Phenylcarbamoyl Derivatives of Insulin

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Much research has in the past been devoted to the chemical modification of insulin, mainly because of its relative stability and unique physiological action. The advent of refined separation techniques has made it possible to investigate further the complexity of such protein derivatives.

Acta Chem. Scand. 8 (1954) No. 9
graphy in ultraviolet light. An estimate of the ratio of the two hydantoins was obtained by cutting out the spots, eluting with 0.1 \( N \) alkali and determining the optical density of the eluates at 239 \( \mu \). The method is not very exact \((\pm 10\%)\) owing to a rather high and varying absorption of the paper blanks. The results obtained are summarised in Table 1.

**Table 1.**

<table>
<thead>
<tr>
<th>Peak</th>
<th>Gly.hyd. + Phe.hyd. moles per mole of derivative</th>
<th>Molecular ratio Gly.hyd.: Phe.hyd.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>theoret.</td>
<td>found</td>
</tr>
<tr>
<td>( I )</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>( D_1 )</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>( D_2 )</td>
<td>2</td>
<td>0.85</td>
</tr>
</tbody>
</table>

\( D_1 \) is obviously a mixture of the two monosubstituted derivatives, consisting mainly \((\geq 90\%)\) of the mono-derivative substituted on the N-terminal glycine \((D_{1,\text{ Gly}})\). The yield obtained of Gly.hyd. (about 63\%) is consistent with the fact that Gly.hyd is unstable under the strongly hydrolytic conditions of the cyclisation, 57\% of Gly.hyd being recovered after 18 hours. The corresponding figure for Phe.hyd. is 90\%. This difference in stability of the two hydantoins indicates that the actual molecular ratio of \( D_{1,\text{ Gly}} \) to \( D_{1,\text{ Phe}} \) is even greater than 10. It will be noticed that the yield of Gly.hyd. from \( D_1 \), calculated on the basis of a minimum molecular weight for insulin of 12 000 would be \( > 100\% \). The present experi-

![Fig. 1. Elution chromatogram of partially substituted insulin. Absc.: Number of fractions (23 ml). Ord.: Optical density at 277 \( \mu \).](image)

**Acta Chem. Scand.** 8 (1954) No. 9
with that obtained from $D_1$. The possibility that $D_4$ is a mixture of di-derivatives must not be overlooked. It seems reasonable to suppose, however, that the derivative substituted on both N-terminal aminogroups is the major component.

The course of the substitution may be presented as follows:

$$
\begin{array}{c}
\text{k}_1 \quad \text{k}_2' \\
I \quad \downarrow \quad \downarrow \\
D_{1,\text{Gly}} \quad D_1 \\
\text{k}_2 \quad \text{k}_1' \\
D_{1,\text{Phe}} \quad D_1
\end{array}
$$

where $k_1$ ($k_2'$) and $k_2$ ($k_1'$) represent the (second order) rate constants for substitution at the N-terminal glycine and phenylalanine residue, respectively. Examination of Fig. 1 shows that the amount of each component is approximately $I = D_1 = 3 \cdot D_4$ and from Table 1 is obtained $D_{1,\text{Gly}} \gg D_{1,\text{Phe}}$. These facts cannot be reconciled unless it is assumed that $k_2' > k_2$, that is: the amino group of the N-terminal phenylalanine must react faster in the mono-derivative $D_{1,\text{Gly}}$ than in insulin itself. The supposition that this summaskings is an intermolecular effect caused by a higher degree of dissociation of the mono-derivative into subunits as compared with insulin, is not substantiated by ultracentrifuge experiments. The possibility remains that the effect is of intramolecular origin. This would seem to require the distance between the groups involved to be rather short, or in different terms: The A and B chains of the insulin molecule would appear to be parallel rather than anti-parallel. This last conclusion is in agreement with the allocation of the cystine bridges recently published by Sanger.

Further details will be published in Compt. rend. trav. lab. Carlsberg, Sér. chim.

The author is greatly indebted to Professor K. Linderström-Lang for encouragement and helpful discussions during the course of this work and to Nordisk Insulin Laboratorium for financial support and for a gift of crystalline pork insulin.


Received October 6, 1954.

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**Additional Note on α-Aminopimelic Acid in Green Plants**

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In an earlier paper we reported the detection of α-aminopimelic acid in *Asplenium septentrionale* by the paper chromatographic method. We have now been able to isolate α-aminopimelic acid from the same plant by cutting out the spot in question from some paper chromatograms before the treatment with ninhydrin (thick Munktell 20 paper was used). The pieces of paper were extracted with water, the extract evaporated to dryness, and the solid rest extracted with a mixture of abs. alcohol and ether in order to remove browncoloured impurities. The white microcrystalline substance (2 mg from 280 g fresh *Asplenium*) which on the paper chromatogram gave only one spot identical with the spot of α-aminopimelic acid, had a melting point of 204°C. Synthetic α-aminopimelic acid had a melting point of 203°C and the mixture of both 204°C. 1.4 mg of the isolated substance used 0.76 ml 0.01 N NaOH (0.304 mg NaOH), calculated for α-aminopimelic acid 0.80 ml (0.320 mg NaOH).

The isolated α-aminodecarboxylic acid is thus α-aminopimelic acid. Its $R_F$-value in phenol + NH$_3$ is 0.40 using Whatman 4 paper; in butanolic acid the $R_F$-value corresponds to that of γ-aminobutyric acid.

We have found free α-aminopimelic acid by the paper chromatographic method also in the seeds of a legume tree, *Ceratinia silicula*.


Received October 6, 1954.