha,\alpha$-di(dl-alanylalanyl)propionic acid hydrochloride are also hydrolyzed by rat kidney, the former substrate yielding a molar ratio of ammonia to pyruvic acid close to unity, the latter a ratio close to 1.6 (2). On the other hand, $\alpha,\alpha$-di(acetamino)propionic acid, $\alpha,\alpha$-di(chloroacetamino)propionic acid, and $\alpha,\alpha$-di(dl-chloropropionylamino)propionic acid are completely resistant (1, 2).

Investigations on this novel class of substances have been carried further by observing whether there is any relation between the integrity of the $\alpha$-amino groups of di(glycylamino)propionic acid and susceptibility to enzymatic attack by a variety of rat tissues. Several new derivatives of di(glycylamino)propionic acid have been synthesized.

EXPERIMENTAL

$\alpha,\alpha$-Di(N-methylglycylamino)propionic Acid Hydrochloride—10 gm. of $\alpha,\alpha$-di(chloroacetamino)propionic acid (1) were dissolved in 100 cc. of a 25 per cent aqueous solution of methylamine, and the solution kept at 40° for 2 days. On evaporation in vacuo a yellow oil was obtained, which, on being shaken with absolute alcohol for several hours, yielded a mass of fine white crystals. The material was filtered at the pump, dissolved in hot water, and recrystallized by the cautious addition of hot alcohol. The product crystallized in tufts of long needles. M.p. 215° with decomposition; yield 3.5 gm.

$$C_{6}H_{13}O_{4}N_{4}\cdot\text{HCl}.$$ Calculated. C 38.2, H 6.8, N 19.8, Cl (ionic) 12.5


$\alpha,\alpha$-Di(N-dimethylglycylamino)propionic Acid Hydrochloride—The preparation of this product followed essentially the same procedure as that described for the monomethyl derivative above, except that a 25 per cent aqueous solution of dimethylamine was employed. The product crystallized in tufts of long needles. M.p. 215° with decomposition; yield 3.5 gm.
lized in the form of long needles. M.p. 219° with decomposition; yield 5.2 gm.

C₉H₉O₄N₂·HCl. Calculated. C 42.5, H 7.4, N 18.0, Cl (ionic) 11.4  
Found. " 41.8, H 7.3, " 17.7, " " 11.2

α,α-Di(chloroacetylglucylamino)propionic Acid—20 gm. of α,α-di(glucyl-  
amino)propionic acid hydrochloride (1) were dissolved in 100 cc. of chilled  
2 N NaOH and, with further cooling, treated alternately and with shaking  
with 30 gm. of chloroacetyl chloride and 200 cc. of 2 N NaOH. At the end  
of the reaction the mixture was treated with 5 N HCl to pH 2.0, filtered,  
and evaporated in vacuo to dryness. The residue was extracted with hot  
acetone and filtered. To the cooled filtrate ethyl acetate was added drop-  
wise. The first oily precipitate was discarded, and with further addition  
of ethyl acetate a yellowish, granular precipitate appeared. The material  
was filtered off and washed several times with dry ether, which removed  
the last traces of color. The product was redissolved in acetone and treated  
again with ethyl acetate. Repetition of this procedure yielded 12 gm. of  
a white, powdery product which possessed no definite crystal form; m.p. 98°.

C₁₀H₈O₅N₂·HCl. Calculated. C 35.6, H 4.3, N 15.1, Cl 19.1  
Found. " 35.7, " 4.4, " 14.4, " 18.1

α,α-Di(glucylglucylamino)propionic Acid Hydrochloride—2 gm. of di-  
(chloroacetylglucylamino)propionic acid were dissolved in 40 cc. of 28 per  
cent ammonia and kept in a sealed flask for 36 hours at 40°. At the end  
of this period the solution was evaporated at 20° to dryness, and the residue  
was washed several times with alcohol to remove ammonium chloride. The  
compound was then taken up several times in the minimum amount of  
water and precipitated each time with an excess of absolute alcohol. A  
white powder was finally obtained which had no definite crystal form.  
Yield 1.1 gm.; m.p. 164° with decomposition.

C₁₀H₈O₅N₂·HCl. Calculated. N 22.7, Cl (ionic) 9.6  
Found. " 21.9, " " 9.3

The enzymatic susceptibility of the di(acylamino)propionic acids was  
tested in the manner described (1, 2). All substrates were stable in aque-  
ous solution and in the presence of boiled tissue preparations. The digests  
consisted of 1 cc. of freshly prepared aqueous rat tissue extract, 2 cc. of 0.15  
m M borate buffer at pH 8.1, and 1 cc. of either water or 0.025 M substrate  
solution. The extracts were prepared by grinding the tissues with clean  
sand and homogenizing with distilled water, followed by light centrifuga-  
tion. The rate at which ammonia and pyruvic acid appeared over that  
of the controls, during the period when such a rate was nearly linear, was  
taken as a measure of enzymatic activity. 1 mole of ammonia appeared  
per mole of substrate hydrolyzed (1). The temperature of digestion was
37°. Solutions of di(chloroacetylglucylamino)propionic acid were brought to pH 7.0 with dilute NaOH before addition to the digests. The data are given in Table I.

DISCUSSION

Di(glycylamino)propionic acid, in effect, may be considered as glycyldehydroalanine to which a mole of glycynamide has been added at the double bond (1). For this reason, and because the participation of dehydropeptidase has been suspected in the enzymatic degradation of the di(acylamino)propionic acids (1, 2), a comparison of the susceptibility of analogously

**Table I**

**Enzymatic Hydrolysis of Di(glycylamino)propionic Acid and Derivatives in Rat Tissue Extracts**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Kidney</th>
<th>Pancreas</th>
<th>Spleen</th>
<th>Brain</th>
<th>Liver</th>
<th>Hepatoma*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha,\alpha)-Di(glycylamino)propionic acid†</td>
<td>50</td>
<td>15</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>(\alpha,\alpha)-Di(N-methylglycylamino)propionic acid†</td>
<td>24</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>(\alpha,\alpha)-Di(N-dimethylglycylamino)propionic acid</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(\alpha,\alpha)-Di(chloroacetylglucylamino)propionic acid</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(\alpha,\alpha)-Di(glycylglycylamino)propionic acid</td>
<td>38</td>
<td>14</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

* Primary tumors induced in Osborne-Mendel rats by feeding p-dimethylaminoazobenzene; donated by Dr. J. White.
† Pyruvic acid determined in the digests as heretofore (1) was practically equimolar with the ammonia evolved.

constituted dehydropeptides of alanine and di(acylamino)propionic acids is of interest (3).

For both dehydropeptides and di(acylamino)propionic acids, the most active normal tissue is kidney, followed in descending order by pancreas, spleen, brain, and liver. The susceptibility of both glycyldehydroalanine and di(glycylamino)propionic acid is higher in extracts of the hepatoma than in those of normal liver. The susceptibility in normal tissue extracts of both N-methylglycyldehydroalanine and di(N-methylglycylamino)propionic acid is roughly half that of their respective unsubstituted parent compounds, while in the hepatoma extract the susceptibility of the N-methylated compounds practically vanishes. No comparison can be made of the N-dimethyl analogues because of the failure to synthesize N-di-methylglycyldehydroalanine (4). Like chloroacetylglucyldehydroalanine,
di(chloroacetylglcylamino)propionic acid is not apparently enzymatically hydrolyzed. When, however, both compounds are aminated, to form the respective glycyglycyl derivatives, the latter are again enzymatically susceptible. These findings may be related to the absence in tissues of an enzyme capable of hydrolyzing the bond between chloroacetic acid and glycine and to the presence of a peptidase which rapidly hydrolyzes the bond in glycylglycine (cf. (3)).

The enzymatic hydrolysis of di(glycylamino)propionic acid has been considered to involve either one or perhaps both of the following mechanisms (1): (a) an initial enzymatic hydrolysis at one of the two glycylamino linkages, leading to the formation of glycine and the unstable α-amino-α-glycylaminopropionic acid which spontaneously decomposes to ammonia, glycine, and pyruvic acid, or (b) an initial attack at the bond between a glycylamino residue and the tertiary carbon atom, leading to the formation of glycine and glycyldehydroalanine, the latter being subsequently hydrolyzed by dehydropeptidase to glycine, ammonia, and pyruvic acid. The end-products of the hydrolysis are presumably the same by either mechanism. The similarity in susceptibility of analogously constituted dehydropeptides and di(glycylamino)propionic acid derivatives in normal tissues and in the hepatoma appears to support mechanism (b), but this similarity, although interesting, may possibly be deceptive. Studies of variously constituted substrates with different tissues are capable of providing some illumination but are not decisive. It appears highly reasonable that the hydrolysis of the di(acylamino)propionic acids occurs in two consecutive steps, and that the initial reaction requires in the susceptible substrate a basic α-nitrogen atom to which at least 1 hydrogen atom is attached. The problem concerned with the course of this hydrolysis will be solved when both steps are separated and individually characterized. Work in this direction is being pursued by parallel studies on saturated peptides with purified tissue fractions.

**SUMMARY**

α,α-Di(N-methylglycylamino)propionic acid hydrochloride, α,α-di(N-dimethylglycylamino)propionic acid hydrochloride, α,α-di(chloroacetyl-
glycylamino)propionic acid, and $\alpha,\alpha$-di(glycylglycylamino)propionic acid hydrochloride were prepared, and their hydrolysis in various rat tissue extracts followed mainly by the rate of ammonia evolution over the controls. The monomethylglycyl and glycylglycyl derivatives were hydrolyzed by all normal rat tissues studied, whereas the dimethylglycyl and chloroacetylglycyl derivatives were not attacked. In extracts of hepatoma, the susceptibility of di(glycylamino)propionic acid is higher than in liver, whereas that of di(N-methylglycylamino)propionic acid nearly vanishes. Comparison is made between analogously constituted dehydropeptides and di(glycylamino)propionic acid derivatives, and certain similarities are described.

BIBLIOGRAPHY

DERIVATIVES OF $\alpha,\alpha$-DI(GLYCYLAMINO)PROPIONIC ACID
Jesse P. Greenstein and Vincent E. Price

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